A Cell-Based Model Selection Framework for Explanations of Zebrafish Retinal Organogenesis

by

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Abstract

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Abstract text

Dedication text

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Introductory Notes

Many different, well substantiated explanations for various aspects of eye development exist. A recent, influential explanation for retinal progenitor cell (RPC) function in the zebrafish, with important fundamental and therapeutic implications, uses mathematical modelling techniques to argue that RPC function can be explained in terms of "stochastic" effects internal to the cells (dubbed herein the Internal Stochastic Processes explanation or ISP). This thesis attempts to clarify the meaning of these claims, and to test whether their predictions hold in the little-studied postembryonic period of zebrafish organogenesis. After finding that the ISP is substantially incomplete, a model comparison framework allowing evaluation of the relative quality of quantiative "systems biology" models used to explain retinal progenitor cell (RPC) function is advanced. Finally, this framework is applied to a novel zebrafish RPC mutant, *rys*, to evaluate its utility.

In the first chapter, I review the plethora of mechanisms offered to explain aspects of RPC function, and assess the theoretical motivation for the ISP. I next examine the origins of the simple stochastic model (SSM) used by the ISP in the classic work of Till and McCulloch, identifying the traditional source of the ISP's model-system conflation. I argue this has resulted in the preoccupation with the stochastic features of the model, over the sources of variability in RPC lineage outcomes. I subsequently assess the collection of models advanced to support the ISP and find that their behaviour is largely produced by model elements with no clear biological referent. Finally, I use evidence collected from postembryonic zebrafish retinal development to demonstrate that these models are severely underfit to the phenomenal evidence, rendering them incapable of explaining the quantitatively and ethologically most important period of retinal organogenesis, driven by the circumferential marginal zone (CMZ) RPC population.

The second chapter develops a global model selection framework, motivated by the issues observed in the first chapter's examination of ISP models. By adding a measure of retinal spatial structure to the usual considerations of RPC lineage outcomes, I permit the use of information-theoretical techniques to evaluate the relative quality of a variety of models and their components.

The third chapter

Terminology and Style

Throughout Chapter 1, I have used the apellation "Harris" when referring to the output of William Harris' research group. This is a large body of research spanning several decades, and involves many co-authors. My use of "Harris" here is a convenience, as Harris is the only common author across the period in question, and presumably the agent carrying these ideas forward from project to project. It is not intended to slight or minimize the contributions of any of the other members of Harris' group (many of whom are now senior scientists in their own right). I have occasionally referred to the output of other

labs by the senior scientists' surname in an identical manner, where appropriate.

I have used the term "differentiation" solely in its conventional English sense, meaning the distinguishing of two objects by the development of differences between them. The word typically refers to the process by which cells take on a particular specified fate. I have referred to this exclusively as "specification", which I understand to mean the assumption of a stable macromolecular identity or "fate". This term is intended to correspond exactly with the appearance of stable markers of cell type. In many cases relevant to this study, cells may be "differentiated" (ie. from one another) without being specified, as in the case of a progenitor cell which differs from its neighbours in RNA transcription profiles, without any of these cells yet being specified. In general, I have eschewed the use of terms like "commitment", since it remains unclear after many decades of use what the biological referent of these terms might be.

There are a number of specialised philosophical terms that appear in quoted material in the theoretical appendix. I have made occasional use of some use of them to save space. These are as follows:

iff - "if and only if"

explanans - the fact to be explained

explanandum - the explanation itself

Unless otherwise noted, formatting of quoted material is preserved, so that italic emphases appear in the original. An exception to this is citations in original material, which have been removed wherever irrelevant or uninformative (ie. numbered references). I have indicated my own editorial comments and alterations to quoted material with [square braces].

Use of Hyperlinks and Tooltips within and between documents

For the reader of the .pdf document, I have made use of hyperlinks so that key concepts which are referred to in the data chapters may be defined seperately in the prolegomenon. These links appear in an plum color so as not to disrupt the reader. In order to facilitate returning to the appropriate section on different .pdf readers, I have, at the end of each prolegomenon section which is referenced in a data chapter, enumerated which data chapter sections have the relevant links.

I have also embedded tooltips that allow a .pdf reader to place the mouse cursor over any abbreviation to display the full term, to assist with the large number of technical terms used.

Chapter 1

Systems Approaches to Retinal Organogenesis

1.1 The Harris ISP Explanation

This chapter comprises a critical examination of the best-developed theory of *D. rerio* retinal progenitor cell (RPC) function, and a broader, global general modelling approach motivated by the examination's findings. The theory in question originates with pre{:eminent retinal biologist William Harris' research group, referred to hereafter the Internal Stochastic Effects (ISP) explanation. This theory purports to explain the function of zebrafish RPCs in terms of the stochastic effects of two particular transcription factors.

The ISP explanation for RPC function is of fundamental theoretical and practical interest. It arises from a concerted effort by the Harris group to make sense of the difficult problem of explaining how a complex tissue like a retina can arise from a field of similar proliferating cells. In 2009, Harris coauthored a detailed review chapter documenting the bewildering array of macromolecules and cellular processes thought to be involved in RPC function [AH09]. This enumeration contains many caveats and notes that the effects of particular macromolecules routinely differ between developmental stages, cell types, organisms, and so forth. At this time, no clear, detailed, comprehensive models of RPC function had been advanced, and the review is typified by statements like "It is difficult to reconcile all the studies on the initiation and spread of neurogenesis in a single model."[1] It is therefore remarkable that, over the next nine years, the Harris group would go on to promulgate a simple stochastic model of zebrafish RPC function invoking only two named macromolecules.

Harris thus seems to be making a bold attempt to cut the Gordian knot of conflicting evidential threads and advance a simple, comprehensible, "mind-sized" model of RPC function. In effect, Harris' explanation for zebrafish RPC function is a microcosm of the broader promise of "Systems Biology" to make sense of the contemporary welter of conflicting datasets. By using sophisticated mathematical methods drawn from information and complexity theories, the apparent confusion will be clarified and underlying molecular mechanisms will be revealed. Given Harris' track record and preeminence in the

[1] This was in no sense a problem with Harris' understanding. A similarly high-level review coauthored by Pam Raymond [AR08] concluded, with regard to models of photoreceptor fate specification: "The data reviewed in the preceding sections indicate that a one-size-fits-all model is not possible..."

field, we have good reason to take seriously the possibility that he has succeeded, and therefore should examine whether this is the case. As I believe Harris' effort has gone somewhat awry, it will be useful to begin by summarising the state of the art at the time of his 2009 review (as well as relevant subsequent additions), in order to appreciate the scope of his theoretical maneuver, and possible alternatives to it.

1.2 Explanations for RPC function in 2009 and the drive to unification

In many ways, molecular biologists have been attempting to explain the same remarkable features of retinal progenitor cells for decades. Animal retinas, having relatively well-understood functions and highly stereotypical structures, seem like highly tractable tissues for typical molecular biological explanations. With well defined cell types present in tightly regulated, neurotopologically limited proportions and organisations, it is no surprise that both theoretically-inclined molecular biologists and clinically-inclined regenerative medical practitioners have been keenly interested in the the retina[2]. The high level of regular, easily detected order in eyes seemed to suggest a similar level of order and regularity in the macromolecular processes which underlay the formation of the tissue. Retinal biologists have long offered explanations suggesting that RPCs are more-or-less identical and go through highly stereotypical macromolecular processes. It is, however, the persistently observed departures from this (by now, obviously naïve) conception that have occupied most of our attention.

In general, we may crudely gather the RPC-related phenomena studied by biologists under the headings of proliferation, specification, and organisation. A molecular biologist guided by a preference for cognitive simplicity (that is, Occam's Razor) will reasonably suppose that the simplest explanation for the regularity of retinal development is that any given RPC is executing the same strict "developmental program" as its neighbours. In other words, any one RPC lineages' proliferative and specificative outcomes are the same as any other RPC lineage. If every progenitor produces a similar number of cells, and the progeny are specified in similar proportions, well-understood principles of cellular adhesion could understandably give rise to the characteristic organisation we observe in animal retinas. However, by the 1980s, vertebrate lineage tracing experiments were routinely revealing a surprising degree of inter-lineage variability in many neural progenitor systems, not least of which was the retina. In their classic 1987 paper, David Turner and Connie Cepko, using retroviral lineage labelling techniques, demonstrated that individual RPC lineages in rats had a wide range of proliferative and specificative outcomes [TC87]; Harris' group confirmed this result in *Xenopus* the subsequent year [HBEH88], suggesting this variability was a common feature of vertebrate RPC function.

Indeed, at this point, we find fairly clear accounts of what retinal biologists took their theoretical options to be in explaining this variability. As Harris' 1988 states:

> Changes in cell character associated with cell type diversification may be controlled in an autonomous way, reflecting either a temporal program inside the cell (Temple and Raff, 1986), the asymmetrical segregation of cytoplasmic determinants (Strome and Wood, 1983; Sulston and Horvitz, 1977), stochastic events inside the cell (Suda et al., 1984), or some combination of these processes. Alternatively, cell type may be controlled in a nonautonomous way, as in cases in which the extracellular environment (Doupe et al., 1985) or cellular interactions (Ready et al., 1976) elicit or limit cell fate. With its multiplicity of cell types, the vertebrate

[2]Indeed, if central neuroregenerative medicine is to become a clinical possibility, it seems likely that the theoretical and practical issues are most likely to be resolved in eyes before other areas of the CNS.

> nervous system would seem to require the ultimate sophistication in its means of cellular determination. [HBEH88]

It is striking, then, that Harris' review of the literature two decades later describes the situation similarly:

> Once differentiation is initiated, regulatory mechanisms within the retina ensure that progenitors retain the capacity to undergo more divisions, in parallel with churning out differentiated cells, and that progenitors cease dividing at variable times. There is still debate about the extent of early programming that allows progenitors to step through a series of stereotypical divisions and the extent of regulation from within the whole retina. The production of differentiated cells alters the retinal environment with time...
>
> Moreover, cells from the same clone do not all differentiate at the same time, suggesting three possibilities: a stochastic mechanism for the decision to differentiate, exposure of the two daughters to different environments, or asymmetric inheritance of determinants. [AH09]

In the same paper, he states that the "simple structure and accessibility of the retina make it a useful model to study cell division and differentiation, and as a result most aspects of this have been studied, from lineage tracing of progenitors, to the morphological aspects of division, to the molecular mechanisms involved." Thus, by Harris' own account, some twenty years of additional research into almost every variety of macromolecular explanation for a huge range of RPC-related phenomena had not provided any means to narrow down the possibilities he had already laid out in 1988. We still have Raff's temporal program ("early programming ... step[ping] through a series of steretypical divisions"), asymmetric segregation of cytoplasmic inheritants during mitotic events, "stochastic" events internal to the cells, and possible "environmental" extracellular determinants. While the number of particular macromolecules functionally implicated in proliferative, specificative, and organisational RPC phenomena had greatly expanded, this had not provided any means to differentiate between these theoretical options. This is only one example of a general phenomenon experienced by molecular biologists, in which enumerationist research programs, directed at producing more and more facts about macromolecular involvement in cellular phenomena, have failed to generate additional theoretical understanding. Harris' ISP explanation therefore represents an example of what I have termed in the Theoretical Appendix the "Systems Biology Encounter", in which biologists apply the analytical and interpretative methods of the physical and mathematical sciences in an effort to resolve the problems posed by biological complexity.

Before proceeding to the ISP itself, let us briefly summarise the diversity of phenomena implicated in RPC function by 2009, as well as the panoply of mechanisms offered as explanations. In doing so, it will become clear what has been elided in the ISP explanation, and what may need to be restored in any alternative modelling approach.

1.3 Canonical vertebrate RPC phenomena: the RPC "morphogenetic alphabet"

The majority of our knowledge of RPC behaviour stems from histological observation employing a limited number of techniques. Simple observations of mitotic figures in a variety of animals had, by the 1950s, revealed the surprising diversity of RPC proliferative phenomena across vertebrate clades. However, it was the advent of lineage tracing techniques, particularly those marking single clonal lineages in whole

retinae, and the extensive use of these techniques in the 1980s-90s, that formed most of the basic body of observations that any macromolecular explanation is now called upon to account for.

Since the majority of vertebrate retinas of biomedical interest are mammalian, and these retinae are fully formed in an early developmental period, RPC behaviour has been best-studied in an embryonic and early developmental context. Here, vertebrate RPCs are derived from the eye field population of the early neural plate and later neural tube. This population is separated into left and right eye primordia, which in turn pouch outwards toward the ectoderm, and, in conjunction with the lens placode (itself induced from the ectoderm), form the optic vesicle. The primitive eye is formed when this vesicle completes a complex morphological folding process, resulting in the cup-shaped structure of the retina [Cav18]. During this process, the cells of the neural retina are differentiated from the overlying retinal pigmented epithelium (RPE). Sometime after the formation of the retinal cup, RPCs begin to exit the cell cycle and are specified as retinal neurons. Studies of this early period revealed numerous difficult-to-explain features of RPC behaviour. Following Larsen's observation that tissue form is attributable to only six behaviours [Lar92], which she describes as the "morphogenetic alphabet", I have categorised RPC phenomena as relating to proliferation, specification, migration, growth, death, and extracellular matrix formation[3]. Since the vast majority of reported phenomena fall under the first two categories, those belonging to the last four are described collectively.

1.3.1 Proliferative phenomena

Clonal lineage tracing experiments have reliably found that vertebrate RPCs give rise to highly variable numbers of offspring over the collective proliferative "lifetime" of their descendants. The most dramatic of these findings found that rat RPC lineage sizes vary across two orders of magnitude in vivo, from 1 to over 200 [TSC90][4]. The physical organisation of these clones is complex; as detailed below, RPC progeny may appear in any of the 3 retinal layers in a wide variety of specified fate combinations, and may engage in short-range migrations to appropriate positions for their specified fates. Most clonal lineages are "extinguished"; that is, after some time, all of its members have become postmitotic. However, it has long been noted that not all cells produced by RPCs are strictly postmitotic neurons; specified Müller glia retain the ability to reenter the cell cycle in response to stimuli (normally, retinal damage) [DC00, FR03], and peripheral RPCs remain proliferative in those vertebrates whose eyes grow beyond early development (notably in frogs and fish, while the chick retina has a CMZ of more limited potential [FR00]). Therefore, some clonal lineages may be organised into clumps associated with Müller responses, while others in frogs and fish may continue to be "plated out" in a more-or-less linear manner at the retinal periphery for as long as the lineage "lives" [CHW11]. This ongoing RPC contribution to peripheral neurogenesis has long been recognised, so that by 1954 we find the following remarkable statement casually introducing a study of unusual mitoses in the retina of a deepsea fish:

> It is conventional[5] to hold that the growth of the vertebrate retina is only possible due to the presence, in this tissue, of a peripheral germinal zone. In this region, young elements

[3] I have renamed Larsen's categories to clarify them for the retinal context, but retained her conceptual scheme, which is discussed in more detail in the Theoretical Appendix.

[4] While at least some of this variability must be related to differential integration of lineage markers into "older" (giving fewer offspring) and "newer" (giving more) RPCs, it is generally accepted that vertebrate RPC lineages tend to vary in size by at least one order of magnitude, from less than ten to tens of neurons.

[5] Unfortunately, I am unable to locate the source of this convention, likely due to the poor preservation of many of these older reports. That this required no citation in 1954 suggests the original observations of CMZ proliferation may be in the early 20th century.

> actively multiply, and, by subsequent differentiation, give rise to the diverse nervous and sensory constituents of the retina. [VL54]
>
> [author's translation from the French]

Despite this, the proliferating RPCs in this "peripheral germinal zone" (also known as the "ciliary marginal zone", or CMZ, for its proximity to the retinal ciliary body) have not received the same level of attention as those associated with the central retina. As a consequence, these RPCs have generally, and in particular by Harris, been treated as though they are a type of "frozen" progenitor population, recapitulating spatially, along the peripheral-central axis, the process which RPCs in the central retina undergo in a time-dependent fashion[6] [HP98].

The length of the RPC cell cycle has been of considerable interest, since the evolution of this parameter in time, in conjunction with the RPC population size (the number of cells specified in the eye field), determines the eventual size of differentiated retinal neural population, and therefore the retina. RPC cell cycle length has generally been inferred from clonal lineage size, although it has also been occasionally assayed directly in cumulative thymidine analogue labelling experiments. Vertebrate RPCs have generally been found to undergo a period of relative quiescence, in which the cell cycle lengthens, before the neural retina begins to be specified (in zebrafish, this period is 16-24 hpf). The cell cycle shortens as RPCs begin to exit the cell cycle [HH91, LHO+00]. After the central retina is specified, the RPC cell cycle once again lengthens, and is presumed to continue to slow until RPCs have completed differentiation[7].

Finally, the orientation of the RPC division plane in mitosis has also been implicated in retinal organisation. The orientation of divisions has been associated both with the proliferative and differentiative fate of RPC progeny. For instance, interfering with spindle orientation in the developing rat retina, such that more RPC divisions occur parallel to the neuroepithelial plane (rather than along the apico-basal axis) results in more proliferative and fewer postmitotic, specified progeny[ŽCC+05]. That said, it seems that whatever effects are attributed to mitotic orientation are likely species-specific, as zebrafish RPCs display a different pattern of axis orientation, dividing mainly in the epithelial plane[DPCH03].

1.3.2 Specificative phenomena

Irrespective of their location in the retina, vertebrate RPC lineages have offsping which may enter any of the three cellular layers of the retina. Moreover, single lineages can include any possible combination of cell fates, so that RPCs cannot be said to be of different "types" on the basis of lineage fate outcomes [HBEH88, TSC90, WF88]. While some specified progenitors have propensities to give rise to similar cell types, these relations appear to be species-specific, and do not seem to define separate progenitor pools [AR08]. In general, then, RPCs are taken to be totipotent with respect to the neural retina- all of the cell types[8] of the differentiated retina are derived from similar RPC lineages. Very little about this picture has changed since its initial development, using a variety of lineage tracers (including retroviruses, thymidine analogues, and injectable dyes) and histochemical markers to supplement morphological identification of specified neurons. In particular, sophisticated modern live imaging experiments in zebrafish

[6] Since both early central and later peripheral proliferation and specification are similarly, and non-trivially, organised both in space and time, this idea will be addressed in somewhat more detail below.

[7] This presumption is demonstrably incorrect in the /textitD. rerio eye, see INSERT SECTION

[8] The "cell type" concept is unusually well-defined in the retina, as there are an abundance of distinct morphological and molecular features which differentiate numerous subtypes of the seven general types of retinal neuron.

(many pioneered by Harris), have broadly confirmed the findings of the 80s and 90s in mammalian fixed specimens, explants, and the like [BRD+15].

Of particular note here are the observations of the Raff group [WR90, CBR03], who demonstrated that dissociated rat RPCs, cultured at clonal density, took on morphological and histochemical features associated with different specified neural types in similar numbers and proportions, and on a similar schedule, to same-aged RPCs cultured in retinal explants. These results dramatically suggested that both the proliferative and specificative behaviour of RPCs depended less on intercellular contact, and the complex signalling environment of the developing retina, than on factors intrinsic to the RPCs themselves. These studies contain the essential germ of Harris' eventual commitment to the ise, purporting as they do to "test the relative importance of cell-intrinsic mechanisms and extracellular signals in cell fate choice" and providing convincing evidence for the preponderant importance of the former.

In spite of the apparent ability of RPCs to produce offspring specified to any of the possible neural cell fates, at any time during their lineage history[9], vertebrate retinal development displays a temporal ordering, such that in any particular location, retinal ganglion cells (RGCs) tend to be produced first, followed by the other cell types in what has been described as an overlapping "histogenetic order", pictured in Figure . As noted above, RPCs also exit the cell cycle in a spatiotemporally defined order (from central to peripheral over time), and specification follows the same pattern[10]. This naturally gave rise to some question about the origin of the "overlap" observed in the sequential production of various cell types; conceptually, this overlap could be produced by identical RPCs executing identical rigid specificative programs if they begin to execute it in the spatiotemporal gradient noted above. It is impossible to reconcile this notion with the results of Harris' in vivo zebrafish lineage tracing studies [DPCH03, HZA+12, BRD+15], which confirm mammalian cell culture work in demonstrating that vertebrate RPC lineages do not execute identical (or even vaguely similar) specificative programs.

1.3.3 Other morphogenetic phenomena

The outcomes of RPC proliferation and differentiation have generally been taken to be sufficient to explain the formation of the neural retina. Indeed, after the eye cup has been formed (prior to the differentiation of any neurons), RPCs are, in effect, already "in place". Therefore, there are few documented RPC phenomena outside of the "proliferation" and "specification" categories of the [alhpabet]morphogenetic alphabet, which ennumerates the cellular processes contributing to tissue form and structure. RPCs do not seem inclined to migrate long distances , they seem not to generate much in the way of extracellular material[11], and, in non-pathological conditions, they are rarely seen to die. Still, there are a number of phenomena which appear to be important for proper retinal organisation that fall into these other "alphabet" categories.

The most notable of these is interkinetic nuclear migration (INM), in which RPC nuclei move back and forth between the apical and basal surfaces of the retina. Common in many neural tissues, INM affects both proliferative and differentiative outcomes, but is dissociable from them- both cell cycle

[9] Even in papers arguing for a strict, linear sequence of specificative outcomes in all RPC lineages, the actual data show RPCs occasionally giving rise to "late-born" photoreceptors subsequent to their first division[WR09], giving lie to the notion that the process is particularly strict.

[10] In many studies, cell cycle exit is conflated with specification such that evidence of the former is taken as evidence of the latter. There are, however, reasons to believe cell cycle exit and specification are not the same process, discussed below.

[11] The formation of a laminin-rich basement membrane seems to be necessary for optic vesicle formation [ICW13], but it is not clear that RPCs produce this. ECM function in eye formation remains under-studied.

and differentiation proceed (albeit in a precocious manner) when INM is disrupted[MZLF02]. The environment provided by the apical retinal surface appears to be required for RPC mitosis to proceed[12], and INM seems to consist of a directed, probably actomyosin-mediated movement to the apical surface, followed by an undirected "random walk" away from the apical surface and hence toward the basal retina [NYLH09]. More committed RPCs also appear to actively migrate to positions appropriate for the specified cell type [CAR+15, IKRN16], although it is unclear to what extent this short-range migration is related to INM undergone by more actively proliferating progenitors. It is likely that these short-range migrations of more specified cells are especially significant in the early retina, before the neural plexiform layers (consisting of axons and other neural processes) have begun to divide the cellular layers into bounded compartments.

Notable lacunae in the study of the RPC morphogenetic alphabet include the regulation of cell size and growth, and potential roles for cell death, both of which have hardly been studied at all. While cell size and growth are known to be tightly linked to proliferative behaviour in yeast [YDH+11], any related effects in RPCs have not been elucidated. This may be a significant oversight, given the requirement for RPCs to continuously grow during their proliferative lifespan. Cell death does not seem to play the same "pruning" role for RPCs that it often does in other neural tissues, and observed rates of cell death in normal RPC populations are very low, so few studies have been conducted.

1.4 Macromolecular mechanistic explanations for RPC phenomena

Having surveyed the cellular phenomena pertaining to RPC function in retinal development, let us examine a selection of the many macromolecular mechanistic explanations (MEx) that have been offered to explain aspects of RPC behaviour. Unsurprisingly, given the field's focus on the early events of eye development, these MEx are intended mainly to explain the phenomena associated with this period. Thus, the majority of MEx offered have been concerned to explain tissue-level phenomena like the initial "wave" of cell cycle exit and specification, without necessarily seeking a global explanation for RPC behaviour irrespective of context, so that it is unknown how many of these might pertain to ongoing peripheral neurogenesis or other, adult neurogenic phenomena like those exhibited by Müller glia. That said, we now turn to examine some of the best-developed of these explanations.

1.4.1 Transcription factor networks

Perhaps the most notable MEx offered to explain RPC specification and development is the eye field transcription factor network, or EFTFN. The roots of this MEx are found in the Pax6 "master gene" explanation popularised in the 1990s [Geh96]. This explanation revolved around the observation of the apparently universal involvement of Pax6 gene products in eye formation in model organisms, and the promiscuous inter-species effects of Pax6 (with mouse RNA able to induce ectopic formation of eye structures in *Drosophila* imaginal discs, for instance), so that it appeared to be a highly conserved

[12]Nonapical divisions do occur, notably in specified, but proliferative cells [GWC+07]. The extent to which RPCs depend on the apical surface may depend on how "RPC" is defined. In general, RPCs are no longer considered as such when they acquire characters associated with differentiated neurons (cell type markers, morphological traits, etc.), although it is clear that the acquisition of these characters does not necessarily imply that the cell is postmitotic. Any complete explanation of how RPCs give rise to the structure of the eye must also consider these nonapical divisions.

genetic "switch" for eye development.

Importantly, this explanation purported to resolve what Darwin regarded as a serious problem for his theory, the apparent implausibility of the gradual evolution of eyes (and other "organs of extreme perfection") from some primitive ancestral structure [Dar88, p.143-4]. In particular, Pax6 suggested to some theorists a resolution to the conundrum noted by Mayr and Salvini-Plawen, that differences in eye structure and function across clades suggested the independent appearance of eyes in more than 40 clades [**?**]. Pax6 was thus taken to provide a molecular pointer to a potential common ancestor for all animal eyes [EHML09].

Subsequent investigations revealed that vertebrate Pax6 is a conserved member of a complex network of cross-activating and inhibiting transcription factors, including Pax6, Rx1, Six3, Six6, Lhx2, ET, and Tll [Zub03]. Members of this network tend to promote proliferation and suppress markers of differentiated neurons, and their loss commonly results in the failure to form the eye field at all [AH09]. The expansion of this explanation to include other TFs in a network revealed significant differences between species [Wag07]- while the role of Pax6 seems to be conserved between *Drosophila* and vertebrates, other members of the EFTFN are not. Moreover, the universality of Pax6 was only apparent, and not real, as there are bilaterian eyes whose development is Pax6 independent (including in *Platynereis, Branchiostoma*, and planarians) [Koz08]. This highlighted the great difficulty in connecting morphological characters such as those observed by Mayr with a genetic basis- it is simply not clear that Pax6 conservation points to a common ancestor for all eyes, or even all photoreceptive neurons[13]. Moreover, the expansion of the monocausal "master gene" explanation, to include a network of TFs with broad gene regulatory effects, clearly highlighted the problems of complexity in offering MEx for RPC function. The components of this network interact in complex, context-dependent ways, and while the EFTFN as a whole is taken to promote RPC proliferation and to delay specification[14], its individual components have also been held responsible for the specification of particular classes of differentiated neurons, and remain expressed in those postmitotic cells. Notably, Pax6 is implicated in the expression of bHLH TFs required to specify multiple classes of retinal neuron [MAA+01], and is known to directly activate Ath5, necessary for RGC specification [WSP+09]. The EFTFN has thus been taken as an explanation for the maintenance of the multipotent, proliferative RPC state, but how this network is disassembled, and its components repurposed to promote specification, remains obscure.

The EFTFN is not the only transcription factor network offered as a MEx for RPC function. Another well-developed MEx involves Chx10 (aka vsx2), a transcription factor which was found to be important for normal proliferation of RPCs, its loss causing microopthalmia in the mouse [BNL+96]. Chx10 was subsequently found to repress Mitf, which is involved in RPE specification, and hence to promote neural retinal fates over pigmented epithelial ones [Hor04]; in the absence of Chx10 the early eye cup does not stratify properly between apical pigmented cells and the neural retina. Much like the multifunctional EFTFN components, Chx10/vsx2 has also been implicated in the specification of particular neural fates, notably bipolar neurons [BNL+96] and the regulation of Vsx1 (a paralogue of Chx10), Foxn4, and Ath5, associated with specification of subpopulations of bipolar cells, horizontal and amacrine cells, and RGCs and PRs, respectively [CYV+08, VJM+09].

Clear hypotheses advocating for particular relationships between different TF MEx are rarely stated.

[13] Indeed, the relevant "unit" of homology for evolutionary explanations for eyes remains contested, with some arguing for the cell itself over any particular set of gene sequences [EHML09]

[14] This is sometimes referred to as "promoting RPC fate", since RPCs are taken to be those cells which proliferate but do not yet display markers of specification. Since it is, by now, widely recognised that cells that appear to be well-specified may remain in cell cycle [GWC+07, ESY+17] this terminology should probably be jettisoned.

It is tempting simply to arrange them in some kind of "developmental order", perhaps with the Chx10/Mitf network "downstream" of the EFTFN. That this would be facile is evident from the changing roles of these transcription factors depending on developmental and cellular context. To date, no unifying framework has been applied. Obvious candidates include the "developmental gene regulatory network" concept [LD09], a type of cybernetic explanation which assembles genes into feedback networks. Given the popularity of this type of explanation, it is worth noting that no one has yet had any success in offering one for RPC function.

These transcription factor network MEx frequently incorporate extracellular signals (often as an explanation for the appearance or "set-up" of the TF network), and it is generally recognised that these signals have a profound influence on these networks, and on RPC behaviour generally. We therefore turn to explanations invoking these signalling mechanisms.

1.4.2 Intercellular signalling networks

Virtually every developmentally significant class of signal has been implicated in RPC function, so that Harris, by 2009, simply glosses over a majority of these pathways by briefly summarising them in tabular form and not otherwise mentioning them [AH09]. These include BMP, CNTF, FGF, Glucagon, Hedgehog, IGF, Notch, TGFα, TGFβ, VEGF, wnt, and a host of neurotransmitters. This diversity of signalling pathways has proved to be a formidable problem for integrated explanations, since almost all of these pathways converge on the same two cellular outcomes in RPCs, that is, proliferation and specification. Thus, most signalling MEx for RPC function elide the majority of other signals which are known, or thought, to affect the same processes. That said, let us explore a few of the more detailed signalling explanations.

In developmental terms, the first phenomenon requiring explanation is the appearance of the eye field to begin with- what is it that accounts for the differentiation of RPCs from the rest of the anterior neural plate and tube? Wnt signalling MEx have been offered to explain the appearance of the *Xenopus* eye field. Fz3 signalling seems to promote expression of eye field transcription factors (see below)[RDT+01], while an unspecified non-canonical interaction between Wnt11 and Fz5 inhibits canonical β-catenin signalling through Wnt8b/Fz8a, which would otherwise promote prospective anterior forebrain fates [CCY+05]. Inhibition of FGFR2 signalling, and activation of ephrinB1 signalling have also been implicated in early Xenopus eye field cell movements [MMDM04]. Subsequent experiments determined that the xenopus ADP signalling through the P2Y1 receptor directly activates the eye field transcription factor network (EFTFN), another well developed MEx described below [MBE+07]. More recent experiments in zebrafish suggest that precocious acquisition of neuroepithelial apicobasal polarity, probably driven by interactions with a Laminin1 basement membrane, distinguishes the early eye field [ICW13].

Developmentally subsequent to the appearance of eye field RPCs and their rearrangement into the optic cup, the apparent central-to-peripheral "wave" of RPC exit from cell cycle and specification of early RGCs [HE99], has had detailed MEx advanced to explain it. In both zebrafish and chick retina, FGF3 and FGF8, originating from the optic stalk, initiate this early cell cycle exit and specification [MDBN+05], while inhibiting FGF signalling prevents this from occurring, and ectopic expression of FGF can cause it to occur inappropriately. The progression of this "wave" of cell cycle exit and specification has been separately explained, by Sonic Hedgehog (Shh) signalling from the newly specified RPCs inducing cell cycle exit and specification in adjacent cells [Neu00]. This process is dependent on, and downstream of, the above-mentioned FGF induction [MDBN+05]. The role of Hh signalling has been challenged on the

basis that Hh inhibition in subsequent experiments did not display the same effect size [SF03], and that its effects on Ath5 expression, required for RGC specification, are ambiguous [AH09]. More recent MEx advanced by Harris have suggested that Hh signals may decrease the length of the cell cycle, resulting in increased proliferation and earlier cell cycle exit and specification [LAA+06, ALHP07].

A well-developed "local" signalling MEx (mainly advanced by Harris) invokes the classic Notch/Delta lateral inhibition model, with small fluctuations in Notch/Delta activity giving rise to a positive feedback response that differentiates neighbouring cells. Cells which have high Delta expression tend to be specified as retinal neurons, while those with high Notch tend to remain proliferative, either as RPCs or Müller glia [DRH95, DCRH97]. Such a mechanism could regulate the activity of both early, central RPCs, as well as peripheral RPCs, and may contribute to inter-RPC variability. These differences between RPCs located in different parts of the developing retina have been of significant interest, and it is worth briefly examining patterning MEx that may also explain spatial differentiation between RPCs.

1.4.3 Patterning mechanisms

Among the most interesting features of RPCs is that they reliably give rise to specified neurons, in particular RGCs, that seem to "know where they are" in the retina, enabling them to wire their axons in correct retinotopic order in the superior colliculus (SC) or optic tectum (OT). The most robust MEx explaining this refer to gradients of EphA and EphB receptors expressed in RGCs, and their respective ephrin ligands expressed in the SC or OT. In the retinal RGC population, an increasing nasotemporal gradient of EphA is paired with an increasing dorsoventral gradient of EphB. A corresponding increasing rostrocaudal gradient of ephrin-A is paired with an increasing lateromedial gradient of ephrin B in the SC/OT. This allows for a two-axis encoding of an RGCs' position in the retina [TK06]. As the RGCs' axon pathfinding depends on repulsive effects mediated by Eph receptors, this code is sufficient to allow correct wiring of even single RGCs [GNB08]. This code seems to be established by (FIND MISSING REF TO FACTORS UPSTREAM EPHRIN GRADIENT) in RPCs themselves, prior to differentiation.

Indeed, there are numerous similar observations of expression gradients that create spatial differences between RPCs themselves. Most relevant to the proliferation dynamics highlighted in this chapter is the observation that, in *Xenopus* eyes, a decreasing dorsoventral gradient of type III deiodinase renders the cells of the dorsal CMZ refractory to thyroid hormone (as the deiodinase inactivates TH) [MHR+99]. The effect of this is to set up a differential response to TH in post-metamorphic RPCs, so that the ventral population selectively expands in response to TH [BJ79].

These patterning mechanisms are of particular interest here, in large part because they clearly establish that the RPC population is heterogenous, both with respect to proliferative and specificative behaviours, and perhaps others as well. This is of critical importance for any modelling effort, as virtually all mathematical models used by stem cell biologists (and those used to justify Harris' ISP) assume, at least initially, homogenous populations of stem or progenitor cells. Since RPCs do not meet this condition, special care is needed to use these models.

1.4.4 Chromatin dynamics

In recent years, the great importance of chromatin conformation in RPC proliferation and specification has become more clear. Indeed, chromatin dynamics are now widely invoked in explaining stem and progenitor cell behaviour, and suggested as a target for cell reprogramming [Kon06, TR14]. In RPCs,

detailed accounts of three-dimensional chromatin dynamics have yet to appear. However, a number of studies point to the importance of chromatin state in informing the overall cellular state. In particular, histone deacetylation seems to be important for RPC specification, as the loss of histone deacetylase 1 (HDAC1) in zebrafish results in overproliferation and decreased specification, and correlated increases in Wnt and Notch activity [Yam05]. In mouse retinal explants, pharmacological inhibition of HDAC results in decreased proliferation and specification [CC07]. Additionally, the chromatin remodelling complex SWI/SNF has repeatedly been implicated in RPC function. Notably, one particular component of this complex seems to be particularly associated with vertebrate RPCs (BAF60c, an accessory subunit) [LHKR08]. A switch to other subunits seems to be necessary for specification [LWR+07]. Details regarding the subunits involved in specification and their downstream effects are complex and context dependent, much like the signalling pathways mentioned above.

1.5 Toward a unified theory of RPC function- "blurring" to order

From the foregoing discussion, we can clearly see Harris' theoretical conundrum. Macromolecular explanations for RPC behaviours, like those throughout the molecular biological tradition, have generally been built outwards from particular transcription factors, receptors, etc. The result is an archipelago of MEx, at best connected by tenuous speculation, and in most cases, without any known means to form an integrated model. Furthermore, the degree of complexity and context-dependence evident from the literature might seem to preclude such a model. As we have seen, Harris found that the evidence did not allow for clear discrimination between logically distinct types of mechanisms for producing the observed variability in RPC lineage outcomes.

In this situation, Harris effectively had two theoretical options. The first is simply to "crank the handle"- to generate more and more facts describing the difference particular molecules make to RPC outcomes in dozens of relevant contexts, piling up exceptions and idiosyncracies, in the hope that doing so will eventually bridge the explanatory "islands" of the MEx archipelago. I have referred to this as the Ennumerationist approach and explained why it has failed, and continues to fail, in the Theoretical Appendix.

The second option, the one actually chosen by Harris, is more theoretically sophisticated. As Nicholas Rescher has noted regarding the in-principle limits to scientific knowledge, nature has infinite descriptive complexity- one can always add more detail to a description of a natural system, and no such description is ever complete [Res00, p.22-9]. Moreover, "even as the introduction of greater detail can dissolve order, so the neglect of detail can generate it." [Res00, p.62] As Rescher goes on to comment:

> [W]e realize that in making the shift to greater detail we may well lose information that was, in its own way, adequate enough ... information at the grosser level may well be lost when we shift to the more sophisticated level of greater fine-grained detail. The 'advance' achieved in the wake of 'superior' knowledge can be - and often is - purchased only at a substantial cognitive loss.
>
> ...
>
> It is tempting on first thought to accept the idea that we secure more - and indeed more useful and more reliable - information by examining matters in greater precision and detail. And this is often so. But the reality is that this is not necessarily the case. It is entirely

> possible that the sort of information we need or want is available at our 'natural' level of operation but comes to be dissolved in the wake of greater sophistication. [Res00, p.65-6]

Rescher's greater point is that "blurring" detail, at levels below the phenomenal one under consideration (for RPCs, generally, the cell or lineage), may actually be necessary to produce an ordered explanation that is useful with respect to some objective. Given the number of apparently contradictory MEx for RPC behaviour, we have exactly the kind of situation Rescher is describing- more sophistication, and more detail, has dissolved order, not revealed it[15]. The inability to assemble an unified explanation for RPC function has left us without a good fundamental understanding of how highly ordered neural tissues like eyes can be generated from composites of units with highly variable, temporally and spatially ordered outcomes like RPC lineages. As this is a common feature of vertebrate neurogenesis more generally, the theoretical problem here leaves us without the ability to produce complete models of neurodevelopmental processes in many species. Moreover, in the absence of a clear framework for comparing the explanatory power of the diverse array of MEx so far advanced, practical contributions of clinical relevance have been scanty and tentative, with RPC transplantation, and more recently, gene therapies taking little note of complex MEx for RPC function[CAI+04, GS07, **?**].

In a situation of this kind, it may well be that this type of "blurring" is required, and it seems that is what Harris is attempting in advancing his ISP explanation. Harris is asking, in some sense, whether most of the MEx offered for RPC behaviour are extraneous to an adequate understanding of how RPCs work. By cutting down to the simplest possible explanation, Harris hopes to bring into view order that was previously obscured by detail. There is, of course, a significant danger here: how does one decide what is "blurred out" and what remains? We can easily understand how a practicioner's biases could lead to a sort of relativism, where the "blurring" makes apparent a spurious order that conforms to these biases rather than to reality as such. With this in mind, let us examine Harris' ISP explanation in detail, to see how, and how well, this theoretical operation has been performed.

1.6 Explanatory Strategy and Intent of the ISP

As we have seen in Section, Harris' long-held understanding of the explanatory options for RPC function divides them into four broad categories, which I summarise as follows:

1. A linear algorithmic "program" of proliferation and specification
2. Asymmetric segregation of specificative determinants
3. "Stochastic processes" internal to the cells
4. Influences of extracellular factors

Harris' sophisticated discussions of RPC MEx rarely treat these categories as exclusive, and concede that good explanations for RPC behaviour may involve phenomena from more than one of them. Indeed, the ISP necessarily contains elements that Harris concedes are "linear" and "deterministic" [HZA+12].

[15] At least part of this problem is likely related to the fact that the majority of biomedical findings cannot be replicated [Ioa05]. The finding, mentioned above, that Shh effect sizes on RPC function were not as large as initially reported when subsequently investigated, is typical and symptomatic of this replication problem. "Blurring" may therefore be necessary not only because of fundamental epistemic limits, but also because it is often difficult to distinguish bona fide results and explanations from spurious ones.

Still, his overall strategy for the ISP is, first, to substantiate the predominant influence of one of these categories of phenomena (that is, category 3, internal stochastic processes or effects), and subsequently to specify an actual macromolecular system that could plausibly be such an "internal stochastic process". These two theoretical maneuvers, while tightly linked, serve different purposes within Harris' overall explanatory framework, which must be examined separately.

The ISP explanation for zebrafish RPC function has been developed across three separate papers [HZA+12, BRD+15, WAR+16]. Each builds on the earlier publications, collectively purporting to explain the behaviour of RPCs wherever, and whenever, they may be found in the zebrafish eye. The underlying model is originally derived from an earlier paper pertaining to rat RPCs [GZC+11]. He et al. [HZA+12] and Wan et al. [WAR+16] use essentially the same model and make up the substance of the first of these two maneuvers. Boije et al. [BRD+15] substantially modifies this model, specifying the activity of two known transcription facts (Ath5 and Ptf1a) as the model's biological referents. This paper constitutes the second theoretical thrust.

The first maneuver intends to provide support for the contention that RPCs are a group of equipotent cells which give rISP to variable outcomes that depend on independent "stochastic" processes within each of these cells. This support is to be provided by demonstrating that a suitably configured Simple Stochastic Model (SSM) of an RPC, numerically simulated many times by Monte Carlo methods to represent a population of RPCs, produces similar outcomes to populations of RPCs in vivo. This explanatory strategy is common in the stem cell literature, the original example having been published in 1964 by Till, McCulloch, and Siminovitch[16]. The ISP therefore represents an example of a traditional scientific logic- an explanatory pattern deployed by stem cell biologists in diverse contexts, and widely accepted because of its ongoing use in the literature.

As I have argued in Section **??**sec]TMS, this explanatory logic is fundamentally defective. It fails to clearly identify stochasticity and randomness as properties of the model (the SSM), incorrectly assigning them to the physical processes generating stem cell lineage outcomes in spleen colony assays (the "TMS conflation"). Moreover, as I conclude in Section **??**sec]stochastic, while Harris routinely describes biological processes as "stochastic", and even appears to suggest that such processes do not require causal explanation[HZA+12], in at least one ISP publication [BRD+15], he concedes that stochasticity is a matter of the descriptive level an explanation is concerned with, and is therefore not a property which inheres in biological systems themselves.

What, then, is Harris attempting to achieve by demonstrating that an SSM can produce similar simulated, quantitative outcomes to zebrafish RPC populations? As I have suggested in the conclusion to Section **??**sec]TMS, as a result of years of TMS conflationary models in the literature, "stochastic" has come to vaguely define a category of cellular processes distinct from others that might well be described with stochastic process models. In particular, the recently-introduced concepts of "transcriptional noise", "cellular noise", and "developmental noise" fall cleanly into this category, as discussed in Section **??**sec]noise, and referred to hereafter as "noise explanations". Harris, therefore, is trying to substantiate the involvement of a process something like this in producing variable RPC lineage outcomes, as opposed to more conventionally-understood MEx like asymmetric inheritance (which could nevertheless be described with a stochastic model).

The success of this first maneuver thus depends on two outcomes. Firstly, the output of the SSM should accurately reflect the observed proliferative and specificative outcomes of zebrafish RPC lineages,

[16] Till1964

giving weight to Harris' claim that it "provides a complete quantitative description of the generation of a CNS structure in a vertebrate in vivo" [HZA+12][17] Secondly, the structure of the SSM should provide good reason to believe that one of the Noise explanations is a better explanatory option for RPC lineage outcomes than those identified by the other three categories of theoretical options ennumerated above.

The second theoretical maneuver is the specification of particular biological referents for entities in the model. This offers an opportunity to move beyond a purely conceptual argument about the kind of process that might produce variable RPC lineage outcomes, and to begin the work of explaining how the behaviour of a particular macromolecular system constitutes such a process, so that empirically verifiable hypotheses may be generated. The success of this maneuver depends on the biological plausibility of the identification between model structure and the biological function of transcription factors, Ath5 and Ptf1a, that the model names. A good SSM-based explanation would point the way for further research by identifying *how* so-called "stochastic processes" in RPCs might function, and make some predictions about this.

1.7 Meta-analysis of ISP explanations for zebrafish RPC function: the ISP EHJMEx

1.7.1 Background to the zebrafish ISP: Gomes et al. 2011

As noted, the zebrafish ISP has its direct root in an earlier report, Gomes et al. [GZC+11], a collaboration between the Harris and Cayouette groups. This paper represents a significant elaboration of Cayouette's earlier work in the Raff group, noted above[CBR03]. The same modeller developed the SSMs used in Gomes et al. and in the ISP also developed the model used in He et al [HZA+12]. The latter model would subsequently be deployed in Wan et al. [WAR+16], as described below. The modelling methods in these reports form a reference-chain back to Gomes et. al. It is thus necessary to briefly examine the model used in this paper (hereafter the "Gomes SSM")and its use as an explanation.

The Gomes SSM is explicitly built as, in effect, a null hypothesis. It represents an extreme case in which the specificative and proliferative behaviour of an RPC is totally independent of any other RPC in its lineage. The stated purpose of this model is "to calibrate the data", serving "as a benchmark". The objective of the study is to compare the lineage outcomes of dozens of individual E20 rat RPCs in clonal-density dissociated culture with the model output. Substantial deviations of the observed data from the fully independent events of the SSM can then be interpreted as causal dependencies between RPC outcome and their relative lineage relationships, as might be expected, for instance, in a linear program of specification.

The model covers two of the six "morphogenetic alphabet" categories relevant to eye morphogenesis: RPC proliferation and RPC specification. There are three components comprising the Gomes SSM. Each component consists of a random variable whose value is determined by a continuous or discrete probability distribution. In all cases, these distributions are generated from observations of the dissociated rat RPCs.

The first random variable determines the length of time for any given cell's cycle; the value of this variable is determined by randomly drawing from a continuous log-normal probability distribution. This

[17] This claim is somewhat extravagant; the SSM, by definition, includes no spatial information, so it is unclear how one could be a "complete description" of any spatially organISPd structure. Still, it can be complete with regard to cell population numbers.

distribution is derived from a log-normal regression fit to actually-measured cycle length data. The value of this variable makes no reference either to the SSM "mitosis type", as described below, or the position of the cell within the lineage, so all cells are strictly equivalent[18]. All cell lineages eventually terminate in symmetrical postmitotic divisions, but may, in principle, retain proliferative members for an arbitrarily long period of time.

The second random variable determines fate assignments for postmitotic RPC progeny; the value of this variable can be one of four discrete specified retinal neural fates (amacrine, bipolar, rod, and Müller glia[19]). The probabilities assigned to each modelled fate are derived solely from the proportion of each of these lineages which appear in the overall population of dissociated RPC lineages; these are very similar to those that appear in vivo.

The third random variable is standard to most SSMs, meshing the first two together by determining the proliferative status of a given mitoses' progeny (symmetrical proliferative (P/P), symmetrical postmitotic (N/N), or asymmetric (P/N)). The probabilities assigned to each type of division are again derived solely from the proportion of each division observed in the overall population of dissocaited RPC lineages. These probabilities have no dynamics, remaining constant throughout the simulated RPC lineages.

From an agent model perspective, the logic each cellular agent steps through may be summarised briefly as follows:

1. Determine cell cycle time; wait this length of time.
2. Determine mitosis type; divide.
3. Proliferative progeny loop back to 1. Postmitotic progeny determine fate assignment and exit cell cycle.

The use of the Gomes SSM is quite clear. In an early example of what might be termed an information theoretical approach, the SSM is used to generate sequences of RPC lineage outcomes that are completely independent of an RPCs' lineage history. These are compared by statistical methods to the observed data, with departures being interpreted as signs of lineage-dependent order. Importantly, the stochastic properties of the model are correctly understood to be an epistemic, rather than ontological, feature of the system: "[T]he balance between proliferation and differentiation of a RPC is not influenced by the fate of its parent. In other words, the division mode of a given RPC (P/P, P/D, or D/D) is **unpredictable and, in this sense, stochastic.**" That is, the Gomes SSM is *not* used in a TMS-conflationary argument, although the emphasis on stochasticity makes this point slightly unclear. The structural feature of the model that is of interest is the *causal independence* of the simulated RPC's cycle time, mitotic mode, and specified fate from each other and from the RPC's history. Since the model output resembles that of the observed dissociated RPC lineage outcomes[20] , this provides a argument that the macromolecular mechanisms which generate these outcomes are likewISP independent of lineage

[18]Gomes et al. argue that this strict independence of cell cycle length from mitosis type and lineage position reflects similar cell cycle times across cell time in lineage and mitotic type. However, they concede that "[w]hen we measured the difference in cell cycle times of all the daughter cells of P/P divisions ... we found a standard deviation ... significantly smaller than ... the standard deviation of all cell cycle times, which would be expected if their division times were uncorrelated. This result suggests a degree of synchrony in the timing of division of sister RPCs that might contribute to the general timing of retinogenesis."

[19]These are the only lineages produced by E20 rat RPCs.

[20]Aside from synchronisation between sister cell cycle times, Gomes et al. notes a small number of lineage pairings that appear more frequently than the SSM predicts.

history. The model itself is purely abstract, not being intended to explain the function of the system, but rather to produce output of a system governed by causally independent events for comparison to real data.

Before moving on to see how the Gomes SSM was developed into the zebrafish ISP, let us note a few important aspects of its output. Firstly, it does not, and cannot, produce outcomes that are histogenetically ordered. As RGCs are absent from the fate outcomes of E20 rat RPCs, the most notable feature of this ordering, early RGC appearance, is not modelled. Since it is carefully fitted to this fairly narrow developmental window, and the experiments are conducted in dissociated cell culture, it is unsurprising that there was no time-dependent variation in cell cycle length. The Gomes SSM, therefore, provides strong evidence for the lineage-independence of late-born retinal neural type specification, as well as good evidence for the independence of cell cycle length and mitotic mode from lineage history, within this context. The biological implications are made amply clear by the text of the paper itself: "these results suggest that RPCs are not counting cell cycle time or rounds of division to coordinate when to exit the cell cycle and differentiate," thus providing evidence against any proposed mechanism involving such a timer.

1.7.2 Explaining RPC lineage size in the zebrafish: He et al. 2012, Wan et al. 2016

Fresh from the success of the well-received Gomes et al. report, Harris rapidly turned his attention to the potential to corroborate those findings in a distant vertebrate phylum, zebrafish. However, this required both substantial modifications to the Gomes SSM, and to the explanatory logic connecting the SSM to observed RPC outcomes. This modified model (hereafter "He SSM") was deployed in He et al. and Wan et al. in 2012 and 2016, respectively [HZA+12, WAR+16]. In the former report, the He SSM is used to explain proliferative outcomes of RPC lineages (i.e. clone sizes and mitotic modes) in the context of the early embyronic phase of central proliferation, before the remnant proliferative population of the peripheral CMZ becomes the sole source of new retinal neurons. The latter report deploys the He SSM to explain these outcomes in CMZ-derived RPC lineages, effectively purporting to explain RPC function throughout the life of the organism. Clearly, this is an ambitious increase in the developmental scope of phenomena the model's output must bear comparison to, relative to the Gomes SSM.

In order to understand how this has affected the He SSM, let us first take note of a passage from the model's Methods description:

> [W]e will suppose that RPCs form a functionally equivalent, equipotent cell population with evolving proliferative potential, which is decoupled from the particular specification of individual cell types. Through temporal and spatial correlations, we expect to capture many aspects of the data, including correlations that might otherwISP require a causative hypothesis. Any residual correlations between lineage and clone size are therefore a reflection of the histogenesis of cell types or a signature of early fate specification.

While echoing the general logic of Gomes et al., in effect, that only deviations from model output are attributable to "histogenesis or a signature of early fate specification" (Option 1 theories), the model is described as simulating equipotent cells with "evolving proliferative potential ... decoupled from .. specification of .. cell types." This is, in fact, a description of the significant differences between the Gomes and He SSMs. The He SSM simulates cells whose mitotic mode probability distribution changes over time ("evolving proliferative potential"), and does not simulate any specified neural fate outcomes

("decoupled from specification"). In effect, one of the Gomes random variables (that referring to specified cell fate) has been dropped, the variables referring to mitotic mode and mitotic p has been given a time dynamic.

This time dynamic is built into the model by periodising TiL, creating three periods in a lineages' history. Cells in each of these periods refer to a different mitotic mode probability distribution when dividing. We should pause here to note that something very strange has happened; as noted, the logic of the Gomes SSM suggested that "RPCs are not counting cell cycle time or rounds of division to coordinate when to exit the cell cycle and differentiate". The He SSM assumes by its structure that RPCs *do* count cell cycle time (in this case, denominated in TiL (h)), and that cell cycle exit (and, implicitly, differentiation, because the mitotic mode concept conflates cycle exit with specification) *is* coordinated with reference to this TiL value!

In other words, despite its close links with the Gomes report, the He SSM is no longer a "baseline" model, representing equipotent RPCs whose proliferative and specificative outcomes are uncorrelated with their relative position in the lineage. Rather, it has *incorporated* the structuring concept the Gomes SSM was deployed to argue against, while maintaining the rhetorical stance that "histogenesis of cell types or ... early fate specification" only requires explanation to the extent that the observed data depart from the stochastic model (i.e. "residual correlations").

It is clear why the incorporation of a temporally dependent mitotic mode probability distribution was necessary: unlike the Gomes SSM, the data the He SSM is fitted to include obvious histogenetic phenomena, including early RGC specification, due to the early developmental context. As noted, the Gomes SSM cannot produce *any* ordering of RPC outcomes, which is why it is useful- as a baseline of a system in which each parameter is totally uncorrelated with any other, we can estimate how ordered various RPC processes are by the extent to which observed lineage outcomes differ from model output. The He SSM does not incorporate explicitly specified cell fates, but, because early RGC specification is associated with P/N mitoses, with the nonproliferative offspring becoming the RGC, it is fitted to data that necessarily reflect RPC histogenetic ordering.

Therefore, Harris has two options here: (A) maintain the SSM as a purely conceptual construct intended as a reference of a fully uncorrelated system, and concede that there *are* substantial "residual correlations" associated with histogenesis or early fate specification; or (B) change the role of the SSM from "benchmarking data" to actually explaining the data in terms of the properties of the underlying macromolecular processes. Harris has chosen (B), so that the SSM is now an MEx for a phenomenon, and not a mathematical artefact to be used in a statistical comparison.

All models are intended to reflect some feature of the structure of the underlying biological processes. In the Gomes SSM, this feature is the lineage-independence of the SSM's random variables, which is taken to reflect the causal independence of the biological process which give rISP to observed unpredictably variable outcomes. Since the He SSM is no longer lineage-independent, and it does not specify any actual biological entities, Harris is forced into a TMS-conflationary argument. While conceding that the He SSM represents a mixture of stochastic and linear, programmatic model elements, the rhetorical emphasis is solely on the stochastic elements, with their presence being described as sure evidence for stochastic biological processes. The adoption of TiL-dependent mitotic mode is argued to reflect something other than histogenetic ordering, being "decoupled" from the specification of individual retinal neural types. As I have shown above, no such decoupling exists between P/N mitotic mode and RGC fate specification; the He SSM admits the very "linear, programmatic" elements that timed competency hypotheses of

histogenetic ordering, which the "stochastic process" SSMs were intended to disprove.

Moreover, the SSM is no longer being used as a baseline, representing fully lineage-independent RPC proliferative and specificative outcomes. Instead, it is being used in a TMS-conflationary argument, suggesting that because the SSM uses a random variable to determine mitotic mode, the model fit implies that the underlying macromolecular process is itself "stochastic". The SSM is thus used to *explain* the function of RPCs rather than to provide an idealISPd, random sequence of lineage outcomes, in order to measure the extent to which RPC lineage outcomes deviate from it.

1.7.3 Are there alternatives to the He SSM?

1.7.4 Does the He SSM adequately explain zebrafish eye morphogenesis?

1.7.5 Can the He SSM explain both central and CMZ contributions to the retina?

1.8 Traditional roots of the ISP: Till and McCulloch and the simple stochastic model (SSM)

At the core of the ISP are variants of the most common model used by stem cell biologists, the Simple Stochastic Model or SSM, described in more detail in Section 2.5.2.1. The SSM consists of a Galton-Watson branching process, a stochastic process originally intended to model the lineage extinction of surnames. Wikipedia describes a stochastic process as "a mathematical object usually defined as a collection of random variables" [**?**]. In the case of branching process models applied to proliferative cells, the random variable determines the mode of division of each cell within the lineage, with this mode being defined by the proliferative state (construed in the model as being either mitotic or postmitotic) of progeny. For any given division, a cell may produce two mitotic, one mitotic and one postmitotic, or two postmitotic progeny, and each of these division modes is given a defined (often, but not always, static) probability. Given these values, the history of a cell lineage may be simulated; the output of many of these simulations pooled together, usually by what is referred to as the "Monte Carlo method"(described in Section 2.7.4), allows the statistical properties of the dynamics of population of simulated cells to be estimated.

The use of SSM derivatives in the ISP is significant for Haris because he understands "stochastic processes" to be one of four explanatory options for RPC function, enumerated above in Section 1.2, which we may summarise here as: (1) "linear program of proliferation and specification"; (2) "asymmetric segregation of determinants"; (3) "stochastic processes internal to the cells"; and (4) "extracellular factors". Harris' basic theoretical strategy is to argue in favour of (3) by demonstrating that an SSM describing the behaviour of RPCs is sufficient to explain the lineage outcomes (cell number and specified type) which determine the composition of the population of cells available for retinal organogenesis.

The astute reader will have noticed that "stochastic processes internal to cells" is incomprehensible in light of the commonplace definition given for stochastic processes above- "mathematical objects". We may be inclined simply to interpret "stochastic processes" as "biological processes well-described by stochastic process models", but there are at least two problems here. Firstly, stochastic process models are also appropriate for use in modelling theoretical options (2) and (4), and (2) is also a process internal to the cell. Secondly, Harris routinely refers to phenomena themselves as "stochastic", even implying

degrees of stochasticity, and moreover takes the inclusion of a stochastic process in ISP models to *explain* (rather than merely describe) the observed variability in RPC lineage outcomes: "we show here that the high degree of variability in the lineages of RPC cells can be explained using a simple stochastic model based on these fixed probabilities of TF expression." [BRD+15]. In the same report, we find a concession to the insistence of statisticians that the properties of mathematical objects used in models do not reflect those of the modelled systems, followed by language which strongly suggests that "stochastic" is nonetheless understood to apply both to the description of the system as well as the system itself:

> It is important to note that, in this regard, whether or not a process is stochastic or follows some complicated deterministic rules is a matter of the level of description. Complex systems in which many variables interact often produce data that can best be described in terms of probabilities even though at the level of individual elements each of the interactions may be determinative. Statistically, however, stochastic processes produce robust and well-behaved distributions, as does the nervous system. This, we propose, is therefore a possible basis for understanding how it is that even though there is a high variability in the size and composition of individual clones, the total number of differentiated cells and the relative proportions of each cell type are almost invariant from one zebrafish retina to the next. [BRD+15]

As I have argued in greater detail in Section 2.6.2, the orthodox view that stochasticity is exclusively a property of models (in effect, models using random number generators are "stochastic"), and not of the underlying modelled system, is correct. That is, the suggestion implicit in the words "even though ... each of the interactions may be determinative", that there is an *alternative* whereby the interactions comprising the behaviour of the modelled system do not have specific, determined causes, must be rejected[21]. Stochastic process models are good descriptions of biologically variable processes because they have variable output, but "stochasticity" by itself cannot explain *how* the biological process comes to vary. While the ISP is concerned to explain the variability of RPC lineage outcomes, Harris has confusingly highlighted the *mathematical* property of the model which produces the variable output resembling that of the *biological* system. To wit, there is no "random number generator" in a cell- the task of the explanation is to identify the biological components and their interrelations making up the system whose output *resembles* that of a computer's random number generator..

The use of the SSM to describe and explain the behaviour of stem cells has a long pedigree in the SCBT, as evidenced by the classic modelling effort of Till, McCulloch, and Siminovitch[22] [TMS64], explaining the variability in the size of ectopic spleen colonies formed by hematopoetic stem cells in irradiated mice by means of a Monte Carlo-iterated simple stochastic model (SSM). This

1.9 Meta-analysis of ISP explanations for zebrafish RPC function: the ISP EHJMEx

Harris' ISP explanation for zebrafish RPC function has been advanced across three separate papers [HZA+12, BRD+15, WAR+16]. Each builds on the earlier publications, collectively purporting to explain the behaviour of RPCs wherever, and whenever, they may be found in the zebrafish eye. In stark contrast with the various MEx described above, bristling with contextual caveats, the ISP explanation is much

[21] The notion that probability theory or quantum mechanics entail the existence of causeless, "random" processes is argued against in Section 2.6.2

[22] It is worth noting that the relevant modelling calculations were performed by U of T computer scientist L. Cseh. The outsourcing of modelling labour to computer science or physics departments remains common, appears in the ISP papers, and is worth considering as a contributor to the model-system conflation I argue has repeatedly occurred.

more like the universal, exceptionless laws characteristic of the physical sciences[23]. Because the three papers applying the ISP explanation to zebrafish RPCs use closely related models, each derived from another, often with subtle modifications, and these are in turn derived from an earlier, fourth, publication applying the ISP to mouse RPCs [GZC+11], the papers and the models they present must be treated together as one extended theoretical unit. In order to perform a rigorous analysis of this metascientific "unit", I have adapted the work of the philosopher of stem cell biology, Melinda Fagan, who has advanced a perspicacious and useful account of mechanistic explanations used in the stem cell biological tradition (SCBT). Fagan's work, and my own modifications to it, are described in detail in Section 2.3.2. It suffices to say here that I am treating this extended theoretical "unit" as a series of related MEx, developing over successive publications, composed of both statistical generalisations of cellular behaviour and particular, interdependent macromolecular components, which I refer to as an extended heterogenous joint MEx (EHJMEx)[24]. Having said this, let us examine the gist of ISP explanations in general, before conducting a detailed examination of the three zebrafish-specific models and their murine forerunner.

As the name I have applied suggests, ISP explanations seek to account for RPC function as the effects of processes which are intrinsic to RPCs, and which are in some way "stochastic". While the latter concept requires some exegesis, the former is straightforward enough. As noted in section 1.3.2, the notion that the most significant proliferative and specificative phenomena associated with RPCs are produced by mechanisms intrinsic to the cells derives much of its empirical support from Raff's work. This story began developing with the observation that co-culturing E15 rat RPCs in dissociated pellet cultures with P1 cells did not accelerate the appearance of the first rods derived from the E15 progenitors (which occurred at a similar time as *in vivo*), suggesting that the specificative "schedule" of these cells is at least partially intrinsic[25] [WR90]. The scope of these observations were dramatically expanded by Raff's subsequent work, intended to address the relative significance of intrinsic versus extrinsic processes in RPC function by comparing clonal RPC lineages in fully dissociated clonal-density cell culture to those in intact explants [CBR03]. The remarkable finding of this study was that dissociated E16-17 rat RPC lineages produce very similar numbers and types of retinal neurons, albeit without morphological or molecular markers of mature neurons. This observation provided strong evidence for the predominant importance of RPC-intrinsic processes in determining both the proliferative and specificative outcomes for RPC lineages, since the complex, spatially organised context of intact explanted tissue seemed to only be required for the maturation of neurons, and was not required to regulate proliferation or the initial commitment to an appropriate distribution of lineage outcomes. As these are the two most obvious and critical parameters that must be achieved for RPCs to produce a functional retina of the appropriate size, the suggestion that both are largely determined by intrinsic processes was a striking confirmation of Williams and Goldwitz's much earlier suggestion [WG92], against the prevailing view of the day, that lineage had a greater role to play than cellular microenvironment in RPC contributions. Interestingly, Raff's interpretation of their 2003 data was that RPCs were most likely stepping through a linear, programmed developmental sequence rather than undergoing shifts in the probabilities of variable

[23] It is extremely unusual for biological explanations to pertain universally, even within species, which has long concerned theorists wishing to establish biology as an autonomous science on par with physics [Sch93, p.120]. Melinda Fagan's work, discussed in the theoretical appendix, has highlighted, rather than suppressed, the uniquely contextual and interdependent features of biological explanations.

[24] Readers not wishing to delve into the theoretical background here may usefully analogise the EHJMEx to a succession of different mechanisms employed in the development of some organism, although the developing entity here is the theory itself.

[25] Co-culturing with P1 cells, did, however, significantly increase the proportion of E15 RPCs specified as rods, resulting in Raff's suggestion here that both intrinsic and extrinsic factors are important.

outcomes over time. It is notable that this interpretation arises not from the data collected in their study, but from considerations of a single unusual clone reported by [TSC90], and by analogy with drosophila neuroblasts. In retrospect, these arguments for programmed RPC outcomes do not seem particularly strong, and it is perhaps unsurprising that these studies are remembered mainly for highlighting the importance of RPC-intrinsic processes.

The concept of "stochasticity" has a long and fraught pedigree in the SCBT. We find it deployed in an identical manner in the early 1960s as today, as in the classic modelling effort of Till, McCulloch, and Siminovitch[26] [TMS64], explaining the variability in the size of ectopic spleen colonies formed by hematopoetic stem cells in irradiated mice by means of a Monte Carlo simple stochastic model (SSM). "Stochastic" is used in an ambiguous manner here, and, importantly, we find precisely the same ambiguity in Harris' a half century later. This ambiguity arises from the application of the term "stochastic" to the biological *process* under investigation, to the process' *outcomes*, and to the *model* constructed to describe the process. This is particularly obvious in this early work, entitled "A Stochastic Model of Stem Cell Proliferation", which states that "variation [in clonal lineage size] may be generated by a well-known probabilistic ('stochastic') process, the 'birth-and-death' process", and that this "process is operative when an entity, for example, a single cell, may either give rise to progeny like itself ('birth'), or be removed in some way ('death') and these two events occur in a random fashion." [TMS64] As the significance and import of the ISP directly depend on what the meaning of "stochastic" is, and since the manner in which Harris uses this concept is directly descended from the usage of Till et al., we must sort through this ambiguity before proceeding.

We may clarify the matter by returning to the biological issue at hand, which Till et al. frame in the terms of classic cybernetic control theory:

> [T]he development of a colony involves processes of differentiation occurring among the progeny from a single cell. Analysis of the cell content of the resulting colony might be expected to cast light on any control mechanisms which act during colony formation. If rigid control mechanisms are operative, acting on cells of a relatively constant genotype, all colony-forming cells might be expected to behave in a similar fashion, and colony formation should be a relatively uniform process, giving rise to colonies with very similar characteristics. Alternatively, if control is lax, colonies with widely differing characteristics might be expected to develop. Results which may bear on this problem are available from experiments in which colonies were analyzed for their content of colony- forming cells. It was found that, while most colonies contained these cells, their distribution among colonies was very heterogeneous, with many colonies containing few colony-forming cells, and a few containing very many. This result suggests that control is lax. [TMS64]

This makes quite clear that the essential question here is how the process which produces stem cell proliferative and specificative behaviours is structured. We may think of Waddington's topological model of cellular specification, itself inspired by cybernetic theory . Rigid and lax control schemes would, if modelled in this topological fashion, present themselves as very different "landscapes". The abstract concept At the logical extreme, rigid control of stem behaviour would be represented by a single deep channel, down which the "ball" (behaving something like a ball bearing) representing "cellular state" rolls, reliably, at the same rate, in every single instance. Conversely, extremely lax control would be

[26] It is worth noting that none of these investigators actually performed the relevant modelling calculations, leaving these to the U of T computer scientist L. Cseh. This division of labour remains lamentably common, and is likely responsible for many of the problems biologists have in understanding the meaning of their mathematical models.

represented by a landscape resembling a Galton board[27], a broad, flat slope with many pegs which may bump the ball this way or that, tending to cluster the balls in the general center of the slope but never preventing some of them from ending up at either extreme (a properly constructed Galton board produces a Gaussian distribution of balls at the bottom of the slope). This metaphor immediately reveals that, *contra* Till et al.'s interpretation of

1.10 Boije et al. 2015 HJMEx Analysis

The ISP EHJMEx has its most specified HJMEx form in [BRD+15]. Here, the activity of two independent transcription factor systems is related to mitotic mode and offspring fate by empirically-derived probabilities. Generally speaking, Boije et al. are offering a MEx that is framed in less ambitious rhetoric than some of the other MEx in the overall ISP EHJMEx. Let us briefly examine their conclusion, which forthrightly lays out the scientific logic of the paper:

> **(1)** Whatever the molecular mechanisms may be in the case of the zebrafish retina, we show here that the high degree of variability in the lineages of RPC cells can be explained using a simple stochastic model based on these fixed probabilities of TF expression.
>
> **(2)** It is important to note that, in this regard, whether or not a process is stochastic or follows some complicated deterministic rules is a matter of the level of description.
>
> **(3)**Complex systems in which many variables interact often produce data that can best be described in terms of probabilities even though at the level of individual elements each of the interactions may be determinative.
>
> **(4)**Statistically, however, stochastic processes produce robust and well-behaved distributions, as does the nervous system.
>
> **(5)** This, we propose, is therefore a possible basis for understanding how it is that even though there is a high variability in the size and composition of individual clones, the total number of differentiated cells and the relative proportions of each cell type are almost invariant from one zebrafish retina to the next. [BRD+15]
>
> [Paragraph broken up into numbered sentences]

Rephrasing this argument about the value and relevance of the proffered explanation in HJMEx terms, with some additional commentary and with reference to the Prolegomenon, helps to clarify the logic here:

1. The overall phenomenon (observed variability in cellular fate distribution of RPC clonal lineages), P, may be taken to be produced by the activity of a mechanism S Φ-ing, described by a HJMEx consisting of a Simple Stochastic Model, which incorporates a basic model of combinatorial TF expression as one of its components x ϕ-ing. This component may be taken to be polysemous- that is, the abstraction of TF expression and it intermeshing properties with the SSM cell fate model may refer to any number of actual mechanisms of combinatorial TF expression, assortation at mitosis, genetic regulatory effects, and so on.

2. The imputing of "stochastic" or "deterministic" properties to a system is a function of the level of detail of the description, chosen by the experimenter. That is to say, whether or not a system is described as stochastic or not is *solely* a function of whether a human experimenter produces a MEx incorporating stochastic statistical generalisations.

[27]The Galton board, named after its inventor, Sir Francis Galton, is significant because the statistical methods Till et al. use to solve the 'birth-and-death' process were also invented by him.

3. Complex systems, like populations of stem cells, often produce data that is practically speaking[28], describable at a population level (hence the use of the SSM component).

4. This is a tautology following from **(2)**. Whether or not a biological system is described as "stochastic" or "deterministic" is solely a function of the choice made by the experimenter to describe it at some level with a statistical generalisation or not. We know that biological systems produce robust outcomes. "Robust and well-behaved distributions" are *descriptive properties of population outcomes*. We use statistical models for biological systems that produce robust outcomes because they are useful to describe such systems, *not* because the underlying system is *ontologically* stochastic, as already conceded in **(2)**.

5. HJMEx which incorporate stochastic components may be useful explanations of P (restatement of **(1)**).

It is worth pausing to consider this concluding argument carefully, since it calls into question the *biological* significance of the use of statistical generalisations in HJMEx. This is particularly notable in an overall EHJMEx which defines its contribution to the understanding of RPC lineage commitment by its use of these generalisations.

(2) accurately reflects the epistemic dimension of these components- whether a mechanism is described by a statistical generalisation or some deterministic molecular interaction will largely depend on experimenter goals and the level of biological organisation under consideration. However, this is followed by a tautology, **(4)**, which strongly suggests that the epistemic and ontological dimensions have been confounded. Although the authors recognise that the selection of stochastic or deterministic descriptions is contingent on the type of MEx being offered, they seem to insinuate that there are *actually* "stochastic" and "deterministic" molecular processes. For instance, **(3)** seems to suggest that an "actually deterministic" complex molecular process can be adequately explained with statistical generalisations, so that the good fit of a stochastic model is consistent with either:

(a) the actual macromolecular zs μ-ing, polysemously generalised with a statistical model process, are a deterministically interacting set of macromolecular components whose behaviour is conveniently summarised with the model *or*

(b) the actual macromolecular zs μ-ing, polysemously generalised with a statistical model process, are *actually* stochastic, so that the model accurately reflects the way the molecular components "really are"

As indicated in Section **??**, I take the view of the great statistician Jaynes, in insisting that the use of statistical generalisations in explanations says nothing about the *actual randomness* of some process. It is trivial to dismiss the view of Boije et al. on the basis that it is not Bayesian, or even that it does not clearly distinguish between unpredictability ("stochastic" processes) and randomness

[28] Boije et al. say "best described", but this can only refer to pragmatic considerations, as it is obvious that if the system is *actually* deterministic, a description of the rules governing the interactions of "individual elements" is a better MEx than a couple of molecularly polysemous statistical generalisations that only loosely refer to those elements.

Chapter 2

Theoretical Appendix

2.1 Purpose and Use of Theoretical Appendix

I have chosen to include an introductory chapter to this thesis in order to clarify the reasoning of the arguments presented in the data chapters. As even the literature reviews introducing the data chapters are structured according to the methodological considerations outlined here, reference to this chapter has been provided to elucidate concepts that have extensive background content necessary to understand their reference or employment. It has been written to be read as a single document if desired, but it may also function as a hyperlinked reference for the reader primarily interested in the observational work in Chapter 2 and Chapter 3 and less interested in the theoretical model selection framework. Such a reader may simply proceed to Chapter 2 and read only those sections of this chapter that clarify unfamiliar concepts or explain seemingly contentious or unusual statements.

To the extent that this prolegomenon chapter has any independent function, it is to prosecute a series of philosophical and methodological arguments that outline the "worldview" guiding the analyses presented. While I hope to pre-empt genuinely mistaken arguments regarding terms and concepts used in the data chapters, I also intend to articulate what I believe is a reasoned, consistent, and reflexive meta-method for evaluating molecular biological explanations. I found this necessary because of the conceptual complexity of the work being conducted by my colleagues.

Indeed, it was the process of working through the scientific logic of the formal models presented by the eminent Canadian biologist William Harris, as explanations for retinal formation by multipotent retinal progenitors, which led me directly to writing this chapter. This work is the most directly relevant to my own studies, being conducted in zebrafish eyes and largely concerned with the same sorts of phenomena. In exploring these models and their implications, I noticed that they could not explain aspects of retinal formation that I considered significant and had been observing for some time (notably, the continued growth of the retina beyond the larval period and the proliferative dynamics of the niche through to late maturity).

While I understood the general logic of the models as presented, when I attempted to represent them as agent models (which I am more familiar with, and find easier to code, than the probability distribution function (PDF) models used by Harris' group) I became successively aware that I could not understand particular points of the models (which were documented to varying degrees of thoroughness), and that by "translating" the logic of the PDF models into agent models, I had made plain to myself numerous

interesting features of Harris' argument that were not plain from the text.

Moreover, Harris' models were generally presented as a running line of argument in primary papers. The necessarily compressed language of primary reports made for problems in understanding why particular lines of argument were being pressed, particularly with regard to the concept of "stochasticity" or "randomness", which is used in a variety of senses throughout the relevant literature. Attempts to understand *why* the claims were significant immediately implicated reams of literature on empricism, randomness, chance, statistical induction. Investigations into the grounds on which one might prefer one type of model and what sort of an explanation these complex mathematical constructions were forced me into the philosophy of science, and so on.

If one objects that these are not properly "biological" considerations, I can only respond by saying that this is no longer the case, if it ever were. What I have called here the "Systems Biology Encounter" (SBE) has made plain the reality described by the great German-American philosopher of science Nicholas Rescher as follows:

> The ramifications and implications of philosophical contentions do not respect a discipline's taxonomic boundaries. And we all too easily risk losing sight of this interconnectedness when we pursue the technicalities of a narrow subdomain. In actuality, the stance we take on questions in one domain will generally have substantial implications and ramification for very different issues in other, seeming unrelated domains. And this is exactly why systematization is so important in philosophy - because the way we *do* answer some questions will have limiting repercussions for the way we *can* answer others. We cannot emplace our philosophical convictions into conveniently delineated compartments in the comfortable expectation that what we maintain in one area of the field will have no unwelcome implications for what we are inclined to maintain in others. [Res05, p.97]

The introduction of "Systems" methods (mainly drawn from various branches of the complexity sciences) has forced us to contend with the "implications and ramifications" of the methodological, epistemological, and metaphysical contents of the scientific traditions they are drawn from. If we do not understand what some sophisticated mathematical method assumes about the system to which we apply it, we are bound to make errors in doing so, and we cannot know what "limiting repercussions" the use of these methods to answer some questions will have for future investigations[1]. To fail to take heed of this is simply to concede that we do not really care about ensuring that what we say makes sense, that it is not contradictory, spurious, or simply meaningless. Refusing to make this concession, and lacking any intuitive genius that would allow me to procede without an carefully laid plan, I have made resort to borrowings from philosophers, mathematically- and theoretically-inclined biologists, statisticians, and so forth, to formulate one. I have attempted to do so in a disciplined way; my intent here is not to obfuscate with unnecessary philosophical speculation, but rather to clearly document the conceptual background used to tackle this particular problem in its context.

The meta-method I outline in this chapter assumes that Paul Feyerabend's view of science as a constellation of different *traditions* as substantially correct. This is no longer a particularly contentious view- for the molecular biologist confronting the profoundly foreign, esoteric, and opaque utterances of mathematicians and physicists from the complexity sciences, it is a lived reality. I also accept Feyerabend's contention that historical scientific development occurs *counterinductively*, when traditions

[1] These "limiting repercussions" are not limited to a restriction in the kinds of methods that can consistently be used, given some particular "systems biological" approach. If we make no effort to understand these repercussions, we run the risk of having an internally contradictory or degenerate research program, with all of the associated wastages and opportunity costs.

show up one another's implicit natural assumptions and metaphysical content. By doing so, scientific traditions make available new ways to think about natural phenomena. I have used this basic view to structure my approach- my objective is to proceed in a way that maximises the counterinductive potential of the historical moment.

The meta-method itself consists in analysing the appropriate "metascientific unit" of molecular biological practice, the Extended Heterogenous Joint Mechanistic Explanation (EHJMEx), Melissa Fagan's JMEx concept [Fag15b] slightly modified, and extended in time to allow accounting for the development of explanations over several different primary papers. By doing this with a counterinductive general model in mind, I hope to reveal some of the "metaphysical ingredients" implied by Harris' explanation and its models, and to suggest how different ones might provide better approaches.

The general modelling approach I have chosen conceives of cells as a type of semiotic agent. This agent-based modelling approach allows the traditional models of population-level stem cell modelling to be expressed as a subset of the larger global model. This in turn permits models with different global metaphysical implications to be compared for local explanatory value. Because the claims I make revolve around the validity of these comparisons, I have explained and defended this at some length.

I have subsequently documented the most important mathematical, methodological and metaphysical "ingredients" present in Harris' explanations and in my own. I have attempted to place these within the context of the SBE and offer some remarks regarding the epistemological basis of statistical generalisations of complex systems to make this more meaningful for the biologist reader.

Finally, I have made an attempt to assess the relative sustainability of the different approaches to systems modelling implicated by this discussion, as first attempt to guide a research program with an explicitly scientific, realistic futurology in mind, relying heavily on Nicholas Rescher's explications of the practical and in-principle limits of scientific and technical progress.

I have sought to make this chapter useful for my own future reference, and for any colleagues coming out of various parts of the molecular biology tradition, who are by now confronted with an astonishing variety of ways to interpret biological phenomena, and few clear guidelines on how they might structure their research programs in light of the SBE. It is nevertheless, by necessity, confined to considerations relevant to retinal stem cells in zebrafish. Due to the limited space and time available, I have made what historians and philosophers of science probably should consider gross oversimplifications. I consider my overall line of reasoning here to be merely one way to "tell the story" of what is happening to us as biologists, in a way that seems to provide a productive way to think about this problem.

As I have indicated in this chapter, I intend it in the spirit of what Feyerabend called "open exchange"- I do not intend to dictate the terms of future scientific exchange, or to replace one model with another, but rather to suggest one possibility for how we might compare explanations and responsibly guide research programs given the complexity of the present and the uncertainty of the future.

2.1.1 Metaphysics, Epistemology

Throughout this chapter, I have used the terms "metaphysics" and "epistemology" in reference to assumptions, axioms, postulates, etc. about reality and knowledge, respectively. The asking of a question within the natural sciences always has background propositions from both of these domains that are required to make sense of any answer. That is, in order to perform any experiment, we must start with some idea about what sort of thing a phenomenon could consist of (eg. we decide a cell consists mainly of macromolecular consituents arranged in space-time, which informs the methods we use to study cellular

life), as well as an idea of what a good answer might be (we must have a sense of how our explanations correspond to reality) [2].

As Nicholas Rescher notes, speaking here of metaphysical issues specifically:

> Metaphysical issues are thus 'basic' or 'fundamental' because they are the product of a methodological stance that facilitates empirical inquiry rather than being a product of our observational study of nature. What those principles of traditional metaphysics do is to provide question-generic presuppositions of factual inquiry. Natural science (physics, as the Greeks called it) provides the specific answers to our specific questions. But metaphysics is a matter of 'first principles': it sets out the presuppositional framework within which those answers are developed. [Res00, p.4]

Rescher further subdivides metaphysics into two broad categories:

> Presuppositonal inquiry in relation to science has two aspects, depending on whether we ask, 'What presuppositions are called for if we are to do science *at all*?' or 'What presuppositions are called for if we are to do science the particular way in which the course of experience has ultimately taught us to proceed?' Those initial most fundamental principles are fixed. But then the less fundamental implementation is something else again, something learned, something in relation to what the case of experience costs. At this less fundamental level the presuppositions and methodological principles of natural science must be retrospectively informed and restructured in the light of the deliverance of scientific agency itself. (At this stage we can usefully resort to Otto Neurath's graphic image of the boat refitted and repaired while sailing in the open sea.) At this level of consideration metaphysics not merely underpins but also reflects science. [Res00, p.5]

It is the latter, lesser category of metaphysical proposition I am interested in here, and it is this sort of theoretical "at-sea" refit that I am attempting to conduct by considering the metaphysical and epistemological implications of biological theory in the light of our lived experience of scientific practice.

2.2 Study Structure in the Light of Tradition

A scientific report is, by its nature, a retrospective structuring of an investigation. Structuring a scientific study requires defining the goals and methods of the study. To the extent that the products of a research program (eg. the intellectual output of a number of labs over some period of interaction) are structured by some common set of rules for determining their goals and methods, these rules turn out to be surprisingly difficult to define. As the Kuhnian philosopher of science Philip Kitcher noted about the teaching of classical genetics:

> Neophytes are not taught (and never have been taught) a few fundamental theoretical laws from which genetic "theorems" are to be deduced. They are introduced to some technical terminology, which is used to advance a large amount of information about special organisms. Certain questions about heredity in these organisms are posed and answered. Those who understand the theory are those who know what questions are to be asked about hitherto unstudied examples, who know how to apply the technical language to the organisms involved in these examples, and who can apply the patterns of reasoning which are to be instantiated in constructing answers. More simply, successful students grasp general patterns of reasoning which can be used to resolve new cases. [Kit84]

[2] The callow materialist position, common among younger students in the molecular biological tradition, that metaphysics is valueless obfuscation of "what's really there" thus defeats itself immediately, as one must decide "what's really there" before designing experiments to elucidate how what's there *works*. The prevalence of this intellectual pathology should be treated as a serious epidemiological issue within our discipline.

It is the effective use of these patterns of reasoning that constitutes the good or proper practice of biology. The neophyte biologist, if he inquires into the general structure of those patterns, is likely to be met with what Paul Feyerabend referred to as the "fairy-tale" underlying the special credibility afforded natural scientists:

> Scientists have ideas. And they have special methods for improving ideas. The theories of science have passed the test of method. They give a better account of the world than ideas which have not passed the test. [Fey93]

This is an appealing myth, particularly for the learner motivated by a search for the truth, which may account for its use in redirecting the student to immersion in experimental practice. We often teach that there is actually only one method, "The Scientific Method" (hereafter Method), which consists in iteratively falsifying hypotheses about phenomenal reality, allowing a model of that reality (in the form of an logical argument constructed of these hypotheses) to be refined over time so that the model is, by correctly applying this procedure, inexorably brought closer to reality[3].

The conscientious student, looking for serious academic treatments of the Method, is immediately forced to contend with a bewildering array of perspectives on what constitutes the Method and what procedures are legitimately employed in properly Scientific practice. Most of these are produced from accounts of the development of physics and its auxiliary sciences. Many insist that the Method consists of some formal criterion or procedure which is plainly not in use in biology or in scientific practice at large. The wide disagreement on the very concept of any such Method has, at least, the salutory function of disabusing the student of the notion that scientific practice could possibly be governed by the well-understood "special methods" of Feyerabend's "fairy-tale" scientists.

Deprived of any "special method" recipe to apply to his problem, the biologist only has resort to the patterns of reasoning in their field. These are normally understood heuristically or intuitively- we know how to make arguments about molecular systems without any training in formal logic, and without necessarily offering any account of *what we are doing* when we are making the argument. This type of understanding is sufficient when one is simply applying these patterns to new cases. What are we to do when confronted with claims by a colleague who is using novel, foreign, imported, etc. scientific methodologies? We may attempt to "muddle through" by "seeing what works", but this concedes entirely too much to influences we generally understand to be orthogonal to systematisation of knowledge about nature. If "what works" is largely defined by what gets published, the result of "muddling through" without clear method will frequently be shaped more by the parochial interests involved in the scientific process than by the drive for better biology[4].

The student may therefore return to academic discussion of scientific practice looking not for any recipe-Method, or indeed systematic prescriptions about how to structure studies and analyse data, but

[3]This instruction, strangely, often fails to note the origin of this notion with Karl Popper and its subsequent uneven reputation as a good description of, or prescription for, scientific activity. This idea now often goes by the name of "evolutionary epistemology", with the general idea being that incorrect hypotheses are selected against in a progressive refinement of knowledge. To whatever extent this happens, the selective procedure is not iterative application of a particular methodology or set of rules.

[4]For instance, both the academic careers of scientific personnel staffing with granting agencies and editorial boards, and the commercial success of manufacturers selling expensive scientific equipment, reagents etc. depend directly on the perception that the methodology used by the personnel with the equipment and reagents is in some way *orthodox*. This perception may be justified with reference to some philosophical rule to establish the boundary between science and non-science, such as Popper's "pseudoscience" demarcation. In the particular case of stem cell biology, as Melissa Fagan has noted, even basic conceptual divisions (eg. "adult" vs. "embryonic" stem cells) can become politically charged [Fag13, p.47]. Given the lucre associated with promising approaches to stem-cell based regenerative medicine, the influence of these "orthogonal influences" is particularly strong in the SCBT- the perception that a particular theoretical approach undergirding some therapy is mainstream, well-supported science is critical for the therapy's adoption.

simply for points of agreement on what science is and what scientists are doing. As a full survey of this literature would extend across a huge range of studies in a variety of disciplines, I have instead focused on one informative point of agreement arrived at in the philosophy of science. In the second half of the 20th century the observation was made by Thomas Kuhn, Paul Feyerabend, Imre Lakatos, and others, that science plainly did not proceed by iterative hypothesis falsficiation, as asserted by the more credible scientific realists of the first half of the 20th century. Kuhn, Feyerabend, and Lakatos conceived of different scientific "paradigms", "traditions", or "research programmes", respectively, making competing claims on truth in a historical process of scientific development. Rather than collectively producing one huge cultural artefact called "Science", it became clear that the only way to explain the apparently "revolutionary" character of science, as well as the chaotic succession of different scientific theories, was to understand science as the activity of people committed to a plurality of different methodologies interacting in particular historical contexts.

These ideas and their relation to one another took time to digest, but by the end of the 20th century, most philosophers of science agreed that scientific theories must be analysed in their historical, social, and intellectual context, and that diverse scientific schools of thought advance theories and models as competing explanations for phenomena, rather than proceeding by any mutually agreed upon Method. Scientists participate in and draw from these schools as they perform their work, convert from one school of thought on an issue to another as the latter becomes more fleshed-out and persuasive, and so on. From a biologist's perspective, this is a realistic description of scientific practice as a type of ecosystem, rather than the rote application of some logician's rules. The agreement of the philosophers suggests that this is genuinely a better description of what scientists are actually doing than the quasi-mythological "received view" of the past, particularly in the context of the bitter disputes on virtually every other topic.

I have therefore chosen to define a framework derived from these basic insights in order to concretely examine what I take to be the leading research program in my field. In order to structure this examination, I have chosen to make use of a number of concepts from the philosophy of science: Feyerabend's "tradition", Fagan's "Joint Mechanistic Explanation (JMEx)", the latter modified by Schaffner's "extended theory". I briefly describe some of the relevant background of these concepts with occasional clarifying examples from our field. I then make modifications to these concepts for my own use and demonstrate how they inform the structure of this study.

2.2.1 Feyerabend's Scientific Traditions

The central, seminal insight of Paul Feyerabend's *Against Method* is that the observed succession of scientific theories occurs by counterpositional advancement of incompatible opposing theories, because only counterinductive comparisons *between* theories are capable of showing up their implicit assumptions and allowing them to be challenged. Feyerabend explains:

> ... it emerges that the evidence that might refute a theory can often be unearthed only with the help of an incompatible alternative: the advice (which goes back to Newton and which is still very popular today) to use alternatives only when refutations have already discredited the orthodox theory puts the cart before the horse. Also, some of the most important formal properties of a theory are found by contrast, and not by analysis. A scientist who wishes to maximize the empirical content of the views he holds and who wants to understand them as clearly as he possibly can must therefore introduce other views; that is, he must adopt a *pluralistic methodology*. He must compare ideas with other ideas rather than with 'experience' and he must try to improve rather than discard the views that have failed in the

competition.[Fey93, p.20]

Against Method takes as its historical exemplar Galileo's advancement of the heliocentric Copernican model against its orthodox Aristotlean competitor championed by the Catholic Church. Although we commonly think of the so-called Copernican Revolution as the replacement of an obviously defective theological explanation by a properly formed theory from the empirical sciences, Feyerabend shows that this conceals the actual means by which Galileo makes his persuasive case for the (itself badly defective) Copernican model. Centrally, it is process of comparing the heliocentric and geocentric theories that makes the implicit, unstated "natural assumptions" of the geocentric theory clear. Feyerabend elaborates on the kind of structures that counterinduction can reveal:

> Methodological rules speak of 'theories', 'observations' and 'experimental results' as if these were well-defined objects whose properties are easy to evaluate and which are understood in the same way by all scientists.
>
> However, the material which a scientist *actually* has at his disposal, his laws, his experimental results, his mathematical techniques, his epistemological prejudices, his attitude towards the absurd consequences of the theories which he accepts, is indeterminate in many ways, ambiguous, *and never fully separated from the historical background.* It is contaminated by principles which he does not know and which, if known, would be extremely hard to test. Questionable views on cognition, such as the view that our senses, used in normal circumstances, give reliable information about the world, may invade the observation language itself, constituting the observational terms as well as the distinction between veridical and illusory appearance. As a result, observation languages may become tied to older layers of speculation which affect, in this roundabout fashion, even the most progressive methodology. (Example: the absolute space-time frame of classical physics which was codified and consecrated by Kant.) The sensory impression, however simple, contains a component that expresses the physiological reaction of the perceiving organism and has no objective correlate. This 'subjective' component often merges with the rest, and forms an unstructured whole which must be subdivided from the outside with the help of counterinductive procedures. (An example is the appearance of a fixed star to the naked eye, which contains the effects of irradiation diffraction, diffusion, restricted by the lateral inhibition of adjacent elements of the retina and is further modified in the brain.) Finally, there are the auxiliary premises which are needed for the derivation of testable conclusions, and which occasionally form entire *auxiliary sciences.*
>
> ...
>
> Consideration of all these circumstances, of observation terms, sensory core, auxiliary sciences, background speculation, suggest that a theory may be inconsistent with the evidence, not because it is incorrect, *but because the evidence is contaminated.* The theory is threatened because the evidence either contains unanalysed sensations which only partly correspond to external processes, or because it is presented in terms of antiquated views, or because it is evaluated with the help of backward auxiliary subjects.
>
> ...
>
> It is this *historico-physiological character of the evidence*, the fact that it does not merely describe some objective state of affairs *but also expresses subjective, mythical, and long-forgotten views* concerning this state of affairs, that forces us to take a fresh look at methodology. It shows that it would be extremely imprudent to let the evidence judge our theories directly and without any further ado. A straightforward and unqualified judgement of theories by 'facts' is bound to eliminate ideas *simply because they do not fit into the framework of some older cosmology.* Taking experimental results and observations for granted and putting the burden of proof on the theory means taking the observational ideology for granted without having ever examined it.

[Fey93, p.52]

Feyerabend argues that it was the cognitive contrast between the Copernican model and orthodox Aristotlean geocentric and geostatic conceptions which showed up precisely this kind of implicit assumption. In this case, the assumption in question gave rise to the unchallengeable "fact" that the Earth could not be moving rapidly through space because objects are observed to fall straight down- if the Earth were in motion, falling objects would appear to be moving in a slanting trajectory.

> We start with two conceptual sub-systems of 'ordinary' thought ... One of them regards motion as an absolute process which always has effects, effects on our senses included. The description of this conceptual system given here may be somewhat idealized; but the arguments of Copernicus' opponents, which are quoted by Galileo himself and, according to him, are 'very plausible', show that there was a widespread tendency to think in its terms, and that this tendency was a serious obstacle to the discussion of alternative ideas.
>
> ...
>
> The second conceptual system [the Copernican model] is built around the relativity of motion, and is also well-entrenched in its own domain of application. Galileo aims at replacing the first system by the second in *all* cases, terrestrial as well as celestial. Naive realism with respect to motion is to be *completely eliminated*.
>
> ...
>
> Viewing natural phenomena in this way leads to a re-evaluation of all experience, as we have seen. We can now add that it leads to the invention of a *new kind of experience* that is not only more sophisticated *but also far more speculative than* the experience of Aristotle or of common sense. Speaking paradoxically, but not incorrectly, one may say that *Galileo invents an experience that has metaphysical ingredients*. It is by means of such an experience that the transition from a geostatic cosmology to the point of view of Copernicus and Kepler is achieved.
>
> [Fey93, p.69]

In other words, the conceptual frame of the Galilean observer has shifted so that fundamental, common-sense natural perceptions (objects which are not observed to be in motion cannot be in motion) are overturned, and "experience now ceases to be the unchangeable fundament which it is both in common sense and in the Aristotelian philosophy. The attempt to support Copernicus makes experience 'fluid' in the very same manner in which it makes the heavens fluid, 'so that each star roves around in it by itself'. An empiricist who starts from experience, and builds on it without ever looking back, now loses the very ground on which he stands." [Fey93, p.72]

Thus, the Copernican Revolution succeeded because the counterinductive comparison of the older Aristotlean explanation with Galileo's theory revealed the flawed natural assumption of the geostatic model- the assumption that all motion is operative, that objects not in apparent motion for some observer are indeed at rest in an absolute sense. Only by thinking of motion as a relative phenomenon that only produces observable effects when bodies are moving *with respect to one another*, does the problem with the assumption of absolute motion become obvious.

This success was not the result of any of the usual "rules" offered as candidates for the mono-Method. Galileo's theory substantially contradicted the available evidence, erroneously asserted the reliability of telescopic observations, made extensive use of ad hoc hypotheses, and was advanced by propagandistic and even dishonest means. Much of this was unavoidable. Ad hoc hypotheses are necessary for new theories because the auxiliary sciences associated with them have not been developed-

scientific development is intrisically uneven, obligating the use of these makeshift theoretical devices. Without a certain level of dishonesty and rhetorical sleight of hand on Galileo's part, the Copernican program would have succumbed to the greater development and argumentative weight of the scholastic tradition. As Feyerabend notes, if any of the typically suggested Method criteria were applied, the Church would have won the debate and we might still have an Aristotlean cosmology!

For Feyerabend, then, science, like other human social practices (including the arts, administration, and so on) consists of a variety of interacting traditions with differing assumptions, methods, sensory interpretations, and so on. The development of science is thus "not the interaction of a practice with something different and external, *but the development of one tradition under the impact of others.*" [Fey93, p.232]

2.2.2 Classical and Molecular Genetics: Shared Traditions, Plural Methodologies

To bring the implications of Feyerabend's views into some familiar relief, let us briefly examine a case from the field: the "molecularisation" of zebrafish genetic experimentation. Because the zebrafish genome took some time to be released in reliable revision, the use of the Mendel/Morgan "classical genetic tradition" (CGT) was widespread in mapping experiments before this. One might locate an allele of interest by recombination experiments, mapping its position in (by now unfamiliar) units of centimorgans to reflective relative recombination frequency between loci. This is, of course, increasingly unusual, as the practices of the Watson/Crick "molecular genetic tradition" (MGT) are more and more accessible due to the previously unavailable genomic data[5]. The zebrafish geneticist now has access to two traditions to perform their work, and will make recourse to either as they assess circumstances warrant (without any reference to an explicit external rule about which tradition should supercede the other in particular cirucmstances). For instance, if I am planning to cross two fish that are heterozygous at some allele, I will use typical CGT assumptions to calculate the expected number of various offspring genotypes. Even if I was able to offer a reasonable description, in molecular terms, of zebrafish meiosis, recombination, fertilisation, and so on, I would never choose to do so- an MGT explanation is unnecessary where Mendel's laws suffice, and will begin to lack explanatory power the moment I apply it to another species, unlike the Mendellian CGT framework.

One might object that the claims of the CGT tradition "reduce" in some meaningful way to the MGT tradition, or "cover" for it, so that CGT theories are in fact just abstractions of MGT theories. In other words, the fact that MGT claims are not useful and lack explanatory power in some areas does not change the fact that MGT *could* provide good theoretical explanations for all of the claims of CGT. As Kitcher shows, this is not correct[6]. The CGT description of the *process* of meiosis as a particular

[5]It can be an edifying experience to ask a student to "convert" between CGT units like centimorgans and MGT units like megabases. At the very least, the cognitive discomfort produced will tend to reveal a problem in the question's assumptions to the student. At best, one can gain a practical understanding of how scientists make sense of and experience "switching" between different traditional explanatory frames.

[6]Kenneth Schaffner later insisted that Kitcher was wrong, and that CGT theories are reducible to MGT theories, because the MGT theories establish direct linkages between DNA sequences and proteins, and thus are formally equivalent to the classical concepts of genes and phenotypes [Sch93]. This now seems obviously mistaken on at least 2 counts: (1) there is no stable molecular entity implicated by "the gene": its local context in terms of primary sequence (promoters etc.), and tertiary arrangement in a nuclear "transcription factory", all contribute to the complexity of the causal locus involved in transcription alone, and (2) there are very few phenotypes of interest that are caused by stable, uncomplicated interactions between DNA and protein such that classical genetic phenotypes reduce cleanly or usefully to descriptions solely in terms of macromolecules. Moreover, this does not address Kitcher's point: there *are* phenotypic traits which are *not* describable in terms of their protein constituents, and that there *are* higher-level processes which may be generated

type in which "paired entities (i.e. chromosomes) are separated by force so that one member of each pair is assigned to a descendent entity"[Kit84, p. 349] (which Kitcher calls a PS-process), is not describable solely in terms of the molecules participating in the process. Because a PS-process like meiosis can be realised in any number of ways at a molecular level, PS-processes cannot be accounted for in a general way by MGT theories. The manner in which different traditions frequently address different levels of biological organisation in similar non-interchangeable ways is discussed further below.

We can extend this example to see that the typical biologist has access to a great plurality of methodological traditions. Biologists are intuitively used to "translating" between traditions and to switching freely between them when translations are cumbersome or useless. Where there are no deep conflicts between the implict aspects of these traditions, no counterinductive process occurs. That is, we do not "refute" CGT with MGT in the same way the Copernican Revolution "refuted" Aristotlean geostatic theory because MGT rarely makes the structure of CGT seem untenable to us; an attempt to replace CGT with MGT would surely be quixotic. We simply appropriate the methodology of the tradition which is most appropriate for the problem at hand.

2.2.3 Sense of "Tradition" in this study

Thomas Kuhn was soundly criticised for the vague sense in which he used the term "paradigm"[Sch93, p.206], which left him unable to offer coherent explanations. Feyerabend was far more precise, but, as a philosopher rather than a practitioner, never had need (or desire) to guide a scientific project from within the worldview he developed. Having erected an unassailable proof that no special Method existed, and that subscribing to one would have prevented the central Galilean vignette of naive philosophy of science's core Enlightenment self-narrative, he effectively retired[7].

When Feyerabend wrote of "scientific traditions", he meant scientific practice in its broadest possible sense, a social practice with "historico-physiological character". That is, scientific theories are conditioned by the historical moment, and by the involvement of the observer and their social, cognitive, and biological baggage in the phenomenon under study. For the purposes of making Feyerabend's arguments against the existence or advisability of a mono-Method, this was entirely adequate, but I intend only to deal with a limited subset of these materials. The "Stem Cell Biological Tradition" defined below, in its full Feyerabendian sense, includes rumours circulated at stem cell conferences, methodological tips and tricks passed on by observation of careful practice (that could never be learned from any protocol), the commercial aspirations of particular academics, and so forth, in addition to all of the "properly scientific" materials implicated by all of the publications that have ever used the "stem cell" concept or a recognisable homologue. While my use of the term acknowledges this broader contextual sense, I will only look at the explicitly documented "traditional background" of particular, formally advanced explanatory statements or models. The objective here is mainly to account for molecular biological explanations occuring within a "composite tradition" with diverse, only partially overlapping internal "subtraditional" currents.

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by any number of configurations of molecular consituents and are thus better understood as types of processes and not as specified molecular systems.

[7] Feyerabend's chilly reception and subsequent exile is difficult to understand in retrospect, as the basic framework of his argument is now generally understood to be correct in some sense or another. Whether one ascribes the initial poor reaction to his combative personality or flamboyant political statements, his ideas are by now pervasive, even normative. The appearance of "post-colonial" biological studies would have been very unlikely in the Popperian scientific world of the 1960s. Curiously, the Feyerabendian molecular biological traditionalist will note that this may actually constitute an intra-traditional "colonial" venture on the part of critical theorists and continental philosophers.

Therefore, in general, I have not intended to draw any hard divisions between subtraditional currents in the molecular biological tradition (MBT). While I have pointed out how MBT explanations may include components drawn from irreducibly separate subtraditions (the irreducible CGT explanation for PS-processes discussed above), I have only mentioned the philosophical, methodological, etc. implications of MBT subtraditions where necessary, as the primary concern is the extra-traditional background of the "systems" modelling approaches encountered in the data chapters. For instance, in the case of the Galton-Watson branching process model, my definition of this as an artefact (one can think of a probability distribution plot figure in a paper) from the Probability Theory Tradition (PTT) means that this is a traditional statistical solution to an early biological lineage model, which stands in for the large-scale behaviour of a simple model of lineage growth and extinction, in a larger MBT mechanistic explanatory framework (the MEx, Mechanistic Explanation).

I have also made reference to the "Stem Cell Biological Tradition" (SCBT), which is intended to convey the last approximately 6 decades of research practice organised around the stem cell concept[8]. The question of the relationship between the MBT and SCBT is not simple. Till and McCulloch were not offering molecular mechanisms for the phenomena they were documenting [MT60], and could well have been described as operating in an earlier Cell Biological Tradition (CBT).

My conception of this relationship is that, generally, SCBT practice has been "molecularised"; most of the explanations that SCBT practitioners care about are molecular MEx, and the SCBT is therefore a subtradition of the MBT. Nevertheless, the multilevel character of all MBT explanations means that SCBT practicioners routinely use CBT concepts where a specified molecular system would be useless or inappropriate. An example is the ubiquitous abstraction of mitotic phenomena as a class of cell behaviour in SCBT explanations (referred to simply as "mitosis", "proliferation", "renewal" etc), rather than making any attempt to specify this central concept in explicitly molecular terms.

Therefore, in this sense, SCBT practitioners are used to "slotting in" explanatory components from a variety of traditions in order to offer a broader MEx for some phenomenon (regeneration, repair). We are in this sense classic Feyerabendian "Epistemological Anarchists", using whatever explanatory frames suit our purposes. This habit has, in the context of the SBE, shifted from predominantly "intra-" to "inter-traditional anarchism", with the result that the required level of sophistication required to advance a good molecular MEx has increased steeply.

The SCBT is a particularly notable subtradition of the MBT because of its explicitly therapeutic, and therefore ethical and normative orientation. That is, while the broader developmental biology tradition does not have a unified therapeutic goal, the SCBT is structured by the objective of interacting with stem cell phenomena to medicinal and therapeutic ends. This particularly ethical aspect of the SCBT compels me to make some brief remarks about how my own study's design is be guided by this ethical orientation.

2.2.4 Ethical Aspects of Participation in Scientific Traditions

A commonplace in most scientific traditions is that the natural sciences are methodologically incapable of answering moral questions, or offering guidance on what we should value. There is no scientific principle from which we may deduce that we ought not increase the virulence and deadliness of common laboratory pathogens, but most of us choose not to do this, at least in part because we regard it as

[8] I include anti-stem cell and stemness theories in this tradition, since they are marginal heresies to a hegemonic orthodoxy and not meaningfully independent.

ethically dubious. We have noted Feyerabend's demonstration that Galileo, in his dispute with the Church hierarchy, engaged in lies and deception in order to advance his views, in addition to the various theoretical sleights of hand performed. Obviously, there is a difference between creating bioweapons and lying to administrators who bear strong views about the contents and meaning of your work, but we may nonetheless regard Galileo's conduct as somewhat unsavoury, from the point of view of most of humanity's major ethical traditions. The disturbing suspicion thus arises, for the reader of Feyerabend, that conduct which is broadly considered unethical may actually be a common or even *necessary* mode of scientific development.

I contend that this is not the case, at least with respect to the particular problem at hand. As detailed above, this study is not a result of participants in one tradition striving for orthodox recognition over an opposed tradition. Rather, it is the participants of one tradition (broadly, molecular biologists, more particularly, stem cell biologists) adjudicating internally how to make sense of the SBE. This process will implicate the participants' extra-scientific interests, and may well be heated, but theres is no reason to assume that resort to skulduggery will be necessary. By explicitly committing to an exemplary set of ethical principles, I hope to discipline my own extra-scientific commitments by explaining how they give rise to particular objectives of the study.

The particular ethical principles that I take to be relevant for this study's methodology are as follows:

1. **Principle of Charity**

   As a broad range of traditions have been brought together by my colleagues to produce the scientific reports examined critically in this study, it is inevitable that some inconsistencies will be found. Moreover, although I have generally selected papers with formal models for consideration, the narrative rationale provided for the models in primary reports is often fragmentary and confused. I therefore endeavour to understand the arguments I analyse in their strongest possible form wherever more than one interpretation is possible. Finally, I make efforts to *improve* the models I am proceeding counterinductively against on the basis of my criticisms, in order to anticipate good counterarguments from my colleagues.

2. **Respect for Traditional Scholars**

   Following again from the diversity of traditions employed by my colleagues, it is generally necessary to accept mainstream, consensus views on issues outside of my formal training. I prioritise the views of scholars on methods from within their tradition over the views of scholars who are commenting on and using exotic extra-traditional methods, particularly those drawn from the mathematical and statistical traditions. In making borrowings from the philosophy of science, I have done so with the intent that the philosophers in question would find nothing objectionable in way I have used their work, but with the guiding principle that the philosophy of science is an incomplete auxiliary science with respect to my tradition (the MBT)[9], and must be modified to the minimum degree necessary to fit the study.

[9]To the extent that this requires any justification, Feyerabend's remark that the philosophy of science in 1975 was a "bastard subject" with "not a single discovery to [its] credit", might be uncharitably updated to note that it remains to be clearly explained how the situation has changed. The philosophy of biology is a marginal specialisation within this tradition, with little general agreement about how biological theories are structured or function. For the purpose of the study, this is clearly an auxiliary science which has been left behind by the uneven development of traditions, and requires a certain amount of ad hoc tinkering to fit the problem.

3. **Commitment to Traditional Integrity and Stewardship** In taking heed of the historical nature of the MBT, I am expressing my concern that the tradition both remain stable and consistent. The ultimate failure of the 20th century molecular informational paradigm, apparent by the early 2000s, raised the prospect that molecular biology might be substantially incomplete in ways that seriously threatened its explanatory power. Since I am broadly sympathetic to the view advanced by Michel Morange, to the effect that the apparent "death" of molecular biology is a superficial phenomenon concealing biological practice's deep commitment to this tradition [Mor08], I suggest that the construct known as "systems biology" is in fact the molecular biology tradition under the impact of a group of other scientific traditions.

   To the extent that we believe that our tradition has enduring value for humanity, we should be concerned about its integrity in this encounter. We can easily conceive of haphazard adoption of bits and pieces of complexity theory introducing fundamental contradictions into the tradition's base of theory, rendering it useless or counterproductive. Moreover, the nature of the encounter raises the risk that we will be left with methodologies and auxiliary sciences that are much more "expensive" than before, in ways that our society can scarcely afford at this historical juncture. I take it to be the self-evident telos or function of a tradition to sustain itself- a tradition which pays no mind to its own sustainability is likely to terminate in (historically speaking) short order, potentially depriving future generations of the use of that body of thought.

   Such a loss might be particularly devastating if it included the SCBT, with all of its potential therapeutic implications. Understanding, diagnosing, and correcting corruption of subtraditional knowledge should be a priority as a result. I therefore use the criteria of effects on the stability and consistency of the tradition to weigh the future consequences of pursuing particular theoretical constructs as research programs.

4. **Commitment to Open Exchange** Feyerabend describes two possible ways of collectively deciding traditional issues, which he calls "guided" and "open" exchanges:

   > In the first case some or all participants adopt a well-specified tradition and accept only those responses that correspond to its standards. If one party has not yet become a participant of the chosen tradition he will be badgered, persuaded, 'educated' until he does and then the exchange begins. Education is separated from decisive debates, it occurs at an early stage and guarantees that the grown-ups will behave properly.
   > ...
   > An open exchange, on the other hand, is guided by a pragmatic philosophy. The tradition adopted by the parties is unspecified in the beginning and develops as the exchange proceeds. The participants get immersed into each other's ways of thinking, feeling, perceiving to such an extent that their ideas, perceptions, world-views may be entirely changed - they become different people participating in a new and different tradition ... An open exchange has no organon though it may invent one, there is no logic though new forms of logic may emerge in its course.

   This description puts me in a slightly awkward position. I have already adopted as a central concern the integrity of a particular tradition, and I suggest a very specific method to guide an inter-traditional exchange. With respect to the SBE, I certainly appear to be attempting to engage in authoritarian, top-down exchange mediated by a particular molecular biological perspective that I happen to favour.

It is worth here recalling that Feyerabend's central concern was to demonstrate that scientific traditions should not be socially privileged over others on the basis that they use a particular, special Method or regime of reason, because they demonstrably do not. He did not intend to enumerate the conditions required to establish an inter-traditional open exchange in a free society.

While the participants in the open exchange may not specify a particular tradition to provide an "umbrella" for the proceedings, they nevertheless are steeped in particular traditional "ways of thinking" and "world-views". Feyerabend suggests that it is immersion into foreign traditions that produces the beneficial novel traditional structure at the end of an open exchange. I believe this presupposes traditional actors who are unusually aware of their own philosophical commitments and therefore competent to represent their own tradition and its implications to others from other traditions. I freely confess to spending most of my biological career without any such deep competency; a persistent, general, communal deficiency in this regard would seriously compromise the agency of MBT practitioners in the SBE.

I therefore offer this work as a type of personal "intra-traditional" prerequisite to successful open exchange, intended to contribute to the development of the internal discourse required for reasoned participation in the broader SBE. Of course, the SBE already being well underway, we do not have the luxury of treating prerequisites as preliminaries to a later event; rather, this is remedial education that must be conducted on the fly, as it were- Otto Neurath's at-sea refit of my own "scientific metaphysics" is precisely what I am trying to do.

2.3 EHJMEx as a Subtraditional Metascientific Unit of Molecular Biological Theory

From the practitioner's perspective, we are interested in thinking about the "metaphysical unit" of scientific practice on a finer scale than allowed by Feyerabend's concept of a tradition. If we take the practice of molecular biology to be about generating explanations for biological phenomena in terms of macromolecules and their organisation, we want to know enough about the structure of these explanations to allow us to compare them. While we know that we are looking for the "underlying molecular mechanisms" of (usually) cellular phenomena, it is not always clear how we might go beyond intuitive preferences for one proposed mechanism or another in, say, deciding what assay to perform next. We may reason in terms of plausibility or agreement with evidence, but we rarely do so formally[10]. Moreover, it is sometimes unclear what kinds of descriptions are mechanistic explanations (MEx) and when we can agree such a description has explained some phenomenon.

A deliberate counterinductive evaluation of two "units" of biological theory that is productive- one that allows us to imaginatively explore the metaphysical implications of two scientific models for explaining some phenomenon- requires a definition of the unit. Feyerabend's description of scientific tradition suggests that we start by looking at "local" traditional practice. I have thus chosen here to rely heavily on a general conception of the "locally accepted method" proffered by the philosopher of stem cell biology, Melinda Bonnie Fagan (Fagan hereafter), who has extremely usefully summarised these ideas in her recent book "Philosophy of Stem Cell Biology: Knowledge of Flesh and Blood", as well as a number of

[10]It may not even be advisable to do so in many cases, as, for instance, we often become aware of problems with particular lines of research which are not disclosed in published reports.

other useful publications[Fag13, Fag15b, Fag15a]. I have modified her idea by extending it temporally (following Schaffner's Extended Theory concept[Sch93, p.211]) and by placing it within the what I take to be the extended context of the Molecular Biological Tradition conceived of in Feyerabend's terms- explicitly including its auxiliary sciences and metaphysical implications.

2.3.1 Law-based and Causal Mechanistic Explanations

Fagan is not the first philosopher of science to note the local currency afforded to MEx in the MBT. Indeed, she has thoroughly addressed the inadequacies of earlier theorisations of biological MEx. In general, philosophers have previously advanced law-based and causal theories to explain what biologists are doing when they offer MEx.

2.3.1.1 Law-based MEx

Law-based theories suggest that MEx operate by explaining a phenomenon in terms of universal, exceptionless laws of broad scope, given some initial set of conditions. I take it to be obvious that this is not what biologists are doing- MEx simply do not express this kind of law, and I have personally never encountered a biologist who understood MEx in anything like "universal, exceptionless" terms, even as a "regulative ideal" toward which MEx are asymptotically refined. Mechanistic diversity underlying broad classes of phenomena is understood to be normative, so "nomological" law-based concepts are of little use to us.[11]

2.3.1.2 Causal MEx (CauMEx)

Theories of MEx that are underlain by some understanding of causal relations described by the mechanism are more influential than law-based ones within biological practice. The most typical theory of causality taken to underlay these "Causal MEx" (CauMex, com-ex) is an interventionist one, typically expressed in Fagan's description of "manipulability theory". Indeed, biological studies are often divided between "interventional" and "non-interventional", largely along the lines proposed by James Woodward. As Fagan notes of Woodward's description:

> This theory analyzes causality as a relation between values of variables, X and Y. X causes Y if and only if there is a possible manipulation of some value of X, under idealized experimental conditions, such that the value of Y changes. Woodward's is a counterfactual account of causality, hinging on what *would* happen to the values of variables in an idealized experiment, or *intervention*. An intervention I on variable X with respect to variable Y is a causal process that determines the value of X in such a way that, if the value of Y changes, then the change in Y occurs only in virtue of the change in X.
>
> The concept of an intervention provides a regulative ideal for experiments aimed at discovering causal relations: approximate the conditions of ideal experimental interventions. Insofar

[11] Fagan refutes recent advocacy of the law-based view. Schaffner previously advocated for a more sophisticated conception in which biological explanations were only "universal" or "exceptionless" in the specific context where they were formulated (species, tissue, cell type, etc). Biological explanations might then consist of laws which support counterfactual refutation. As Fagan points out, MEx are not even expected to be locally universal explanations for phenomena. One of the best-understood MEx, that of asymmetric mitosis in drosophila Germline Stem Cells (GSCs), only applies in full to about 80% of GSC divisions which "have a plane of division parallel to the hub-GSC binding site." [Fag13, p.97] A proponent of the idea that biological theories make use of "laws" might have resort to "legal pluralism", in which they assert that more than one law may pertain to a particular case, depending on some contextual factor. I doubt a concept of this sort can really be any more useful than Fagan's Joint MEx description adopted here.

> as they meet this standard, experiments reveal genuine patterns of counterfactual dependence among sets of entities and their properties, i.e. causal relations in the world. Because invariance [in the correlative relationship between X and Y] is required only under *some*, not all, interventions, causal explanations need not include general laws. The range of interventions under which a dependency relation between values X and Y holds may be broad or narrow. Within their range, 'fragile' dependency relations are no less causal than those with a much wider range of invariance: universal causal laws. Experiments, if correctly designed, reveal relations between values of variables that are invariant under some interventions. The relevant counterfactuals for causal relations are tested by realizing particular values of X and observing the values of Y under controlled circumstances that fall within the range of invariance for X and Y. [Fag13, p.99]

I quote this description at length because it well-describes the essential orthodox view implied when we teach students the canonical experimental typology of the MBT. This is a highly plausible account of how these experiments "work" to explain the underlying system. We can think of a typical dose-dependence curve in pharmacological research: we apply some hormone over a range of physiologically-reasonable doses X to some cells, and observe the response of some functional readout Y (say, the cell-normalised luminance of a luciferase reporter downstream of a receptor for X). If there is a invariant relationship between X and Y over some reasonable dose range, we may postulate that the application of dose X causes the luminance of the cells Y by resulting in hormone binding, activation of a promoter element, etc. If we perform a series of other interventions we can test counterfactuals about this relationship: we may try to observe the movements of GFP-tagged signalling proteins during the application of dose X, or apply inhibitors of enzymes, antagonists of receptors, and so on, to implicate the other elements involved in the causal chain we suppose makes up the mechanism. Therefore, CauMEx do seem to be describing useful features of our explanatory practices in the MBT.

Fagan provides this formulation (M) to summarise the conventional understanding of this view:

> (M) A mechanism S consists of multiple diverse components (x's) engaging in causal relations or activities (ϕ's) such that x's and ϕ's are spatially and temporally organized so as to produce some overall phenomenon. [Fag13, p.95]

Of course, mechanistic explanations are multi-level, so components may themselves be composed of sets of these causal relations, and the overall mechanism S may in turn be a component of a higher level set of relations which describes a broader S, and so on. This overall view of CauMEx explanations is summarised in Figure 2.1.

Of this view of CauMEx, Fagan writes:

> An account is full if and only if it describes all *relevant* components of a mechanism. Causal relevance is in turn analyzed in terms of mutual manipulability. A working component (x ϕ-ing) is relevant to an overall mechanisms behavior (S Ψ-ing), if the latter can be manipulated by intervening on x's ϕ-ing *and* x ϕ-ing can be manipulated by intervening on S Ψ-ing. A component is irrelevant to a mechanism if neither x's ϕ-ing nor S Ψ-ing can be manipulated by intervening on the other. These two sufficient conditions link the hierarchical structure of MEx to experimental practices in neuroscience, which employ both top-down and bottom-up strategies to detect components of mechanisms. [Fag13, p.100]

Therefore, we seem to have an appealing account of what biologists are doing with MEx that is neatly connected to our experimental practices. However, Fagan describes three fundamental problems with CauMEx.

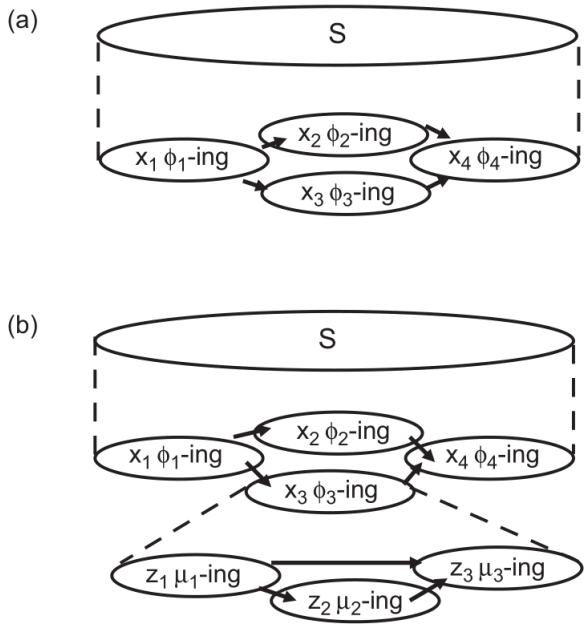

Figure 2.1: The consensus view of mechanisms, excerpted from [Fag13, p.96] (a) Two levels: component xs and overall mechanism S. (b) Downward expansion: three mechanistic levels.

1. **Ambiguity**. The overall mechanism S is a causal explanation: mitosis (S) is a process of cellular reorganisation resulting in two cells being produced from the one mitosing (the phenomenon P) (Fig. 2.2 (a)). We infer the contribution of S's components x (eg. proteins involved in spindle formation, cytoskeletal components, etc) to P by examining the effect of altering their activities ϕ (such as by mutating a spindle formation protein of interest) on P (Fig. 2.2 (c)). However, usually, we take the overall explanatory bricolage we assemble of inter-component causal relations to explain S, not only P. That is, the proteins involved in spindle formation are part of a mechanism that explains the mechanism of the mitotic process itself (S Ψ-ing), and not only its phenomenal manifestations while Ψ-ing (chromosomal reorganisation, the appearance of mitotic furrows, etc.) or after S has finished Ψ-ing (the fact of there now being two cells).

2. **Directionality**. The assumed *direction* of causal relations in the MBT is usually implied in our casual speech: we are looking for "underlying" molecular mechanisms, which is to say, we take any "chain" of causal relations to flow upwards from a bottom level of macromolecular components. Generally speaking, "overarching" mechanisms in which the whole S feeds back onto its component xs in some way are not produced. Moreover, it is unclear in what sense one could impinge on the overall mechanism S and "cause" a change in one of the components x in a "downward" sense- as Fagan lucidly notes, intervention into a mechanism *is* impinging on its components x[Fag13, p.103]. We are establishing a "constitutive" relationship between S and its components x, not a causal one. This makes a nonsense of the "mutual manipulability" interpretation of top-down and bottom-up approaches, since there is no manipulation of x by virtue of an intervention into S as a whole [12].

[12] As Fagan correctly notes, top-down experimental approaches are *exploratory*, not *explanatory*.

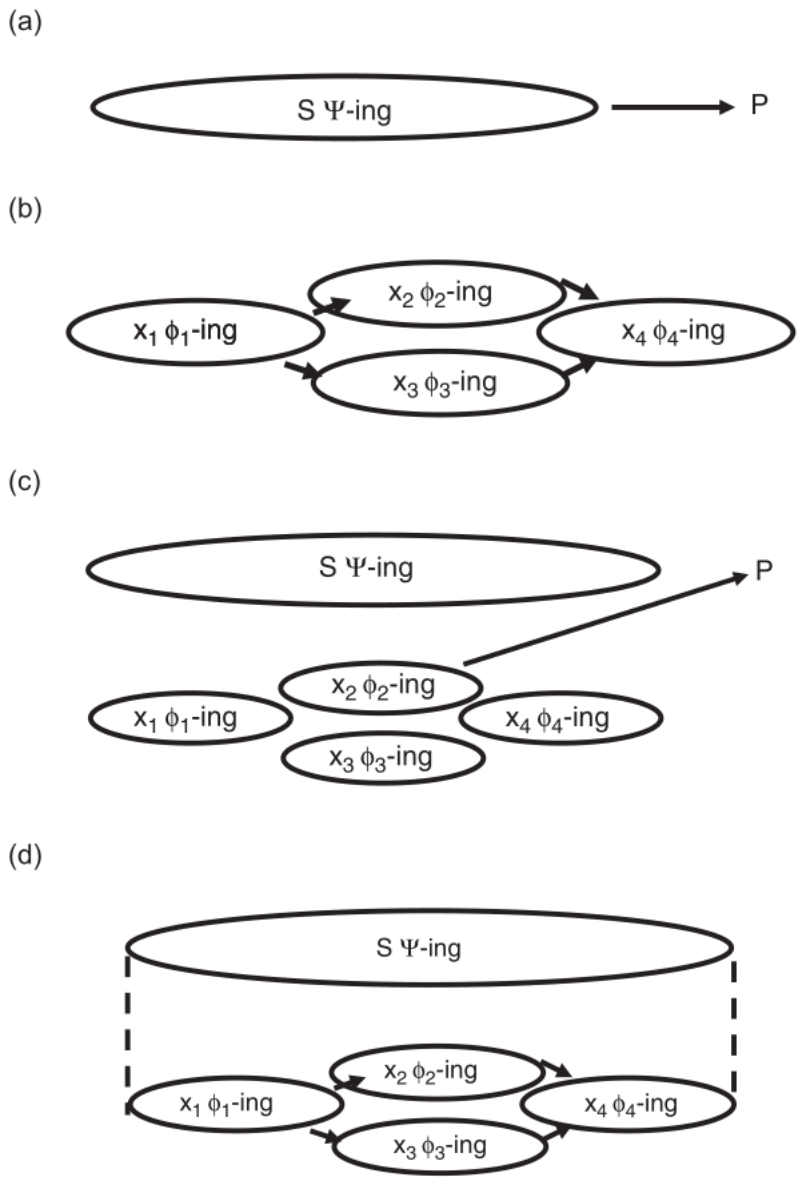

Figure 2.2: Levels of causal relations in MEx, excerpted from [Fag13, p.102]. (a) System-level: mechanism S Ψ-ing produces phenomenon P. (b) Component-level: component x's causally interact (ϕ-ing) to effect one another. (c) Inter-level: component ϕ-ing x's make a difference to Ss outcome (P). (d) Constitutive MEx: component x's interact (ϕ-ing) to constitute mechanism S's Ψ-ing

3. **Modularity**. In order for components to be defined in terms of manipulability theory, they must be independent. If we offer a mechanism consisting of generalisations about the behaviour of $x_{1,\ldots,n}$ macromolecular processes, in order for x_1 to be differentiable from x_2, it must be in some way causally independent- we must be able to intervene into x_1 (eg a generalisation about DNA synthesis) without affecting x_2 (eg a generalisation about membrane fusion complexes). Without some form of "modular independence" between mechanism components, there is no reason to consider them separate, yet causally linked, "components" of a larger overall mechanism.

   However, as Fagan notes, the "interventionist" causal paradigm implies explanations that "reveal what a phenomenon of interest depends on, answering what-if-things-had- been-different? questions about the values of variables within a fixed range of invariance. But MEx are prima facie concerned with a different kind of question: how-does-it-work?" [Fag13, p.106] In this case, the causal relations that form "the ways we conceptualize them as distinct, relevant components ... *are the very same* causal generalizations that figure in MEx." [Fag13, p.106] That is, the causal relations that provide the rationale for the division of a mechanism into independent components are *also* those that we take to be causing the activity of the mechanism- the components are thus not merely *conceptually* but *"actually", causally* separable, like components of a machine. Obviously, we understand this not to be the case. Fagan concludes:

   > For physiological, cellular, and molecular mechanisms, as we understand them, the behavior of isolated components is not a good guide to their behavior together, and their behavior in one context is not a good guide to their behavior in others. If this line of reasoning is correct, then the causal-mechanical account of MEx leaves out some- thing important: *inter*dependencies among components of biological mechanisms. [Fag13, p.106]

   As most modestly observant MBT benchworkers have noticed by now, components of biological mechanisms are not literally interchangeable gears that can be removed from one machine and slotted without modification into another "geartrain" (mechanism) missing a similar component [13]. *How* x ϕs depends on *how* it is intermeshed with other xs and what their ϕing consists in. A gear only depends on the other components to the extent that it requires their presence and their intermeshing in one particular way within some tolerances[14] Indeed, the nature of the interdependencies between molecular components are critical to the "how-does-it-work" explanation. As indicated in the section on hierarchy theory, understanding these interdependencies is especially important in "multi-level" explanations, where xs ϕ-ing at different dynamical scales do not *directly* interact in the same manner that same-level xs ϕ-ing do.

[13] There are a few theorists who still don't understand this, but the situation has improved. We are, now, only rarely subjected to the hallucinations of "synthetic biologists" insisting that trees will be reprogrammed to grow chairs directly on their branches, for instance. The persistence of this fixation can be attributed to the mind-boggling success of the standardisation and modularity practices of modern logisticians, and their mechanical and electrical engineering colleagues. It is worthwhile to remember these are extremely recent developments- an artefact as modern as the Rolls Royce Merlin engine, the machine that won the Battle of Britain, did not have interchangeable parts (to the profound horror of Allison engineers in the USA tasked with setting up domestic American production of the engine). Each part was literally hand fitted for each engine by an English craftsperson. We should note that the very English metaphor for biological "design practices" is "tinkering" and not "process systems engineering".

[14] There must be at least b microns of backlash for the gears to ϕ together (rotate mutually around their axes), or they will lock and abrade each other when the machine applies force by Ψ-ing, until abrasion establishes b microns between them and they are free to ϕ or the mechanism fails (probably jammed with powdered gear teeth). Biological components "intermesh" in a much greater diversity of ways and with far looser coupling ("slop") than mechanical ones, although every real mechanism needs a little slop in it, as the gear example indicates.

Therefore, Fagan has gone on to advance what is, in my view, a much improved conception of biological MEx, the Joint MEx, to which I have made some minor additions, as described below[15]

2.3.2 (Extended) Heterogenous Joint Mechanistic Explanations ((E)HJMEx)

While by "Extended Heterogenous Joint Mechanistic Explanation" I mean a unit of theory consisting more or less of a diachronic series of Fagan's "Joint MEx", I have a slightly different understanding of the "explanatory components" of JMEx (jay-mex), which I have differentiated with the term HJMEx (hedge-mex). I will first clarify how I understand Fagan's JMEx improvement over the Law-based and Causal concepts. I will then define HJMEx and then explain what it means to "extend" one for analytical purposes, producing a EHJMEx (edge-mex)[16].

2.3.2.1 Joint MEx (JMEx)

Fagan has described a similar improvement to the typical Causal MEx view on three occasions: first in 2012 [Fag12], later in her 2013 book [Fag13], and most recently in a 2015 paper [Fag15b]. I have adopted the general sense of the framework described in these works, which I have called the JMEx, for Joint Mechanistic Explanation.

Fagan's terminology varies as she emphasizes different aspects of her overall idea. [Fag15b] seems to use "collaborative" interchangeably with "joint," which was the preferred term in [Fag13]. "Collaborative" seems to be intended to emphasize the amenity of the concept to explanatory pluralism- this is one reason I have selected it, but it is less descriptive of MEx structure. I have therefore adopted the shorter "Joint", since a commitment to explanatory pluralism *within* MEx is part and parcel of the the entire foregoing description of the MBT, and respecifying it is redundant.

Fagan indicates that both CauMEx and JMEx are species of "Constitutive" MEx, which is to say that the mechanism consists of components that are together taken to constitute some kind of productive causal regularity which generates a phenomenon. Because a mechanism's components must be linked *somehow* if they are not just to be considered separate systems, some theory of causality is required to establish what these linkages might consist in. JMEx's account of "intermeshing properties" is sufficiently general that MBT practitioners with different ideas about causality can talk to each other about these properties without needing to carefully specify either a particular local causal account with significant problems (manipulability theory) or a fully worked-out global theory of causality.

Fagan's solution to the problems with Causal MEx dispenses with the problems her analysis of them revealed. Let us recapitulate Fagan's formulation of Causal MEx (M) and follow it with her JMEx formulation (JM) for comparison[17].

> (M) A mechanism S consists of multiple diverse components (x's) engaging in causal relations or activities (ϕ's) such that x's and ϕ's are spatially and temporally organized so as to produce some overall phenomenon. [Fag13, p.95]

[15] Fagan is an explanatory pluralist herself; in [Fag15b] she explicitly states that she does not seek to replace "causal mechanistic" conceptions with her own JMEx. However, the conceptual problems with causal explanations she catalogues in [Fag13] remain unresolved. If there are "good" CauMEx, it seems to me they need a much deeper theory of causality than interventionist accounts provide. For the time being, Fagan's work is adequate to stand on its own as an account of the "local explanation" in the SCBT.

[16] While "fanciful" naming conventions and illegible acronyms are time-honoured traditional practices of the MBT, I apologise to readers for indulging out of a need to conserve space and stably refer to particular concepts.

[17] I have used [Fag15b] (JM) as it contains the [Fag13] definition but is more precisely worded. I have changed "system M" to "mechanism S" to conform with the other citations of [Fag13] used here, but this does not change the meaning in any way.

> (JM) Components $x_1,\ldots x_n$ jointly Ψ as mechanism S if and only if
>
> (i) each x_i has properties that mesh with one or more other x_i,
>
> (ii) $x_1,\ldots x_n$ are spatially organized and their activities ϕ_1-ing, $\ldots \phi_m$-ing causally organized in virtue of their meshing properties,
>
> (iii) $x_1,\ldots x_n$ and $\phi_1, \ldots \phi_m$ so organized constitute mechanism S Ψ-ing, and
>
> (iv) $x_1,\ldots x_n$ and $\phi_1, \ldots \phi_m$ not so organized do not constitute S Ψ-ing
>
> [See footnote 17 for description of clarity edit made to (JM)] [Fag15b]

Fagan elaborates on what would therefore constitute a good JMEx:

> (JM) suggests a basic norm for what I shall term 'collaborative explanation,' namely, that a successful explanation of this kind must show that the components and system of interest (the target of the model) satisfy the conditions of (JM). Given a set of components and their associated activities, and an overall system exhibiting some behavior, an explanatory model of the latter should describe:
>
> (i) the properties of components that allow them to interact in specific ways (I.e., meshing properties, as well as other conditions, such as spatio-temporal proximity.)
>
> (ii) the spatial and causal organization determined by these interactions
>
> (iii) how the overall system behavior is constituted by the organized components, such that
>
> (iv) components' organization makes a difference to the overall system behavior.
>
> [I have expanded Fagan's footnote related to item (i) as a parenthetical comment] [Fag15b]

This strikes me as an entirely adequate definition for MEx, as well as a prescriptive norm for "good" or "complete" explanations that provides a reasonable broad guide to what sort of things MEx should eventually be able to do for the system they explain. Moreover, with JMEx, Fagan resolves all three problems with CauMEx simply by replacing manipulability theory with her conception of Joint relations between MEx components, which I find a highly convincing demonstration of the conceptual adequacy of Jointness.

1. **Ambiguity** is resolved. The component xs of JMEx are understood to be interdependent and linked by descriptions of their intermeshing properties expressed while ϕ-ing (eg. binding, activation, phosphorylation, and so on). It is the assemblage of xs, linked by virtue of these properties, that constitute mechanism S Ψ-ing, so that we are explaining this overall activity of S with our JMEx, which in turn is understood to produce P.

2. **Directionality** is clarified. JMEx "bottom out" at the level of macromolecules, in general, and do not typically reference "lower levels" in the scale hierarchy, like atomic or electronic phenomena. We understand that when we intervene experimentally into the activity of some system S that a mechanism describes, we are not having a "downward" effect on the mechanism's components x in their ϕ-ing, but that intervening into S *constitutes* intervening into some x, all the way down levels of organisation to the MBT's "explanatory foundation" macromolecular level.

3. **Modularity** is dispensed with. Given the "Jointness" account of the intermeshing properties of components x of JMEx, he rationale for their "separateness" does not depend on manipulability theory. Therefore, we are able to account for the interdependency of the mechanism's components and avoid implying that we believe we can intervene into one component in a manner that causally isolates it from the others.

Having summarised Fagan's JMEx concept and its fine qualities, let me pause to point out an interesting feature of JMEx: the "jointness" or "collaborative" account of causal relationships between JMEx components is adapted from social scientific accounts of *shared cooperative activity* (SCA), that is, the intentional, end-direct activity of multiple agents collaborating. As Fagan indicates, her analysis of joint activities "is modeled on (Bratman 1999)[[Bra99, pp.93-108]], with adjustments for non-intentional contexts." [Fag15b] I return to some aspects of Bratman's description of inter-human SCA for useful ideas about agency later, but overall, Fagan's adjustments make perfect sense. She has chosen to "strip down" an account of joint action between people in order to produce one that fits, say, signalling pathways, which clearly have no psychological states, and of which it is meaningless to speak of "intent". Moreover, the "stripping down" leaves an excellent skeleton which can be "refit" in a conservative, non-psychologising manner that admits biosemiotic descriptions of cellular agents, at the appropriate levels and with regard to the appropriate "meshing properties".

This makes JMEx a spectacularly good type of explanation for multi-level mechanisms that incorporate *bona fide semantic agents* like cells. We expect to be able to express JMEx for higher-level phenomena like tissue formation because we can plausibly represent the joint, coordinated activity of the cells that constitute the tissue as agents.

I take a substantially different view of how "systems biology" is related to JMEx, however, which I have explained below. Firstly, while Fagan is clearly a sophisticated philosopher of biology, and a masterful documenter of the "local model terrain" in the SCBT, her particular vision of JMEx that has a two limitations for my purposes. I have addressed each limitation in turn, in the context of my proposed solutions: the "H" and the "E" (proceeding outward from the core JMEx concept) of the EHJMEx.

2.3.2.2 Heterogenous JMEx (HJMEx)

The first of these limitations to JMEx, as presented by Fagan, is related to an epistemological and metaphysical stance shared with the influential master historian of the MBT Evelyn Fox Keller, who offered her own thumbnail sketch of the SBE some 18 years ago:

> "Out of the wickedness of war," wrote Warren Weaver in 1949, in a paper entitled "Problems of Organized Complexity," "have come two new developments . . . of major importance in helping science to solve these complex twentieth-century problems." The first of these was the electronic computer, built to process the masses of data generated by the procedures of modern warfareand, perhaps most famously, to decipher enemy messages encoded in ever more elaborate encryption devices. The second development is most commonly associated with the word *cybernetics*, Norbert Wiener's term for the study of control and communication in machines and living beings. Extrapolating from his experience with "goal-oriented" and "self-steering" devices designed to improve the accuracy of anti-aircraft artillery, Wiener and his followers envisioned the construction of purposive machines that would resemble living organisms in every way. Indeed, these machines would be built on the very principles of circular causality ("in which every part is reciprocally both end and means") that Kant himself had invoked as the defining feature of the organism.
>
> These two developments were clearly related-at the very least, they were related in time, in place, and in the needs from which they arose. Yet despite their persistent conjoining in the popular imagination, despite Wiener's own hopes, and despite even John von Neumann's efforts at integration, conspicuous differences between the two remained. In the one the emphasis was on computational power, while in the other it was on principles of organization andincreasingly over the 1950s and 1960sof self-organization. In fact, in was not

> until the 1980s that the different visions embodied in these two developments would begin to resolve, and the first steps of that resolution came with the rise of connectionism, parallel processors, and neural networks. Yet Jacobs claim that the sequence of DNA could serve as Bernards "invisible guide" depended absolutely on joining together these two still-disparate developments. His metaphor of a program drew directly from Turings original model of a computer (the reader may recall from Chapter 1 his equation of "the genetic material with the magnetic tape of a computer"), but the idea of a purposive machine was borrowed from Wiener's cybernetic vision. The difficulty is that, in locating the program in the genome, much of the cybernetic vision of goal-seeking and self-organization was lost. And so was the recognition of the importance of reliability and with it, an appreciation of the kinds of organizing principles that would be needed to maintain such reliability. Redundancy, for example, is a basic principle of design for building reliable systems, and it is hard to imagine how, were it not for this amnesia, recent findings of extensive redundancy in developmental pathways could have been quite as startling as they have been. [Kel00, p.109-111]

That is, on Keller's view, the "molecular reductionist" trend in the MBT, myopically fixated on Crickian information flow outward from genomes, lost sight of the need to deploy the fruits of the IT revolution and of cybernetic theory to explain "goal-seeking" and "self-organisation", which is to say, agency. The logical solution to this quandary is to do precisely what Fagan suggests- reintroduce the *cybernetic formula*, levering the computational power of modern semiconductor devices to solve systems of ordinary differential equations (SODEs), treating cells as a type of complex chemical system with Dynamical Systems Theory (DST). Both Keller and Fagan believe this is a reasonable way to find the macromolecular basis for Waddington's classic theory of cellular specification. Fagan makes this view explicit in her definition of mathematical "Systems" approches to JMEx:

> A cellular systems model consists of a finite set of molecular elements $\{X_1, X_2 X_n\}$, representing DNA, RNA, proteins, and small molecules. Complexes of multiple components and functionally-distinct forms of one molecule are represented as distinct, so the set may be larger than a simple parts list. Each molecular element in the set is characterized by a value of a state variable $\{x_1, x_2 \dots x_n\}$ at time t. In these models, the cell is defined as a complex system which at any time t is in a state $S(t)$ that is fully determined by the values of a set of variables $\{\mathrm{x}_1, \mathrm{x}_2 \dots \mathrm{x}_n\}$ representing the state of each molecular component. The values of these variables exhibit numerous and diverse dependency relationships. Cell behavior, including development, is conceived as the result of changes in the values of state variables. A set of molecular elements, each described by a state variable, and dependency relations between the values of those variables, comprises a cell network model.
>
> In this way, systems biologists aim to derive predictions about cell behavior, including developmental phenomena, from mathematical descriptions of interacting molecules. Insofar as these predictions are confirmed by experiments, the mathematical models that entail them can be said to explain the phenomena in question. The process of model construction begins with detailed description of a molecular mechanism. This mechanistic description is then simplified into a wiring diagram, which is next translated into a formal framework. Solutions within a formal framework correspond to vectors and attractors, and these vectors and attractors in turn define a 'landscape.' [Fag13, p.204]

Fagan has advanced this prescriptive view of what Systems biological approaches to JMEx consist in on several occasions, so that she has produced a useful summary diagram, reproduced in Figure 2.3. The most obvious feature of the diagram for the student familiar with MBT models is its linearity: there is *only one* Systems approach represented here.

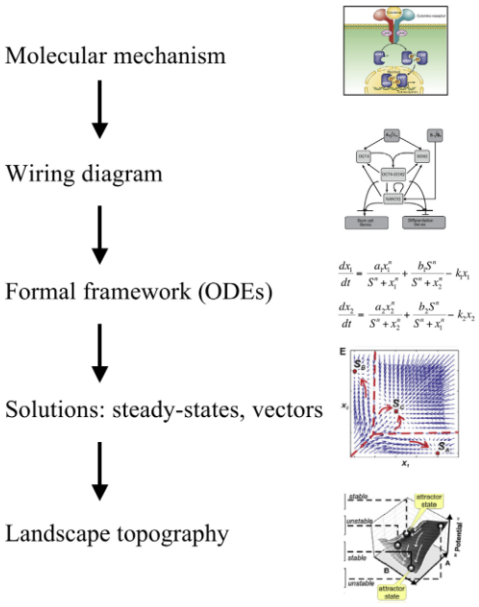

Figure 2.3: Cellular systems model-construction, excerpted from [Fag15b, p.7]. The system of equations and subsequent steps are based on a 2-element wiring diagram.

While most MBT practitioners and students are familiar with this approach, what is most notable about it is that it is, in practice, rarely applied in this form. Part of the reason for this is technical problems in relating complex macromolecular systems to formal SODEs, which I have summarised in the section relating to that method and its theoretical underpinnings. In general, however, I have gone against this "Systems formalisation scheme" for the following reasons:

1. **Actually Existing MEx Pluralism**. Harris' theory, like most others, does not use DST to produce topographies that represent some kind of "Waddington space". While much effort has gone into DST-type formalisations of mechanisms, there are other types of "Systems explanations" that must be accounted for.

2. **Unclear General Utility**. While SODEs offers a plausible formalisation of Waddington's view, it is not at all clear that all or most cellular processes involve transitions between attractors in some state space whose dimensions express protein expression levels or estimates of activity. Waddington's view of the cell is of a probability landscape of *chemical tendencies* extended in time, underpinned by genes and the interactions of their products (see Fig. 2.6). This is the conceptual conceit that allows macromolecular relations to be treated as a system of ODEs; their interactions are understood ordered chemical reactions in cellular "bioreactors". I see no reason to presume that this is an adequate modelling frame to explain phenomena where, for instance, the specifics of local inter- and intracellular spatial organisation is as or more salient than chemical kinetics, as in many emerging MEx related to nuclear behaviours, transcription factories, tissue formation, etc.

3. **Commitment to Open Exchange**. As noted in Section 4, I am taking some care not to dictate what post-Systems biology ought to look like. I do not know what "systems" disciplines will ultimately prove to be successfully integrable into the MBT. Given the potential of, for instance, physical explanations to contribute as components of MEx [Mor11] or of limits on them, it seems unwise to exclude these by adopting a MEx concept that does not allow for their inclusion, or of other methods that may prove useful. I am in particular concerned with the formation of subtraditions committed to particular mathematical approaches and not able to meaningfully compare their model formalisations.

I have, instead, treated "Systems" approaches as methods of generating extra-traditional generalisations about the components of mechanisms, so that a "Systems" JMEx does not necessarily summarize the activity of the entire mechanism (S Ψ-ing) using one particular mathematical method, but may draw from any number of traditions in generalising about x's ϕ-ing.

There are at least two concerns with this sort of ad hoc modification: the possible loss of biological macromolecules as the lowest level of organisation represented in MEx, and the possible loss of the multi-level logical relations constituting the "Joint action" of macromolecular x's ϕ-ing together to constitute an S Ψ-ing. I address these in turn:

2.3.2.2.1 HJMEx may specify their "bottom" macromolecular levels polysemously

Firstly, Fagan's JMEx concept usefully explains the fact that MBT explanations tend to "bottom out" at the macromolecular level. This is generally recognised and is often offered as a reason for considering the MBT to be meaningfully independent from physical and chemical traditions. We rarely make reference to physico-chemical properties of biological molecules, except in defining their activity (ϕ-ing) or their relations (how x_1 jointly ϕs with x_2's ϕ-ing). I do not want to lose this feature of Fagan's JMEx.

At the same time, we often offer generalisations about molecular behaviours that we intend *polysemously*. Let us take the example Fagan offers of the mechanism for *Drosophila* germ stem cell (GSC) specification- as Fagan notes, most (80%) but not all mitoses proceed by employing this mechanism. However, if we want to model the cellular population dynamics of a whole *Drosophila* embryo, we probably will offer a generalisation about a mitosis as a *process*- that is, we are interested in the *total number of divisions*, irrespective of their molecular mechanisms. If we were interested in (a)symmetric divisions, we would actually want to specify something like Kitcher's PS-process concept, which could conceivably be accomplished by any number of mechanisms. Therefore, a JMEx that made reference to "mitoses" in general, or "symmetric" divisions in particular, could well have a description of some x that ϕs on a higher-than-molecular level of organisation, that does not reduce directly to any particular set of molecular zs μ-ing, to use the terminology of Fig. 2.1. Instead, it may refer to any number of MEx, and is thus "polysemous" in the sense that it is constituted by more than one entity at the "macromolecular basement" level[18] One could conceivably add more detail "below" this level if necessary, but in general, there is every reason to expect MEx to have components that are not usefully broken down beyond a certain level, and which, when applied in an abstract or theoretical manner, may not even refer to stable molecular entities at all[19].

However, as I hope is clear, I do not mean to imply that a CGT traditional concept like a PS-process has some reality *independent* of the specific macromolecular arrangements that actually constitute cells dividing and partitioning their contents between two descendants. The fact that the CGT concept does not reduce cleanly to a set of one or more MGT descriptions of macromolecular processes in nuclei may or may not be relevant to the explanatory quality of the JMEx which uses the CGT concept as a stand-in for many different MGT-specifiable cellular dynamics. To a large extent, this depends on what we

[18]This polysemy essentially specifies all actually-present mechanisms S that Ψ such that some P occurs. It is very likely that eg. mitosis, in most contexts, is driven by a smaller number of mechanisms that account for a majority of divisions, but a very large number of mechanisms that account for rare ones (these could even be completely unique). If we are accounting for cell number in a tissue, we do not care if a mitosis was produced by a miraculous, unique mechanistic causal locus or not. A division is a division.

[19]Arguably, a PS-process is a basic requirement for a living thing, defined as a replicating semiotic agent. Semiosis implies an interior and an exterior, so a membrane or wall of some kind, forming a cell. "Replication" in the strict sense must then proceed by duplication of the agent's contents and segregation by dividing its contents into two membrane compartments. To the extent that the selection of macromolecular materials used in living systems is historically contingent, there will be a correspondingly large number of possible material substrates and intermeshing properties that produce such a process.

suppose the intermeshing properties of the mitotic process and the other components of the mechanism to be. That is, if we suppose mitosis (call it x_1 ϕ_1-ing) to be mainly independent from other relevant cellular behaviours we are modelling ($x_{2...n}$ $\phi_{2...n}$-ing), it may not matter whether we have any account of the specific macromolecular arrangements ($z_{1...n}$ $\mu_{1...n}$-ing together), which we take to constitute x_1's activity, to expand our HJMEx "downward".

Moreover, we understand that any time we propose an experiment which intervenes onto some x ϕ-ing, where the HJMEx uses a polysemous generalisation including many macromolecular arrangements of zs μ-ing jointly, that intervention is onto some subset of actually-occurring, specifiable-in-their-particulars macromolecular $z_{1...n}\mu_{1...n}$-ing together. That is, the use of polysemous generalisations in no way implies that an intervention is not "directly" onto the macromolecular materials constituting an organism. For example, if there is more than one macromolecular process which produces mitosis in some cultured cells, and we apply a chemical inhibitor of mitosis, we understand that we are going to intervene into that polysemous distribution of possible macromolecular developments that result in mitosis. Which mechanisms are actually affected depends on how common those macromolecular trajectories[20] are and how the chemical inhibitor affects the components and intermeshing properties of each one. Chemical inhibitors of mitosis that broadly affect commonly used mechanisms would be promiscuous inhibitors of a macromolecularly polysemous behaviour, validly described by polysemous CGT generalisations about mitosis. We may be interested in specific inhibitors if they are available, but inhibitors of rarely employed mechanisms will probably never be discovered in the first place, due to the population-based assays typically used in drug discovery.

Conceivably, this could lead to a class of MBT HJMEx that are composed entirely of generalisations which cannot be reduced to particular biological macromolecular relations at all. This seems like a plausible outcome of the SBE- a class of models that deal exclusively with phenomena at higher levels of biological organisation, polysemously referring to huge arrays of unspecified molecular arrangements (perhaps consisting of statistical generalisations about assemblages of possible mechanisms S that Ψ to produce P). It seems very unlikely to me that these will be anything more than toy models and curiousities without strong grounding in the MBT's foundational explanatory level, however, so this does not seem like a likely risk to MBT traditional stability.

2.3.2.2.2 HJMEx retain constitutional directionality and allow causal nuance

The second potential problem involves the potential confusion involved in incorporating extra-MBT causal generalisations at higher levels of biological organisation. For instance, there is much interest in tension and shear-induced signal transduction involved in morphogenesis [ML15, p.5] as contributors to self-organisation and biological complexity. Therefore, HJMEx might include physical explanations at very high levels of organisation. It is unclear that all such explanations can be understood to proceed from the "bottom up".

Let us take one such HJMEx that already circulates "in the wild": behavioural impingement on osteoblast development and bone dynamics. Bone, being a highly dynamic tissue, actively remodelled in response to stress, has structural and functional aspects that are determined by organismal behaviour, psychological states, etc. This is obvious when we consider what we have to do to "model" the effects of microgravity conditions on bone dynamics in rodents: the hindlimb elevation assay (HEA?), ie.

[20] I mean this in the loosest possible, non-Newtonian, non-quantitative, non-state space sense.

restraining the animal such that its organised behaviour cannot create normal stresses on the bone, which in turn results in bone mineral density loss[21]. This occurs via the sensing of tissue-level strain by osteocytes, which in turn direct the action of osteoblasts and osteoclasts[22] in remodelling the overall structure of the bone to better suit the actually-experienced load.

A component of a HJMEx describing bone remodelling might therefore include a statistical generalisation of behaviourally-conditioned bone stress dynamics (say, a finite element analysis of a rat's bone under normal behavioural strain over some period of time). It is not immediately clear how such a HJMEx should be hierarchically arranged. Conceivably, a description of the rat behaving could be a "mechanistic" system S Ψ-ing, which includes the details of the molecular mechanisms underlaying osteocyte strain sensing as low-level zs μ-ing in mainly-macromolecular MEx. This ambiguity leaves space for those with directional theories of causation to claim that one may intervene onto the rat behaving (S Ψ-ing) and so cause a downward cascade through mechanistic levels and ultimately impinge onto the basement level in this manner, implying a similar problem to the one we identified with the mutual manipulability account of MEx.

I respond to this in two ways:

1. Firstly, HJMEx retain "constitutional" directionality in two senses:

   (a) there is a "basement" level but no "ceiling" ("sky's the limit", organisationally). That is, HJMEx assume that the biological scale hierarchy *necessarily starts* at the level of molecular biological material *simplicter* and proceeds upwards through macromolecular assemblages, organelles, organelle lineages, cells, cell lineages, tissues, organisms, organismal lineages, local ecosystems, planetary ecosystems, and then, hypothetically, interplanetary ecosystems, intersolar ecosystems, and so on[23].

   (b) HJMEx imply that interventions at higher levels *constitute* intervening at the lowest level. If humanity intentionally destroys the planetary ecosystem by raising the average temperature of the planet to the point where plants no longer reliably germinate, this *is* an intervention at the lowest possible biological level of organisation of all organisms. Unloading the rat's limbs *is* intervening onto macromolecular processes in osteocytes.

   This is completely sufficient for understanding the structure of a causal locus as I have defined it, and it retains JMEx's accurate reflection of the "bottoming out" of biological MEx at the macromolecular level.

2. Secondly, HJMEx deemphasize simplistic "directional" accounts in favour of detailed exploration of the intermeshing properties of JMEx at different levels of organisation.

   Let us again take up the example of osteoclast strain sensing. There is a significant problem in the potential representation of a Mechanical Engineering Tradition (MET) finite element analysis (FEA) as a generalisation of the effects of rat behaviour on bone loading. The FEA is a description of the physical dynamics of tissue, measured on a spatial scale of (perhaps) millimeters, and a

[21] I have placed "model" in scarequotes because it is not at all clear to me that you can study the effects of microgravity except in a 1g laboratory. Microgravity-related BMD loss being a behavioural disease of a tiny number of humans (astronauts), taking on this risk with full informed consent, it is hard to see how it can be ethical to torture thousands of rats with these assays in order to "study" this "medical issue".

[22] Osteoblasts lay down bone, osteoclasts destroy it. The local balance of osteoblast/clast activity determines whether bone is plated out or broken down at a given site.

[23] Theories of panspermia typically postulate intersolar ecosystems with planetary ecosystem components connected by their "intermeshing property"- their propensity to fling debris into space under the impact of asteroids, etc.

temporal scale of seconds. Now, macromolecular dynamics occur on spatial scales of nanometers and temporal scales of microseconds! There is therefore a dynamical barrier in this scale hierarchy, discussed further in the section on hierarchy theory.

What is interesting to us then is not a question of causal priority or "directionality" but a question of "how-does-it-work", a request to elucidate the intermeshing properties between the extra-traditional MET FEA and whatever model we have for macromolecular osteoclast strain sensors. The interesting question is *how* information about strain is brought across the dynamical barrier by the strain sensor, and *how* the resultant activities of osteocytes, osteoblasts, and osteoclasts act to alter the bone's structure such that its stress/strain properties evolve under load.

Therefore, HJMEx allow us to accurately represent contemporary "Systems" theories, while retaining the MBT's understanding of the explanatory "macromolecular basement". By emphasizing the intermeshing properties of mechanisms, and their part-whole relations, we can move past problematic causal generalisations into specific descriptions of these properties.

2.3.2.2.3 HJMEx defined Fortunately, little needs to be done to Fagan's JMEx (JM) definition to differentiate HJMEx. I have indicated these clarifications with HJM-n notations.

(HJM) Components $x_1,\ldots x_n$ jointly Ψ as mechanism S if and only if

(i) each x_i has properties that mesh with one or more other x_i,

(ii) $x_1,\ldots x_n$ are spatially organized and their activities ϕ_1-ing, $\ldots \phi_m$-ing causally organized in virtue of their meshing properties,

(iii) $x_1,\ldots x_n$ and ϕ_1, $\ldots \phi_m$ so organized constitute mechanism S Ψ-ing, and

(iv) $x_1,\ldots x_n$ and ϕ_1, $\ldots \phi_m$ not so organized do not constitute S Ψ-ing

HJM-1: Each x_i ϕ_i-ing consists of a description of the behaviour of a biological system at the macromolecular level of organisation or higher.

HJM-2: At any level of organisation higher than the macromolecular "basement", each x_i ϕ_i-ing may describe one or more lower level mechanisms S Ψ-ing polysemously, and need not specify any of these S in any particulars.

HJM-3: Polysemous xs ϕ-ing describe general properties of a population of Ss Ψ-ing that intermesh with other xs ϕ-ing, and these need not arise as a consequence of specifically defined macromolecular mechanisms S.

HJM-4: Polysemous xs ϕ-ing may have general properties that intermesh with zs μ-ing on lower level, such that there is an interlevel exchange of information (boundary conditions are placed on an assemblage of zs μ-ing, for instance), and these need not arise as a consequence of specifically defined macromolecular mechanisms S.

HJM-5: All descriptions x are drawn from some scientific tradition, and so carry with their use an array of traditional natural assumptions, epistemological and metaphysical axioms, methodological considerations, and so on. No x is *a priori* excluded from inclusion in HJMEx on the basis of its traditional identification alone.

HJMEx are therefore somewhat more complex than JMEx, and require careful attention to the physical and biological plausibility of between-component and interlevel relations. That said,the "basic norm" that Fagan suggests for JMEx is upheld for HJMEx. Good HJMEx describe (i) the properties of components that allow them to interact in specific ways, (ii) the spatiotemporal-*cum*-causal organisation produced by these interactions, (iii) the sense in which the overall system's behaviour is constituted by

the components, (iv) in a manner that explains how the organisation of components makes a difference to the system's behaviour.

Having so-defined HJMEx, let us proceed to consider how we might assess the development and deployment of these explanations in scientific research programs.

2.3.2.3 Extended HJMEx (EHJMEx)

I have further modified the HJMEx concept by suggesting extending it in time over its career, iteratively deployed as an explanation in different studies. I have taken this idea from Kenneth Schaffner's idea of the "Extended Theory". As Schaffner says of his own "metascientific unit":

> I am using the notion of an *extended theory* to introduce a diachronic unit that (1) permits *temporal* theory change and (2) also allows for some *logical* changes - namely, some of the assumptions within the diachronic unit will change while the integrity of the whole is preserved. [Sch93, p,211]

Schaffner was working with a substantially different conception of a biological theory[24], but my intent is similar. There are numerous reasons to describe a theory's development in time. I have done so because I was unable to assess, by inspection, the broader significance of changes to components of the theory as expressed in different primary reports over time.

By representing a changing HJMEx over time diagramatically, I am able to better understand the relationship of changing theoretical structure to the model output and explanatory power, since I can compare the output of different HJMEx to empirical data in the global model selection approach. Moreover, this diachronic approach provides insight into the dynamics of a particular theoretical construct over time, so that we can form an idea of whether a given EJMEx is becoming more or less useful, consistent, precise, etc., and possible reasons for this.

2.3.2.3.1 EHJMEx defined There is little additional nuance to EHJMEx, so let us simply define one as a series of HJMEx explanations, arranged in chronological order, such that the EHJMEx reveals the diachronic explanatory dynamics of the changing HJMEx being offered by a particular research program[25]. I suggest that presentations of EHJMEx would normally be marked up in such a way that the changing components of the explanation and the relevant traditional implications are highlighted.

2.4 The Feyerabendian Modeller

If we understand the value of the interaction of scientific traditions in Feyerabendian terms, we become sensitive to the metaphysical contrasts induced by when we cognitively compare explanations produced by two or more traditions. A valid contrast between traditional explanatory frames occurs when we come to correctly understand *how* the two traditions' metaphysical implications differ[26]. If we are pursue this as a deliberate practice, particularly with the regard to the details of (E)HJMEx, it would

[24] In effect, Schaffner wanted to be able to compare Lakatosan "research programmes" consisting of lawlike generalisations arranged in hierarchies of centrality to the overall programme.

[25] One might also conceive of a complex HJMEx phylogeny, branching as different researchers adapt the HJMEx for their own local purposes, rather than a linear sequence.

[26] Invalid metaphysical contrasts can be drawn, as from misunderstanding or malice, and there may even be rhetorical sleights of hand that make these errors seem consistent, so one must be alert during this process. Biologists are better aware than anyone of how easy it is to "fool oneself", hence our attachment to blinding procedures.

be helpful to have a systematic way of making these contrasts. It is difficult to cognitively explore the full implications of some outside-MBT "Systems" artefact, like a solution to a system of ODEs, without an equally systematic exploration of the implication of that explanation *and its alternatives* for the phenomenon at hand.

Therefore, we are interested in an approach that can reveal the *systematic implications* of arbitrary HJMEx for the dynamics of our system. By this, I mean we want to make formal comparisons between model output for some group of proposed HJMEx, with to their explanatory power for some set of real data. We would also like to ensure that the approach is sufficiently general that it can be used to generate comparable output for HJMEx irrespective of the traditional or methodological makeup of their "explanatory components". In other words, if someone comes to us with a verbal description of rules that a retinal progenitor cell might follow on its morphogenetic trajectory towards integrating into the neural retina, this should be comparable to any complicated set of statistical generalisations about the same kinds of cellular behaviours.

2.4.1 Laws, Rules, Models, Games

As should be clear from our foregoing discussion, we are not expecting to encounter enough "laws" in HJMEx to describe the overall explanation as "Law-based". Nonetheless, one obvious consequence of admitting physical explanations into JMEx is that we may encounter traditional claims which include ostensible *bona fide* laws[27], alongside our typical diagramatic macromolecular JMEx. These may be integrated into explanations that involve something like verbally conveyed heuristic rules ("no differentiation for the first 2 rounds of mitosis"), as well as sophisticated mathematical simulations of phenomena, or statistical generalisations about higher-level phenomena (ie. "mathematical models" in general) whose molecular underpinnings are unknown or not relevant.

There tends to be some ambiguity in the way that we use these terms, and a necessary anthropomorphic projection onto a phenomenal reality of transcendent complexity and informational density. If by "law" we mean that the mathematical description of some natural regularity offered by one of the physical subtraditions is *actually existentiated* as some kind of iron fisted algorithmic tyrant, we have a very strange sort of ontology[28] We know, of course, that our physical descriptions of the world are also "models", that Newton's Laws are not God's Laws, nor Nature's Laws, but a set of finely honed mathematical approximations of macroscopic mechanical phenomena local to a particular gravity well. We do not speak of Quantum Laws but of Quantum Theories, of the Standard Model of physics, and so on. We should nevertheless be clear that no reasonable person any longer believes that there is a "true model", a generative algorithm "actually out there", that can be discovered by comparing different candidate

[27]Various traditions may also posit a priori logical or reasoned truths. We tend to agree that typical logical syllogisms do not need justification, but we rarely examine the logical background of our claims (why are they justified? what is their structure?). The science of logic seems underutilised in the biological sciences.

[28]Richard Swenson has memorably described the definition of genes and memes as "selfish", Ideal algorithmic replicators (which cannot be the case, as neither is a valid unit of selection) as a form of "Neopythagorean Reductionism".[Swe97]. This sort of covert Idealism is a byproduct of the unresolved Cartesian fissure mentioned in the discussion of the SBE. It is, ironically (since advanced by militant atheists), the product of Descartes' bifurcated *Christian* ontology. Unable to fully digest Greek philosophy properly rooted within its Abrahamic heritage, the Greek Pauline church was unable to confer upon its Catholic and Protestant progeny a viable monist ontology capable of providing a reasoned approach to study of causal regularity in phenomenal reality, and was forced to divide existence between the fallen "natural" world and an infinitely transcendent "supernatural" plane, interacting indirectly through providence (the workings of which were irretrievably compromised by the identification of First Cause with a particular human being). It was inevitable that this divide would be reproduced in Rene Descartes' ontology as "matter" and "mind", and thenceforth to the absurd spectacle of "scientists" denying the reality of their own minds while covertly presupposing an invisible mathematical realm where algorithms vie for dominance.

formulations until the "correct" one is found, thus settling the issue of what the "true" set of laws, set of rules, model, or whatever "actually" is. Systematised knowledge about phenomenal reality is *made possible* by abstracting away from the unique momentary particulars of each example of some general class, and any type of theory or model involves abstractions. That is to say, any biological explanation is by necessity *not* a complete recapitulation of the system that generated the original phenomenon.

In this thesis I have, for the most part, described EHJMEx as "explanatory programs" or "bodies of theory", HJMEx as "explanations" or "theories", and reserved the term "model" for particular mathematical formalisations used either (a) as components of HJMEx ("component model") or (b) as summaries of the overall output of HJMEx ("summary model") for some parameters of interest. I am not very interested in enshrining any of the diverse components of HJMEx or the explanations themselves as "laws", but we can usefully speak of "rules" that connect them- the intermeshing properties of various HJMEx components will often be simple prose descriptions of how one depends on the other. These "rules" are more like human-generated legislation than Divinely-*cum*-Naturally ordained Law- we do not expect cells to always and everywhere "follow the law", subject to a tyrannical deity or algorithm. They will, of course, break some of any conceivable set of rules if one observes enough cells long enough.

I therefore suggest that we think of HJMEx in this context as a the "rules" to a type of simulated game. This is appropriate, given that systematic Western modelling of complex biological phenomena has deep roots in the evolution of games like Kriegspiel. Indeed, the widely-known toy Conway's "Game of Life" is a type of cellular automaton closely related to the spatially-situated NetLogo models employed in this thesis. Biologists are thus generally familiar with this type of simulative "game". In comparing summary models of our explanations, we are not asking "which of these models is actually true", but "which of these games is most 'realistic'". We mean something more quantitative than this, of course, which is related to information theoretical measures of information loss between empirical reality and model, as discussed below. It is, nevertheless, necessary to differentiate between the *rules* of the "game" (the overall HJMEx offered in some publication), the *specific interpretation* of the rules that gives rise to the actual implementation of the game (eg. the procedural logic of some summary model that we use to elucidate what the HJMEx "game" implies about the system it represents)[29], and actual living phenomena (cells are not referring to a rulebook in their "play"). This is an appropriate perspective to keep, as we take our models seriously as "realistic" simulations of phenomena while not ascribing reality to any of them, since they are, after all, games and not Law. We may consider analogically the type of computer simulation used by modern militaries- while everyone is entirely conscious of the essential irreality of these virtual representations of combat, their "explanatory power" with regard to the technical particulars of warfare is taken extremely seriously.

2.4.2 NetLogo Agent Model Simulation

For the seasoned modeller, this suggests some general procedural or rule-based simulation space. We can simply assemble the rules and statistical generalisations that we find in HJMEx and simulate the behaviour of a cell or population of cells that satisfies this overall systemic description. This need not be particularly sophisticated to begin with, since none of the current explanations in the field involve detailed explanations of the production of eg. cellular morphology, or anything that would require much

[29] Any set of rules requires an interpreter who implements them. Particularly annoying and tendentious interpreters of game "rules" are often called "rules lawyers", and the same sort of hermeneutic or interpretative activity is always present when confronting the necessary ambiguities in a ruleset. Generally speaking, one tries to keep to the intent of the person offering the ruleset, in accord with the Principle of Charity, and avoid "rules lawyering" that tends to distort this.

spatial detail. That said, there is no reason why a simulation framework should not be "portable" to whatever simulation contexts are required, including those that may eventually include fine-grain three dimensional spatial detail (eg. synapse formation, nuclear dynamics, etc.).

There is, fortunately, an accessible, appropriately-scaled, open source, academic modelling software package that is ideal for expressing the kinds of "cellular logics" that we find in Harris' HJMEx- NetLogo. Developed by Uri Wilensky as a revamp of the Logo programming language, it is specifically intended to handle simulations of emergent phenomena which arise from the interaction of many agents with interdependent internal dynamics. It is relatively simple to code and to understand the code of others, and is designed to be "low threshold and no ceiling", meaning we can start with very simple models without fear that we will be unduly constrained in implementing more complex ones later[30]. Furthermore, NetLogo is widely used in theoretical modelling work, and so is a broadly acceptable approach in the spirit of

Because we can simulate arbitrary cellular logics in 2d or 3d with NetLogo, there are few restrictions on the HJMEx which can be used to explain the behaviour of retinal progenitor cells. Both traditional SCBT modelling approaches (clonal model, SSM) are fully expressible in NetLogo, including in their "unrealistic"features (eg. we could decide to simulate "immortal" stem cells that "self renew" without progeny). We can simulate comparable population-level outcomes for HJMEx which draw on different traditions, because the logic of the different explanations is "translated" into the code which drives the model's agents. This feature of simulating agent models means that dissimilar models may be brought into a comparable context, where their relative explanatory value can be assessed given some empirical dataset.

2.4.3 A Global Approach to Model Selection

If we take NetLogo as our modelling frame in which we will express all of our diverse HJMEx, we need a more concrete idea of how to approach this task. I proceed here with a broad suggestion taken from the statistical theory of model selection. As Burnham and Anderson state in the introduction to their helpful text "Model Selection and Multimodel Inference":

> Often, one first develops a global model (or set of models) and then derives several other plausible candidate (sub)models postulated to represent good approximations to information in the data at hand. This forms the *set of candidate models*. Science and biology play a lead role in this a priori model building and careful consideration of the problem. [BA02, p.2]

The development of global models is generally prior to the consideration of any particular dataset or to the use of statistical methods. The selection of a global model is something which is guided primarily by the modeller's objectives and by their detailed area knowledge:

> Building the set of candidate models is partially a subjective art; that is why scientists must be trained, educated, and experienced in their discipline. The published literature and experience in the biological sciences can be used to help formulate a set of a priori candidate models. The most original, innovative part of scientific work is the phase leading to the proper question.
>
> ...

[30] I am indebted to the interesting paper of Rafael Bravo and David E. Axelrod, [BA13], for demonstrating the practical utility of this approach in simulating colon crypts *in silico*.

> Development of the a priori set of candidate models often should include a global model: a model that has many parameters, includes all potentially relevant effects, and reflects causal mechanisms thought likely, based on *the science of the situation*. The global model should also reflect the study design and attributes of the system studied. Specification of the global model should not be based on a probing examination of the data to be analyzed. At some early point, one should investigate the fit of the global model to the data (e.g., examine residuals and measures of fit such as R^2, deviance, or formal χ^2 goodness-of-fit tests) and proceed with analysis only if it is judged that the global model provides an acceptable fit to the data. Models with fewer parameters can then be derived as special cases of the global model. This set of reduced models represents plausible alternatives based on what is known or hypothesized about the process under study. Generally, alternative models will involve differing numbers of parameters; the number of parameters will often differ by at least an order of magnitude across the set of candidate models. Chatfield (1995b) writes concerning the importance of subject-matter considerations such as accepted theory, expert background knowledge, and prior information in addition to known constraints on both the model parameters and the variables in the models. All these factors should be brought to bear on the makeup of the set of candidate models, prior to actual data analysis.
>
> [BA02, p.16-17]

This approach is itself "counterinductive" to usual field practice, which generally emphasizes finding the simplest model that accords with a selected set of data. As Feyerabendian modellers, we should immediately be aware that this stage of consideration necessarily implicates rules, preferences, and natural assumptions that are specific to traditions, to the intellectual and social background of the modeller, and so on. Models are fundamentally tradition-specific artefacts that arise from particular scientific and social practices:

> Models arise from questions about biology and the manner in which biological systems function. Relevant theoretical and practical questions arise from a wide variety of sources (see Box et al. 1978, OConnor and Spotila 1992). Traditionally, these questions come from the scientific literature, results of manipulative experiments, personal experience, or contemporary debate within the scientific community. More practical questions stem from resource management controversies, biomonitoring programs, quasi-experiments, and even judicial hearings. [BA02, p.16]

One continuously cited traditional consideration that we all recognise, but for which no specific rule can ever be articulated, is "biological plausibility", which must obviously enter into biological modelling practices:

> The more parameters used, the better the fit of the model to the data that is achieved. Large and extensive data sets are likely to support more complexity, and this should be considered in the development of the set of candidate models. **If a particular model (parametrization) does not make biological sense, this is reason to exclude it from the set of candidate models, particularly in the case where causation is of interest.** In developing the set of candidate models, one must recognize a certain balance between keeping the set small and focused on plausible hypotheses, while making it big enough to guard against omitting a very good a priori model. While this balance should be considered,we advise the inclusion of all models that seem to have a reasonable justification, prior to data analysis.[BA02, p.17]
>
> [boldface original]

There is no rule or set of rules by which "biological plausibility" might be established. Stem cells are themselves a clear example of this: the modern history of stem cell research describes the successive

expansion of "possible" cell behaviours in an unpredictable and enduringly surprising fashion. Moreover, plausibility judgements are heavily influenced by the sort of thing that one thinks the system to be, by the attributes we take it to have and the behaviours we already know it undergoes.

For instance, while the phenomenon of cells undergoing "fusion", resulting in durable genetic reprogramming of the resultant "offspring" had been described in culture, there was no particular reason to believe that this could, or did, occur in any systematic way *in vivo*. However, there has recently been description of transgenic hematopoetic stem cells (HSCs) migrating to the eyes of mammals in response to retinal damage, where they enter this immunologically privileged site and fuse with Müller glia, resulting in reprogramming and functional repair of the lesioned retina.[PBS+18, SSV+16] There are any number of reasons to believe that the underlying phenomenon (HSCs infiltrating the retina and fusing with retinal glia) is "biologically implausible". Someone with a view of the mammalian retina as a highly static tissue might not have ever considered the possibility. Whether one accepts the authenticity of the report and its interpretation or not, prior to its publication, excluding this type of "extra-organic" invasion by distant stem cells in modelling approaches to the induction of Müller glial-mediated retinal repair, on the grounds that this behaviour was implausible, would not have raised much objection.

We should therefore to seek to be aware of what is implied by our traditional models, and what may be gained by the use of a general model framework in which they can be expressed as submodels. One advantage of proceeding deliberately in this way is that we avoid the common pitfalls of "shotgun" approaches and "data dredging", which tend to produce descriptions of spurious regularities:

> It is not uncommon to see biologists collect data on 50-130 "ecological" variables in the blind hope that some analysis method and computer system will "find the variables that are significant" and sort out the "interesting" results (Olden and Jackson 2000). This shotgun strategy will likely uncover mainly spurious correlations (Anderson et al. 2001b), and it is prevalent in the naive use of many of the traditional multivariate analysis methods (e.g., principal components, stepwise discriminant function analysis, canonical correlation methods, and factor analysis) found in the biological literature.
>
> ...
>
> A model is fit, and variables not in that model are added to create a new model, letting the data and intermediate results suggest still further models and variables to be investigated. Patterns seen in the early part of the analysis are "chased" as new variables, cross products, or powers of variables are added to the model and alternative transformations tried. These new models are clearly based on the intermediate results from earlier waves of analyses. The final model is the result of effective dredging, and often nearly everything remaining is "significant." Under this view, Hosmer and Lemeshow (1989:169) comment that "Model fitting is an iterative procedure. We rarely obtain the final model on the first pass through the data." However, we believe that such a final model is probably overfitted and unstable (i.e., likely to vary considerably if other sample data were available on the same process) with actual predictive performance (i.e., on new data) often well below what might be expected from the statistics provided by the terminal analysis (e.g., Chat- field 1996, Wang 1993). The inferential properties of a priori versus post hoc data analysis are very different. [BA02, p.18-38]

Therefore, we seek to build an a priori, general, global model, inside of which we may specify "sub-models", including Harris', and including those that may include quite foreign explanatory components. The "design principle" of a Feyerabendian global model is about specifying limits within which many traditional explanations can "operate", so that we do not restrict what kind of sub-models we can compare simply because they do not make sense within a *particular* conception of how the behaviour of stem cells ought to be described.

2.4.4 The Global Semiotic Agent Model

While the details of the global model employed in this study are advanced in Chapter 2, we should briefly consider the broad strokes of the picture so that we have an idea of what types of cellular activities we are interested in. Ultimately, we are looking for explanations of a phenomenon P (zebrafish retinal formation and development), produced by a mechanistic system S, which we take to be constituted (primarily) by cellular xs which ϕ together to produce the overall phenomenon P. We want a tissue-level explanation that drills down to the relevant macromolecular underpinnings of our cellular behaviours ϕ.

At the tissue level, we already have a good, general, a priori description of the sorts of cellular ϕ-ing that give rise to tissue morphogenesis offered by our own evolutionary developmental biologist Elly Larsen:

> All morphology results from only six cell behaviors and two tissue strategies [Fig. 1]. Morphological evolution occurs as a result of heritable modification of where and when the six cell behaviors are expressed. The six cell behaviors contribute to many different tissue structures; for example, cell death is important in embryonic processes, such as the removal of cells between human fingers to allow their separation. Matrix secretion is important in forming structures like bone, but is also important in more subtle aspects of development such as providing chemical signs which guide migrating cells. Migrating cells themselves can help form an astonishing array of structures including skeletal elements and muscles. However, the cell behaviors perform their roles according to one of two tissue strategies.
>
> In the self-governing (autonomous) strategy, sheets of cells, known as epithelia, produce a variety of morphological structures, including the four general types: tubes, rods, spheres and sheets. The power of self-governing epithelial sheets to generate structures is well illustrated by imaginal discs in *D. melanogaster*. These are sacs of cells, which originate as out-pockets from embryonic and early larval epithelial sheets. During larval life, localized cell divisions produce disc-specific folds. At metamorphosis, each disc turns into a specific appendage, such as a leg or antenna, chiefly through changes in cell shape.
>
> Unlike the self-governing strategy, a common tissue interaction or conditional strategy employs mesenchyme cell migration and utilizes interactions between mesenchyme and epithelia. Mesenchyme cells originate from epithelia through cell detachment and migrate through the epithelial basement membrane. Their migratory path and cell differentiation is directed by interaction with overlying epithelial cells. In vertebrate limb development, signals from the epithelium influence cell division and differentiation in mesenchyme, while the mesenchyme modulates cell division in the epithelium. Thus, the tissue interaction strategy adds tissue level controls to the development of the four morphological structures from which organisms are created. It is important to realize that the tissue- level controls produced by the tissue interaction strategy may act as developmental constraints to evolution. [Lar92]

Dr. Larsen's view is summarised in her useful figure, adapted here as Fig. 2.4. Larsen is here concerned mainly with explaining the evolution of *morphogenesis*, which is to say, the specialised functional roles that appear in the generated morphology are of less concern than the form-producing, spatial behaviours of the cells that produce them. While the SCBT is, of course, in conversation with the younger evolutionary developmental tradition (EDT), Larsen's perspective on tissue-level interactions is salutory. Harris' HJMEx are mainly about the dynamics of conceptually isolated cells, but what if he is wrong? In order to establish that, say, the inclusion of tissue-level effects improves the explanatory power of one of Harris' models, we have to be able to account for these effects in the first place. Moreover, Larsen has a clear perspective on tissue-level "intermeshing properties": sometimes these are involved in tissue formation (autonomous), sometimes they aren't (interacting). At this point we should scrutinise these "intermeshing properties" more carefully.

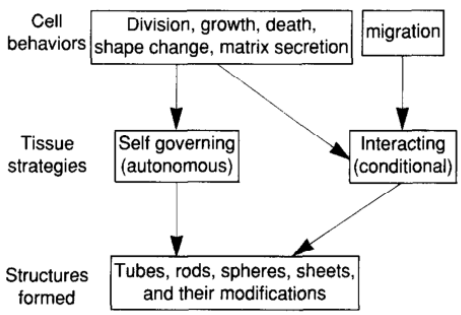

Figure 2.4: Relationship between processes and biological structures, excerpted from [Lar92]. The six cell behaviors and two tissue strategies from which all morphology results.

Since we know already that a tissue-level interactions are constituted by cellular interactions (which are in turn constituted by macromolecular zs μing together in various ways). Let us first deal with the issue of the appropriate level of organisation for intermeshing properties specified by tissue-level HJMEx. We can imagine specifying all of these properties at the "basement" level of macromolecular organisation in physico-chemical terms, and in so doing, specifying the tissue-level system that this web of macromolecular zs μing would constitute. This would be a sort of macromolecular reduction, where we would try to explain Larsen's "morphogenetic alphabet" [**?**] from the ground up. From the discussion above, and the polysemy we expect to encounter in our HJMEx, we can infer that in general, this would be futile. Moreover, "Systems" approaches since Waddington have emphasized the organisation of the cell as a type of cybernetic bioreactor or factory. In Monod's memorable phrase, "You have an exact logical equivalence between these two-the factory and the cell." The various feedback loops at this level are taken to constitute the general entity which is said to "regulate" some cytoplasmic process. Although I think the cybernetic view is mistaken in significant ways, Waddington and Monod obviously understood perfectly well that molecular MEx would not make much sense as descriptions of isolated enzymes or chunks of signalling pathways-*cum*-transcription networks. It may be possible to conceptually, if not experimentally[31], isolate these components and describe them in terms of their feedbacks on one another (as in the *lac* operon), but these components only *do* anything when installed in an appropriately regulated cybernetic cellular factory. Therefore, I take it that there is general agreement that a tissue-level HJMEx will focus on macromolecular phenomena at the level of the cell- where we cannot specify the macromolecular composition of some relevant system, we expect to encounter a statistical generalisation about what we take to be the activity of that macromolecular system operating *at the level of whole cells*.

Let us briefly consider an example: we might postulate that in differentiating, a cell may change its transmembrane protein distribution over time, such that nearby cells are given a differentiation-promoting signal. If we want to represent this in our agent model, will we be looking for a description of the conformational states of transmembrane proteins, and the various models describing their interactions with neighbouring extracellular transmembrane domains? Only to the extent that such a description can inform us of what is happening at the *overall* level of the cell, since we need to minimally hypothesize that such a protein is present in sufficient median concentration across the interacting membrane surface to have the pro-differentiation effect. As we may want to refer to a potentially polysemous macromolecular system (say, several separate pro-differentiation signalling pathways that separately contribute to this effect), the cell forms the "ground floor" above the "macromolecular basement". The fact that SCBT traditional explanations (and Harris' EHJMEx) are organised around the cellular "ground floor" is thus unsurprising- being HJMEx, parts of the "macromolecular basement" are not excavated, and other parts

[31] Obviously, enzyme biochemistry permits partial isolation of some kinds of macromolecular components *in vitro*. The basic idea of synthetic chemistry was that biochemistry would allow the assembly of "biological lego" in this biochemically reductionist sense. The fact that industrial bioprocess research has largely moved on from the idea of synthetic cells and is now focusing on "*in vitro* synthetic biology" is telling, given that this is nothing other than a description of the biochemical roots of the "synthetic biology tradition".

contain potentially chaotic polysemic macromolecular MEx.

We should further consider what this overall picture implies about higher levels of organisation before we move on. There are a few possible conceptual levels that are of less immediate interest: the lineage clearly matters, but in a retrospective, analytical sense, for instance. Tissue-level phenomena may meaningfully "feed back" on the behaving cells which constitute those phenomena. Tissue-level phenomena will generally arise from composite action of cells, such that we expect to see population-level characteristics established at the level of the tissue, constituted by and feeding back upon the behaviour of the cells constituting those populations. If we bring in abstract "tissue-level" effects, like a morphogen gradient, we understand this as a description of a population of cells, either locally or, in the case of tissue-interaction strategies, a second tissue-level mechanism meshing with the first. In the case of two tissue-level HJMEx interacting, we may expect one of these to be a highly abstract generalisation of a paired tissue, like a generalisation of the morphogen gradient, for instance. In this case the intermeshing property may be located in the cells of a local tissue responding to distant production of a signal. We understand that all of these generalisations are "constituted" of cellular xs ϕ-ing, which are in turn made of of macromolecular zs μ-ing. It therefore seems that there is nothing in our selected modelling approach that would contradict the foregoing discussion of what we understand by HJMEx in the MBT.

Having identified in my discussion of the SBE the agency or end-directedness of life as a good candidate for a counterinductive advance, and given my discussion of biosemiotics, I repeat the suggestion here that semiotic phenomena must be accounted for in a more sophisticated manner than allowed for by the cybernetic approach. In particular, it should allow for phenomena to be divided between what the eminent philosopher of science Charles Sanders Peirce identified as "dyadic", or asemiotic, brute facts, and those identified as "triadic", or legitimately semiotic, habitual phenomena. Although considerations of space prevent a recapitulation of this argument here, we may simply note that the NetLogo agent modelling approach does not restrict us from making this kind of representation. This implies that we should be able to compare relatively asemiotic logics like those of Harris' EHJMex to those that include explicit representation of semiotic phenomena, given a global model that includes semiosis, since semiotic phenomena can simply be removed to express Harris' HJMEx as individual submodels within the global one. We therefore move to discuss the specifics of the statistical method involved in doing so.

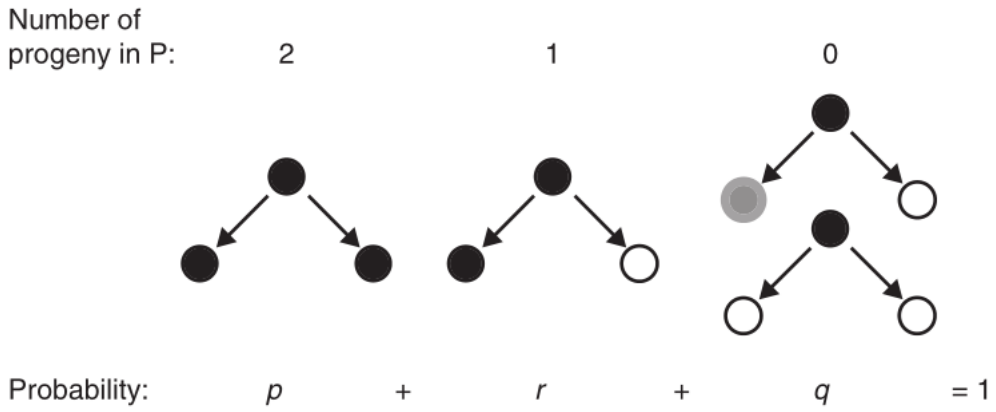

Figure 2.5: Simple stochastic stem cell model, representing probabilities of cell division events, excerpted from Fagan 2013 pg. 61. Black circles denote proliferative cells, while white and grey circles denote different types of postmitotic offspring. "Number of progeny in P" is the number of mitotic offspring produced by each type of division. The probability of each division type must sum to 1, as all possibilities are represented, granting that the division types are defined by the postdivisional mitotic history of the offspring.

2.4.5 Overfitting, Underfitting

2.4.6 The Akiake Information Criterion For Model Selection

2.4.7 Expression of Traditional SCBT Models As Agent Models

2.4.8 From Autoreferential Asemiotic Agents (AAA) to Semiotic Agents (SA)

2.4.9 Cellular Semiosis: Phenomenal Logic and Relation to HJMEx

2.4.10 Bayesian Epistemological View on Statistical Generalisation

2.4.11 Bayesian Epistemological View on Model Comparison

2.5 The Systems Biology Encounter - The Molecular Biological Tradition Under Impact

2.5.1 Historical sketch of the biological encounter with complexity

2.5.2 Explanatory Models in the Stem Cell Biology Tradition

2.5.2.1 Simple Stochastic Models

Referenced on pages: 3,23

The Simple Stochastic Model is schematically summarised in Figure 2.5. This is the basic structure of the great majority of formal models in the SCBT that are derived from post-hoc analyses of populations taken to include stem and progenitor cells. The population-level approach is usually explicit, as no

differentiation is made between types of proliferating cell- in general, no particular cell is identified with a stem cell, nor can any be identified from the necessarily retrospective population data used to infer the parameters of the model.

The central concept of the model is that divisions can be categorised by the number of progeny which remain mitotic after the division. It is important to note that a mitotic event cannot presently be categorized in this fashion except retrospectively. This must be kept in mind when analysing models of this type, as this categorisation does not necessarily imply that there is some mechanism by which the cell specifies the fate of offspring *at the time of mitosis*, although there is extensive evidence for the coupling of mitotic and differentiative processes at the molecular level.

In effect, then, the model compresses the process of differentiation into individual mitotic events. Since the primary distinction between cells in the model is simply whether they are proliferating or not, the model also elides any heterogeneity within the proliferating population. Beyond not identifying particular cells as "stem cells", this may make models derived from the SSM inappropriate for proliferative populations with a large degree of heterogeneity. One may think here of the classic idea of a small number of slowly proliferating "true" stem cells and a larger population of rapidly dividing "transit amplifying" progenitors- this type of internal structure within the proliferating population cannot be represented by this type of model.

As Fagan notes, in the SSM, "relations among p, r, and q values entail general predictions about cell population size (growth, decrease, or steady-state), and equations that predict mean and standard deviation in population size, probability of [lineage] extinction, and features of steady-state populations are derived."[32][Fag13, p.60]

Typically, this type of model has been employed to describe population dynamics of proliferating cells in assays generating ostensibly *clonal* data, where a "clone" here refers to the population constituted by all of the offsping descended from some particular (usually "initial" and sometimes therefore taken for "stem") proliferative cell. This population is the *lineage* generated by some particular dividing cell.

2.6 "Metaphysical ingredients" encountered in MEx

2.6.1 Information theory

The modern field of information theory (Information Theoretical Tradition, ITT) was essentially founded by the American polymath Claude Shannon during his work at Bell labs in the 1940s, building on cryptographic research, but elaborating a novel fundamental theory of communication. Shannon used the thermodynamic concept of entropy to characterise the properties of entities involved in communication (sources, encoders, channels, etc).

It is important to immediately disabuse the reader of any notion that Shannon was at all concerned with meaning, or with the *contents* of communication[33]. Nowhere do the specific identities and contents of messages that habitually enter our minds when we think of biological communication enter Shannon's theory. Communication, for Shannon and for the electrical engineers for whom he was providing his theory, is an extremely broad concept. As Shannon's exegete Warren Weaver indicated in his classic

[32]While Fagan refers to "stem cell" extinction, the model does not specifically define stem cells, nor does it imply intergenerational continuity, such that a particular intergenerationally identified stem cell should be said to have become extinct. The unit which survives or is made extinct is the lineage derived from some particular proliferative cell.

[33]Bioinformatically literate biologists are aware of this, but most of us do not routinely deal with formal definitions from information theory.

introduction to Shannon's work, "it may be desireable to use a [broad] definition of communication, namely, one which would include the procedures by means of which one mechanism (say automatic equipment to track an airplane and to computer its probably future positions) affects another mechanism (say a guided missile chasing this airplane)" [SW63, p.10]. Therefore, Shannon's theory can be used to characterise some aspects of biological systems, but it must be clearly understood that "information" refers to an abstract physical quantity and not to the lay definition concerning the specific contents of some communication.

Broadly speaking, the ITT has been applied to biological systems in two ways: analysis of biological communication and statistical measures of biological order.

ITT analyses of biological communication Shannon's

2.6.2 RPC Fate Specification as a "Stochastic" Process

Much of the complexity of this document is attributable to Harris' regular invocation of "stochastic" and related adjectives to describe the behaviour of RPCs. This is what lead me to characterise the theory primarily in those terms- the Intrinsic Stochastic Effects (ISE) EHJMEx. It may not be immediately obvious why this should be the case; most scientists assume that they know what words like "stochastic" and "random" mean well enough to use them in rigorous technical publications. We may not be aware that there has been a sprawling debate on the meaning of these terms since the earliest statistical formulations begain to appear in the 19th century. However, even the simplest examples (as current today as they were in Laplace's time) reveal how difficult this topic can be.

If we consider the classic example of the coin flip, a process whose outcome we generally regard as being in some way "stochastic" or due to "chance", we immediately face the question of whether these descriptions refer to our inability to know the outcome of the process, or whether they refer to properties of the process itself. In other words, if we could specify the mechanics of the coin toss with sufficient precision, could we predict the outcome? This reflects two possible senses in which we may legitimately describe a process as "stochastic": referring to an epistemic dimension (we may describe some process as stochastic because we are unable to predict its outcome *a priori*), or referring to an ontological dimension (we describe the process as stochastic because this, in some way, describes how it *really is* independent of our knowledge of it).

Complicating matters is the sheer number of implications that we tend to associate with "stochasticity" and "randomness". We may be saying something about the causal structure of an event with deep metaphysical implications. It is common to distinguish between "deterministic" and "stochastic" processes, as though "stochastic" literally meant "indeterministic"- something like the Copenhagen interpretation of quantum physics. We may mean something about the apparent disorderliness of a series of outcomes of some process, with mathematical and information theoretical implications. What is an apparently simple observation- cellular fate distribution in RPC lineages is "stochastic", now seems to require at least a little clarification or interpretation.

Unfortunately, in Harris' case, a fulsome philosophically-inclined review of the ISE EHJMEx is yet to appear. We may attribute some of the difficulty encountered in interpreting the meaning of "stochasticity" to the cramped style necessary in scientific reports. In general, we may say that stochasticity, for Harris, applies to at least the following entities:

1. The population-level phenomenal outcome of the RPC fate specification process (phenomenon P)

2. The overall behaviour of the macromolecular system whose operations produces these outcomes (mechanism S Φ-ing)

3. The particular behaviour of some component of the macromolecular system, eg. stochastic expression of transcription factors (component x ϕ-ing)

4. The statistical generalisations used to characterise relevant aspects of S Φ-ing and x ϕ-ing

It is, moreover, hardly fair to expect Harris to be advancing a coherent theory about the ontological, objective basis of randomness or probability. Indeed, there is no real agreement on these concepts in probability theory or amongst the philosophers. Still, this leaves us in the awkward position of not knowing quite what the leading EHJMEx for retinal formation is actually saying about its explanandum. The EHJMEx is thus at risk of circularity- the explanandum (unpredictable variability in clonal outcomes, P) has as explanans a MEx containing an abstract mathematical model tuned to produce this unpredictable variability. This may, in other words, turn out to be a convoluted case of model overfitting, if the "stochasticity" in question does not have a material biological referent. Before considering this, we need to define our terms more carefully to avoid the pervasive confusion mentioned above.

2.6.2.1 Chance versus Randomness

A commonplace belief is that randomness refers to outcomes produced by chance events. In an extensive and useful discussion, Antony Eagle reviews the evidence for this Commonplace Thesis, or **(CT)**[Eag18], drawing on discussions in the PTT. Importantly, he notes that chance and randomness are not identical, and that one can conceivably exist without the other. This, in effect, disproves the (CT)- it is very difficult to imagine how the two concepts can be directly related in this productive fashion. I will attempt a brief summary of Eagle's argument:

Chance is mainly used to refer to processes. Exemplars are coin flips and die rolls. We can think of these as "single-case" probabilities that we take to inhere in the process. For instance, we may say that an evenly weighted coin has a .5 probability of returning a value of heads on a flip, even if it is only flipped once. That is, probabilities can be taken to be objective properties of individual instances of processes, and not only descriptions of the frequencies of the process' outcomes over many repetitions. This is closely related to the logical concept of "possibility". If something is possible, it has a chance of occurring. However, possibility is a logical binary; something is either possible or impossible. A "single-case" probability is understood as something like an objective feature of a system as a whole given its actual configuration and the relevant natural laws.

Randomness, by contrast, mainly refers to process *outcomes*. That is, randomness is a property of a series of outcomes of multiple instances of some process. It turns out to be challenging merely to define what a "random" binary sequence might be (perhaps generated by a series of coin flips). However, in general, we may say that a random sequence of outcomes is one that cannot be generated by an description shorter than the sequence itself. That is, there is no set of rules that can generate a genuinely random sequence from a shorter sequence. In algorithmic information theory, the length of the ruleset required to produce some piece of information (like a sequence of measured outcomes) is called the Kolmogorov complexity of that object; if the Kolmogorov complexity of the object is equal to the object's length, the object definitionally has the property of algorithmic or Kolmogorov randomness.

Eagle produces numerous examples of the dissociability of these concepts, from which I have selected two concise illustrations:

> Chance Without Randomness
>
> ...
>
> A fair coin, tossed 1000 times, has a positive chance of landing heads more than 700 times. But any outcome sequence of 1000 tosses which contains more than 700 heads will be compressible (long runs of heads are common enough to be exploited by an efficient coding algorithm, and 1000 outcomes is long enough to swamp the constants involved in defining the universal prefix-free Kolmogorov complexity). So any such outcome sequence will not be random, even though it quite easily could come about by chance.
>
> ...
>
> Randomness Without Chance
>
> ...
>
> Open or dissipative systems, those which are not confined to a state space region of constant energy, are one much studied class [of the objects of deterministic classical physics- notably, biological systems are dissipative], because such systems are paradigms of chaotic systems ... the behaviour of a chaotic system will be intuitively random ... [t]he sensitive dependence on initial conditions means that, no matter how accurate our finite discrimination of the initial state of a given chaotic system is, there will exist states indiscriminable from the initial state (and so consistent with our knowledge of the initial state), but which would diverge arbitrarily far from the actual evolution of the system. No matter, then, how well we know the initial condition (as long as we do not have infinite powers of discrimination)[34]., there is another state the system could be in for all we know that will evolve to a discriminably different future condition. Since this divergence happens relatively quickly, the system is unable to be predicted ... Just as before, the classical physical theory underlying the dynamics of these chaotic systems is one in which probability does not feature. [Eag18]

Therefore, the (CT) is untenable. Processes are "chancy"; collections of process outcomes, "trials", or instantiations are "random". It is tempting to say that Harris is explaining random fate outcomes with descriptions of chancy processes occuring internally to RPCs. Let us examine whether this is plausible.

2.6.2.2 Chance in molecular mechanisms

I turn first to consider what it might mean to describe the behaviour of a biological macromolecular system as "chancy". Let us again distinguish between the ontological and epistemic dimensions of this description. There is a sense in which mechanisms S Ψ-ing could be said to be objectively chancy, and one in which the "chancy" outcome reflects our ignorance of some source of variability in the process.

Eagle proffers two common lines of argument in favour of chanciness as an objective property of processes. The first is the notion of the "single-case" probability mentioned above. The examples given are single coin flips, and the decay of single radioactive atoms, which are commonly taken to have chancy outcomes irrespective of anyone's beliefs about them. As Eagle notes, this is closely related to statistician's ideas about stable processes, or trials:

> It is the stable trial principle that has the closest connection with single-case chance, however. For in requiring that duplicate trials should receive the same chances, it is natural to take the chance to be grounded in the properties of that trial, plus the laws of nature. It is quite conceivable that the same laws could obtain even if that kind of trial has only one instance, and the very same chances should be assigned in that situation. But then there are well-defined chances even though that type of event occurs only once.

[34]Note that this condition defines chaotic randomness as an epistemic, rather than ontological, feature of complex systems- a being with infinite powers of discrimination could predict the evolution of a complex classical system with perfect accuracy.

...

> The upshot of this discussion is that chance is a *process* notion, rather than being entirely determined by features of the outcome to which the surface grammar of chance ascriptions assigns the chance. For if there can be a single-case chance of $\frac{1}{2}$ for a coin to land heads on a toss even if there is only one actual toss, and it lands tails, then surely the chance cannot be fixed by properties of the outcome lands heads, as that outcome does not exist. The chance must rather be grounded in features of the process that can produce the outcome: the coin-tossing trial, including the mass distribution of the coin and the details of how it is tossed, in this case, plus the background conditions and laws that govern the trial. Whether or not an event happens by chance is a feature of the process that produced it, not the event itself. The fact that a coin lands heads does not fix that the coin landed heads by chance, because if it was simply placed heads up, as opposed to tossed in a normal fashion, we have the same outcome not by chance. Sometimes features of the outcome event cannot be readily separated from the features of its causes that characterise the process by means of which it was produced.
>
> [Eag18]

Examining the example of the coin toss, we find a fairly simple answer to the question posed earlier: if we knew enough about the mechanics of the toss, could we predict its outcome? The answer is yes, we can- the statistician Persi Diaconis has built a coin tossing machine that reliably produces heads or tails [Kes04]. We therefore know that tightly controlling the mechanics of a coin toss allows us to treat this system as entirely deterministic, without any significant element of chance in the outcome. A coin toss is only chancy when the human doing it does not have full control over the mechanical parameters of the process. Conceptually, there is no *a priori* reason why a coin-tosser should not be able to regularise the angular momentum of their thumb-flick by training with a strain gauge, place the coin on a stable surface allowing flicking, and achieve the same effect as the coin-tossing machine. In this case, Eagle's suggestion that "[s]ometimes features of the outcome event cannot be readily separated from the features of its causes that characterise the process" seems obviously wrong- the "chancy" element of coin tossing is fully separable from the rest of the coin tossing process, and replaceable with a non-chancy component.

If the foregoing argument is correct, it seems that the coin toss is an example of the epistemic, rather than ontological, dimension of chance. The process appears to be chancy, or random, because the human tossing the coin is not able to precisely control the mechanical parameters of the process. Indeed, as Diaconis notes, these epistemically-limited tossers do not actually produce unbiased random outcomes- human coin tosses come up as they were started slightly more often than with the obverse face [DHM07].

Eagle's second example of an "objective single-case chance", is the decay of a radioactive atom. This is a common method of making covert appeals to the second line of argument for objective chance, which is the existence of orthodox quantum theory. There is no known physical process whose parameters are thought to define the lifetime of individual radioactive atoms, in the way that there is a well-specified physical process that produces a particular coin toss outcome. Rather, this is an appeal to the Copenhagen theoretical principle that it is *a priori* impossible to predict the lifetimes of individual atoms. As appeals to quantum theory to ground "objective chance" in biological processes are becoming more common, let us consider whether a quantum theoretical explanation might plausibly underpin the "stochasticity" of RPC fate specification.

2.6.2.3 Quantum indeterminacy - Is relevant to the ISE?

Eagle suggests that, because the Copenhagen interpretation of quantum physics has wide currency among physicists, the theory's implied indeterminacy of physical phenomena at the quantum level could ground "objective chance". While common, this argument downplays the fact that quantum theory is not a homogenous scientific tradition. Unfortunately, a significant misrepresentation of the QTT's internal history has given rise to the impression that the Copenhagen theory is the unanimous or best articulation of quantum theory. We must briefly examine this misrepresentation before we can understand whether Eagle's argument makes sense.

The conventional history of the mid 20th-century QTT holds that John Stewart Bell, in the demonstration of his famous inequality, conclusively proved that deterministic (so-called "hidden variable") theories of quantum mechanics were incorrect. As demonstrated (strangely, without any acknowledgement) in the very pages Eagle's argument appears in, this is a highly partisan and misleading view. Eminent Bohmian theorist Sheldon Goldstein conclusively demonstrates that Bell was an advocate of the deterministic Bohmian mechanical theory, and thought his famous inequality demonstrated that quantum phenomena could not be *local*, not that they could not be *deterministic*[Gol17].

Indeed, Bohmian quantum mechanics are fully deterministic, describe all of the same phenomena as Copenhagen, and in several cases resolve problems that orthodox quantum theory cannot [Gol17]. We are not, therefore, facing unanimous expert consensus that there is objective chance at the quantum level. We rather have a situation where physical phenomena are adequately described by two different traditional theories, one of which takes its statistical generalisations to be descriptions of ontological indeterminacy (Copenhagen), and the other to reflect epistemic uncertainty about a determinate universe (Bohm). Moreover, there is no reason to prefer the Copenhagen approach, given that the Bohmian theory explains Copenhagen's paradoxical results "without further ado"[Gol17][35], deals with empirically verified phenomena of physical and biological interest that Copenhagen does not (eg. electron tunneling), and was the preferred approach of the man who understood better than anyone his own results, JS Bell.

Therefore, Eagle's argument is incorrect. There is no reason to suppose that the existence of quantum theoretical models that posit objective chance is good evidence for the reality of objective chance. Moreover, there are good reasons to suppose that the converse is true. In sum, then, we may say that there is no reason to consider Harris' argument to refer to *objective, ontological* chance, since the arguments for the existence of both single-case objective chance or quantum chance are weak and biologically irrelevant. Clearly, however, the *epistemological* dimension of chance is in play here.

2.6.2.4 Randomness in RPC fate specification

Having dealt with how the concept of chance might apply to Harris' ISE EHJMEx above, let us consider how the term "random" might relate to the process of RPC fate specification and differentiation. As introduced earlier, the technical meaning of "randomness" pertains to sequences of process outcomes. The process outcomes Harris is concerned with are the temporally-arranged fate outcomes of some particular RPC lineage. Therefore, we must ask whether these sequences meet any reasonable technical definition of "random".

[35] Bizarrely, Copenhagen partisans claim that Bohmian mechanics is formally equivalent to the Copenhagen approach. If this is the case, chance is *clearly* a function of model choices and not of any underlying ontological reality. However, Bohmian mechanics is, in fact, substantially more complete than its Copenhagen equivalent, which, by Eagle's (defective) logic, suggests reality is more likely to reflect the deterministic rather than the chancy approach.

Harris' own model proves that RPC fate outcomes are not algorithmically random. That is, the sequence of outcomes has a structure that can be meaningfully compressed by rules which produce typical RPC fate outcomes (Harris' mathematical models are such rule sets). One might object that Harris' meaning is that the particular rules which give rise to cellular fate "choice" in his models involve random number generation. In this case, the claim is trivially about the model and not about the sequence of outcomes that is actually observed in zebrafish eyes. Indeed, all of Harris' later models *axiomatically assume* a tripartite temporal structure to the differentiation process[36]. This is precisely the type of sequential bias which allows efficient compression of a non-random sequence of outcomes by an algorithm. Therefore, Harris himself concedes that RPC fate specification is not an algorithmically random process[37].

We should further note that the question of how ordered, which is to say, non-random processes like fate specification arise in biological systems is a fundamental question of the biological sciences. It has long been recognised that classical and quantum physical systems which have algorithmically random initial conditions do not spontaneously evolve to a state of order. In many ways, then, it is the extent to which variable sequences of outcomes like RPC fate specification *depart* from algorithmic randomness which of interest when we are asking questions like "how does the ordered structure of a retina arise from RPC activity?"

2.6.2.5 Summary: "Stochastic" or "variable"?

Above, I argue that the RPC fate specification process is not objectively chancy (since objective chance is an empirically unsupported concept), nor random (since the sequence of RPC lineage outcomes is structured and therefore non-random). What, then, should we make of the argument that this process is "stochastic"? Let us consider the forceful argument of the great Bayesian statistician Edwin Thompson Jaynes:

> "Belief in the existence of stochastic processes in the real world; i.e. that the property of being stochastic rather than deterministic is a real physical property of a process, that exists independently of human information, is [an] example of the mind projection fallacy: attributing ones own ignorance to Nature instead. The current literature of probability theory is full of claims to the effect that a Gaussian random process is fully determined by its first and second moments. If it were made clear that this is only the defining property for an abstract mathematical model, there could be no objection to this; but it is always presented in verbiage that implies that one is describing an objectively true property of a real physical process. To one who believes such a thing literally, there could be no motivation to investigate the causes more deeply than noting the first and second moments, and so the real processes at work might never be discovered. This is not only irrational because one is throwing away the very information that is essential to understand the physical process; if carried into practice it can have disastrous consequences. Indeed, there is no such thing as a stochastic process in the sense that the individual events have no specific causes." [JBE03]

It is important to emphasize that the utility of stochastic modelling techniques should not be taken to suggest that on some level the modelled phenomenon is actually, i.e. irreducibly, random and without causal structure. When speaking of biological "randomness" or "stochasticity", biologists rarely precisely define what is meant by these terms. This vagueness sometimes arises from or results in a theoretical

[36] That is, an early bias in RPC production is produced in these models by the a priori commitment to a "rule" which results in early RPC production.

[37] Having debunked the lay sense of "random" being equivalent to "chancy" above, there is no reason to consider these other, confused, non-technical definitions of randomness.

deficit where properties of statistical models are understood to directly reflect the system being modelled; the scientist has failed to heed Korbzysky's dictum insisting that "a map *is not* the territory it represents, but, if correct, it has a *similar structure* to the territory, which accounts for its usefulness" [Kor05] (italics in original). The "structural similarity" here is between the model's outcomes and the collection of actually-observed population outcomes, *not* the underlying biological process giving rise to measured outcomes.

However, it would be trivial and a violation of my commitment to the principle of charity to dismiss Harris' argument on this basis. While I do believe that Harris has fallen into the "mind-projection fallacy" James excoriates above, we must ask how this occurred. What was the attraction of this fallacious explanation in the first place? I suggest that Harris appeals to stochasticity as an explanation for *variability* in fate outcomes. Harris describes his work as an attempt to explain how variable clonal outcomes could give rise to an "invariant" retina. We want to know how it is that variable clonal outcomes are produced and what this phenomenon's significance is to overall retinal formation. The value of Harris' ISE EHJMEx, I suggest, lies in its emphasis on the unpredictable variability of RPC lineage outcomes. I have suggested a scheme for identifying possible sources of biological variability in a separate section of this chapter.

2.6.3 Biological Variability

As I have argued in section 2.6.2, there are no good reasons to think that differential outcomes in biological systems are a result of some fundamental, ontological randomness. I share Jaynes' objection to this argument- the result is that the so-described phenomenon is understood to be, on some level, causeless. We are nevertheless left with the fact of unpredictable biological variability. Of course, observations documenting these variable phenomena are perplexing in the context of the pervasive Monodian conventional view, to wit, that cells are logically equivalent to cybernetic chemical factories. In a factory, process control is fundamentally about managing variability. Normally, we want to produce some regular sequence of outcomes for a production line with minimal variability. The chemical factory metaphor requires modification when applied to the context of Harris' work, however[38]. The products we are interested in are cellular fate outcomes themselves. Starting with an aggregate of similar (genetically, if not epigenetically, identical) RPCs, a specification hierarchy emerges. To re-fit the industrial metaphor to the context, the chemical-factory equivalent would be to explain how one can start with a factory that makes machine tools and end with a group of factories that make the diverse chemical products of a highly networked modern chemical industrial sector. In the absence of any central planner, how might the ontogenesis of such an industry take place? What are the factors that give rise to the "decision" to build one type of factory or another, and how do we explain the apparent increase in networked complexity and functional specialisation? What is the source of this apparent scaffolding novelty, and how can we explain the specific outcomes that we observe?[39]

[38]This modification is a logical consequence of the transposition of Monod's cybernetic metaphor for bacterial operons to a multicellular system.

[39]The factory metaphor makes it obvious that this type of question has far more to do with developmental or historical economics than it does with cybernetic process control of petrochemical cracking vessels or fermenters. Aside from Markoff chain theory, biologists have generally avoided methods used to study systems where outcomes heavily depend on the history of the system and related contingencies, probably because of the lack of scientific prestige associated with many of these formalisms ("soft science"). Nonetheless, it is hard to see how one can explain tissue development exclusively in terms of control of cell-internal processes- to attempt this is like trying to explain the historical evolution of the biotechnological industry in terms of the PID controllers it uses to run its fermenters. This will be, at best, a partial and highly misleading account.

Indeed, an explanation of biological variability is, alongside explaining the emergence of biological order, the fundamental task of the biological sciences. Harris has bravely advanced a theory that has, at times, referenced different possible sources for the variability observed in

2.6.3.1 Noise

2.6.4 Time

2.6.5 Semiosis, Meaning

2.6.6 Agency, Will, Choice

2.6.7 Emergence, self-organisation

2.6.8 Scale Hierarchies, Specification Hierarchies

2.6.9 Cybernetics

2.6.10 Waddington's topological model of development

> C. H. Waddington was one (and almost certainly the best remembered) of the embryologists who so profited. He wrote: During the recent war, engineers attained some fa- cility in designing machines to carry out tasks which earlier generations would have considered beyond the capacities of anything but an intelligent being . . . The ideas suggested by these self-regulating mechanisms are both very relevant to biology and rather novel. 28 Waddington himself worked in the Operation Research Section of the Royal Air Force Coastal Command, and it was from his own experience and from that of his friends in self-steering gunnery that he learned to draw the analogy which was to become increas- ingly familiar in the cybernetics of the 1950s and 1960s: The behaviours of an automatic pilot, of a target-tracking gun- sight, or of an embryo, all exhibit the characteristics of an activity guided by a purpose. 29 Indeed, it was at this time, and in this context, that his work on canalization began. 30 In Waddingtons first introduction of the term, he wrote, The main thesis is that developmental reactions as they occur in organization submitted to natural selection, are, in general, canalized. That is to say, they are adjusted so as to bring about one definite end result regardless of mi- nor variations in conditions during the course of the reac- tion. In his view, canalization is built into the organism by natural selection as a consequence of its obvious advan- tages: It ensures the production of the normal, that is, op- timal type in the face of the unavoidable hazards of exis- tence. 31 Canalization was a term Waddington had borrowed from his reading of Alfred North Whitehead, and the con- cept clearly accorded with much of his own prewar thinking about epigenetic landscapes. 32 But it was only after the war that he began to envision the possibility of a theoretical account of such characteristic features of biological organi- zation. An explanation of developmental canalization, he wrote, requires supplementing conventional gene theory with an epigenetic theoryone in which discrete and sepa- rate entities of classical genetics would be displaced by col- lections of genes which could lock in development through their interactions. 33 In other words, an account of develop- mental stability needs to be sought in the complex system of reactions that make up the developmental process. The search for quantitative models displaying such be- havior underlay much of Waddingtons theoretical efforts well into the 1970s. However, he soon concluded that the particular models developed by Ross Ashby and other cyber- neticians on self-organizing systems were not really appro- priate to biological development. Instead, he concentrated on feedback models of cross-reacting systems of metabolic reactions. Yet he did not have a great deal of success with these models. Indeed, it is not his theoretical work but the experimental work from his laboratory in the 1940s and 50s that now, with the benefit of hindsight, attracts the most in- terest. [Kel00, p.117-119]

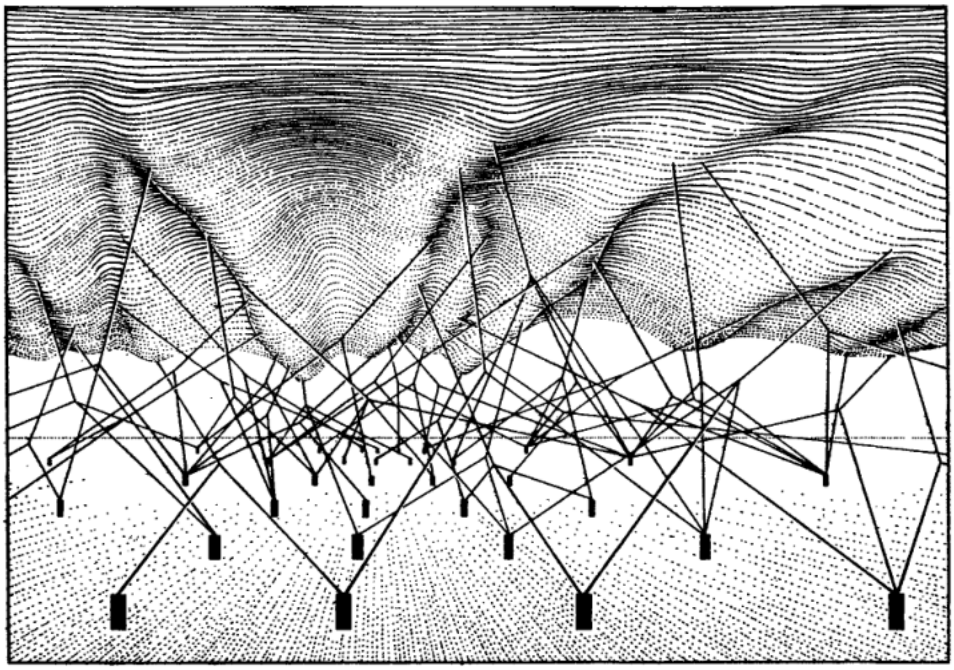

Figure 2.6: The complex system of interactions underlying the epigenetic landscape, excerpted from [Wad57]. The pegs in the ground represent genes; the strings leading from them the chemical tendencies which the genes produce. The modelling of the epigenetic landscape, which slopes down from above one's head towards the distance, is controlled by the pull of these numerous guy-ropes which are ultimately anchored to the genes.

2.6.11 Morphogenetic fields

2.6.12 Physical explanations

2.7 Mathematical concepts encountered in MEx

2.7.1 Dynamical Systems Theory

2.7.2 Probability Distribution Function

2.7.3 Ordinary Differential Equations and Dynamical Systems Theory

2.7.4 Monte Carlo simulation

Referenced on pages: 23

2.8 Implications of Limits in Theory and Practice For Model Selection

2.8.1 Nicholas Rescher's Account of Scientific Progress

2.8.2 Implications of Theoretical Limits of Science

2.8.3 Implications of Thermoeconomic Limits of Science

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