NORTHWESTERN UNIVERSITY

Quantitative analysis of cell fate decisions

A DISSERTATION

SUBMITTED TO THE GRADUATE SCHOOL

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

for the degree

DOCTOR OF PHILOSOPHY

Field of Chemical and Biological Engineering

By

Sebastian Michal Bernasek

EVANSTON, ILLINOIS

June 2019

ABSTRACT

Quantitative analysis of cell fate decisions

Sebastian Michal Bernasek

Organismal development depends upon countless cell decisions to adopt particular fates at the appropriate time and place. These decisions are executed by systems of biochemical reactions called regulatory networks. Elucidating the general principles underlying the structure and function of these networks is vital to understanding all developmental processes, as well as the diseases that arise when they fail.

Prior studies of regulatory networks, and the decisions they implement, have heavily relied upon qualitative analysis of experimental data. It has since become clear that quantitative strategies are needed to unravel the complexities of systems-level behavior. The research enclosed in this dissertation therefore combines chemical engineering, computer science, statistics, and experimental data to quantitatively explore how regulatory networks reliably coordinate cell fate decisions.

The findings are consolidated into three distinct chapters. The first two are anchored to a common model system of the *Drosophila* larval eye. They deploy an assortment of novel computational tools, mathematical models, and statistical methods to derive meaningful insight from experimental measurements of the processes that govern cell fate decisions

during retinal patterning. The final chapter introduces a mathematical modeling framework to lead the development of an exciting new hypothesis; auxiliary negative regulators enable development to proceed more quickly by mitigating erroneous cell fate decisions when cells are rapidly metabolizing.

Beyond their insights into the mechanics of cell fate decisions, these efforts have spawned several computational tools that may prove valuable to the broader community. All of these resources have been made freely available (see Appendix **??**), with the hope that their continued development will contribute toward a more quantitative future for developmental biology.

Acknowledgements

I would like to thank my friends, my family, and the many coffee growers that supported me over the past few years.

CHAPTER 1

Introduction

The natural world presents a stunning variety of multicellular organisms. We tend to distinguish them by the physiological traits that define their stereotyped identities. For instance, we recognize penguins by their unusual stature, black and white fur, long beak, and impressive ability to wobble around on ice. These features are a culmination of many complex cellular processes collectively known as *development.*

Development begins with a single cell, with subsequent growth and division events driving progression toward adulthood. Cells acquire increasingly specific roles and functions as growth proceeds, ultimately giving rise to stereotyped adult morphologies. This process, known as lineage restriction, demands that each cell decides to adopt the correct fate at the appropriate time and place.

Cell fate decisions are remarkably robust, yielding consistent phenotypes amidst the vast array of conditions encountered in natural environments. They are so reliable that we often take them for granted. For instance, it is difficult to imagine a scenario in which we might mistake another human for a penguin. However, they can and do exhibit minor variation that can have dire consequences for human health. Researchers therefore continue to study these complex processes in the hope that they might one day be able to control their behavior; either to exploit them in novel biotechnologies, or rectify the diseases that arise when they fail. Research efforts are predominantly motivated by two fundamental questions. First, how do cells make decisions? Second, how do they make them reliably?

This dissertation addresses subtle aspects of both questions by combining chemical engineering, computer vision, and statistics to extract meaningful insight from experimental data. The remaining sections of this chapter serve to prime the reader with the context needed to situate the presented findings within the broader literature.

1.1. Molecular origins of cell fate decisions

Early experiments in a variety of model organisms demonstrated that cell fate decisions are encoded in the genome. Genetics were therefore believed to provide a predefined road map for the tortuous journey from embryo to adulthood, inspiring researchers throughout the twentieth century to probe the roles of individual genes in coordinating adult phenotypes. Most of their efforts embraced a common philosophy; break something and see what happens. Nowhere is the legacy of this approach more pronounced than in the common fruit fly, *Drosophila melanogaster*, whose genes are predominantly named after the phenotypes that emerge in their absence. Removing *eyeless*, *wingless*, or *hairy* may now seem rudimentary, but these genetic perturbations, and others like them, were vital to the discovery of genetic components and architectures that dictate cell fate decisions in all organisms. They revealed that some genes confer pleiotropic functions across several stages of development, while others are limited to a single context. They also showed that some genes are only essential for proper development when others are absent, rendering them redundant under normal conditions. Moreover, *Drosophila* continues to be a prominent model system for studying developmental processes today, owing to its conveniently short life cycle, wealth of prior knowledge, and deep library of available genetic machinery.

Among these tools, gene-specific reporters have augmented traditional genetic perturbations by providing localized readouts of transcript and protein abundance. Researchers

can now break something and see what happens to specific components of the developmental program. Reporters led to the discovery of transcription factors; proteins that bind the promoter region of other genes in order to modulate their expression. Multiple transcription factors may interact with each other, allowing for the assembly of gene regulatory networks (GRN) that integrate upstream signaling cues to elicit specific changes in gene expression.

GRNs thus arm cells with a chemical mechanism to orchestrate cell fate decisions in space and time. A prominent example occurs during the during the earliest stages of *Drosophila* embryogenesis, where spatial morphogen gradients drive variegated expression of four Gap genes. The expressed proteins trigger subsequent developmental events in a concentration-dependent manner, inducing localized cascades of GRN activity that ultimately give rise to distinct organs. The early landscape of Gap gene expression thereby defines a template for later stages of patterning.

The spatial resolution of GRN activity is thought to be further refined over time. This assertion is in part based on experimental studies of retinal patterning in the *Drosophila* eye, a setting with an enduring experimental legacy that continues to garner attention [1]. This is in part because the temporal history of cell fate decisions is encoded in static images of the eye imaginal disc. During the third larval instar, a wave of differentiation steadily progresses across a disordered pool of multipotent cells [2, 3]. Differentiating cells propagate this morphogenetic furrow (MF) by relaying extracellular cues downstream. Decades of experiments have steadily revealed how cells interpret the signals and commit to particular fates [4]. Fate-specific reporters have shown that R8 photoreceptor neurons are recruited from pools of multipotent cells at the leading edge of the furrow [5]. The first cell to differentiate uses paracrine signaling to inhibit differentiation among its neighbors, resulting in the repeated pattern of regularly-spaced R8 cells that gives rise to the crystalline lattice

structure of the adult eye [6]. The process dynamically transforms a disordered field of cells into an ordered template for subsequent stages of patterning, thus refining the spatial resolution of the developing eye field.

Experiments suggest the race to an R8 fate is non-deterministic. The R8 cell fate transition is driven by the expression of Atonal, a transcription factor initially induced in all cells along the leading edge of the furrow [5, 7, 8]. Stochastic differences in cells reception of signaling cues yields variation in Atonal levels and, consequently, cells propensity to adopt an R8 fate [9, 10]. The eventual R8 cell therefore does not appear to be predetermined. Equivalent mechanisms control sensory bristle formation and the choice between epidermal and neural fates in the neuro-ectoderm [11, 12].

These three examples showcase another core feature of GRNs: their components are heterogeneous, even within the same developmental context. Variation may be attributed to both intrinsic and extrinsic sources of noise, each of which can manifest in the population-wide penetrance of phenotypes [13–16]. The retinal patterning example suggests some systems even leverage heterogeneity, using GRNs to amplify and reinforce stochastic fluctuations to limit signal responses to a random subset of cells [9, 11, 12]. More recently, Pel'aez et al proposed a similar mechanism to explain why subsequent R cell fate transitions coincide with rapid increases in transcription factor expression heterogeneity [17].

These experiments and others like them form our contemporary systems-level view of development, in which complex networks of regulatory interactions guide cells toward the appropriate fates by refining their stochastic behavior over time. However, it is unknown precisely how cell fates are resolved from the blueprints encoded in GRN activity. It is equally unclear how cells integrate stochastic inputs to execute reliable cell fate decisions. These ambiguities persist despite the wealth of experimental data generated throughout the

past century. New approaches are therefore needed to unravel the complexities of GRNs that govern cell fate decisions [18].

1.2. The need for quantitative analysis

Experimental perturbations continue to supply some of the most potent tools in the arsenal available to researchers, but it has become clear that unified and systematic analysis frameworks are needed to tease apart the complex interactions that govern cell fate decisions [18, 19]. Moreover, attempts to either rectify faulty decision mechanisms or engineer new ones, such as for cancer treatment or the generation of induced pluripotent stem cells, demand predictive models backed by quantitative data [20].

The focus has therefore gradually shifted from break something and *see* what happens to break something and *measure* what happens. The transition is supported by simultaneous advances in the resolution with which we can quantify GRN activity during development [21]. The state of the art has progressed from aggregate measurements of transcript and protein content across entire tissues, to *in vivo* measurements of individual cells, to *in situ* quantification of single-cell expression dynamics. These advances bestow mathematical models with the quantitative insight required to generate testable predictions for further experimental validation.

Quantitative measurements and analysis have already begun to illuminate cell fate decisions and challenge canonical theory [22–24]. Petkova et al recently used information theory to show that the Gap proteins provide sufficient spatial context to uniquely define the eventual fates of all cells [23]. They further argued that downstream GRNs likely decode these signals with near-optimal efficiency, implying that several cell fates may actually be determined during the earliest stages of embryogenesis. The study refutes the established

notion that cell fates are spatially resolved as development proceeds, and illustrates the novel insight that may be derived from quantitative data.

Unfortunately, quantification has not been universally adopted by the research community, leading to the sustained prevalence of subjective analysis in the literature. One plausible explanation is that quantification often demands computational proficiency that falls beyond the scope of many experimental labs. Interdisciplinary collaborations are becoming more frequent and should help alleviate this challenge, but they do not provide a permanent solution. These studies could benefit from the introduction of open-source automated analysis software platforms analogous to those that have revolutionized other subdisciplines of biology [25–37]. For example, without the support of automated sequence alignment software, RNA-seq would be inaccessible to all but a few researchers with extensive programming and statistical modeling experience. Similar tools are therefore needed to support quantitative measurements of gene expression during development [21, 38, 39], thus lowering the barrier to adoption of data-driven modeling of cell fate decisions.

1.3. Mathematical modeling of cell fate decisions

Mathematical models have reinvigorated the study of cell fate decisions in a broad variety of contexts. Most modeling efforts fall into one of two categories; those that use a data-driven approach to recapitulate molecular mechanism, and those that provide a sparse representation of systems-level behavior.

The first class of models strive to parameterize specific biomolecular interactions by fitting a model directly to data. They typically describe the time-evolution of transcripts and proteins using systems of coupled ordinary differential equations (ODEs) reminiscent of those familiar to chemical engineers and ecologists. Despite the illusion of mechanistic

detail, these models still deploy a healthy dose of abstraction. None of the commonly used rate represent true elementary reactions, instead opting for empirical representations such as linear degradation and cooperative binding kinetics. Nevertheless, many novel GRN behaviors and functions have been elegantly proposed and tested in this manner [14, 40, 41].

The second class of models forego molecular detail in favor of a coarse-grained representation of a particular phenomenon. These approaches provide a powerful means to identify, characterize, and predict behaviors that span a broad variety of model systems and developmental contexts. Among the common modeling frameworks, control theory has proven particularly fertile for generating and testing hypotheses related to GRN dynamics. Bacterial chemotaxis offers a compelling example in which experimentally derived molecular models were supplanted by a simple integral control framework [40, 42–44]. Analogous strategies have drawn inspiration from several disciplines to identify numerous functions of GRNs [45–49].

Coarse-grained models have contributed a particularly unique perspective as to how cell fates are resolved from spatiotemporal signaling cues. These problems would otherwise be intractable due to the many complex transport processes that shuttle signaling molecules between neighboring cells. Lubensky et al used a comparatively simple reaction-diffusion approach to model pattern formation in the larval eye. They showed that inductive signaling cues could drive cell-autonomous positive feedback to account for the emergence of a hexagonal lattice of R8 cells, as well as the previously-unexplained striped pattern observed in some mutants [50]. Gavish et al used an even simpler model to show that an additional inhibitory signal is necessary to stabilize retinal patterning against minor fluctuations in cells spatial arrangement. The authors then used an experimental technique known as quantitative mosaic analysis to identify the unknown diffusible inhibitor [10].

Mathematical models have also shone light on the regulatory interactions that implement cell fate decisions within individual cells. One study explored how cells generate all-or-none responses to morphogen gradients in the *Drosophila* ventral ectoderm [51]. An ultrasensitive response mechanism was proposed to dictate the expression of Yan, a transcriptional repressor known to impede cell fate transitions [52–54]. A later study proposed that Yan plays a different role in the larval eye, instead forming a bi-stable switch through reciprocal antagonism with a transcriptional activator named Pointed (Pnt) [55]. The authors used a detailed model to demonstrate that the decision to adopt an R cell fate could be triggered by an irreversible transition between two stable states; one characterized by high Yan and low Pnt expression, and the other by low Yan and high Pnt expression. The authors proposed that the switch is flipped via sustained induction of Pnt expression. Both transcription factors are known to be subject to several seemingly redundant sources of negative feedback. The model rationalized the purpose of these inhibitors by postulating that they enforce separation of the two stable states. Shwartz et al extended the model to include autoregulatory interactions that help flip the switch by converting transient inductive signals into sustained Pnt expression [56].

Curiously, a qualitative report later showed that Yan and Pnt are co-expressed in several developmental contexts [57]. Peláez et al then published quantitative measurements that show Yan exhibits apparently mono-stable expression dynamics in the larval eye [17]. These two studies fundamentally contradict the existing model, prompting renewed interest in both Yan and Pnt. Namely, how do they cooperate to mediate cell fate decisions? How do they do so reliably? And why are they subject to so much negative feedback?

1.4. Roles for negative feedback in GRNs

Theory supports many potential uses for negative feedback in developmental GRNs [58]. Perhaps the most obvious application is the maintenance of cell states — that is, rejecting exogenous disturbances and driving cells toward a desired set point [43, 59, 60]. Negative feedback has also been shown to improve information transmission by linearizing input-output relationships and expanding dynamic range in several developmental signaling cascades [14, 41, 61–63]. Yi et al. used a FRET-based reporter of the Gα-subunit to demonstrate dose-response alignment of G-protein signaling activity at the highest level of the yeast mating response pathway [63]. Yu et al. later quantified pFus3 activity to show that negative feedback further increases signal fidelity throughout much of the downstream pathway [41]. These functions mimic important uses of negative feedback in human-engineered systems [64].

Negative feedback is also vital to the reliable execution of cell fate decisions. Rahimi et al showed that WntD-mediated negative feedback enhances the precision of morphogen gradients in the ventral domain of the *Drosophila* embryo [46]. Paulsen et al. showed that the fidelity of BMP4 signaling in the *Xenopus* embryo is improved by concomitant expression of a repressor that suppresses transduction of extrinsic noise. Eliminating the repressor leads to increased variation of pathway outputs, as well as the cell fate decisions they control [14]. These studies provide direct experimental evidence that negative feedback can suppress phenotypic variation.

Experiments in *Drosophila* indicate that short non-coding transcripts, called microR-NAs, confer a similar function by buffering developmental processes against both environmental and genetic variability [65–68]. Li et al. studied the effect of perturbing miR-7 activity during sensory organ development, and found that the microRNA stabilizes both

gene expression and fate commitment decision against fluctuating environmental conditions [67]. Cassidy et al. used directional selection to quantify the heritability of bristle formation defects in miR-9a mutants, revealing that microRNAs can also suppress the influence of intrinsic genetic variation [66]. The same group later showed that miR-9 suppresses the penetrance of genetic variants deleterious to viability at elevated temperatures [65]. Negative feedback mediated by microRNAs has therefore been shown to directly affect the evolutionary fitness of adult organisms by promoting robustness against varying environmental conditions. Precisely how this effect is achieved remains unknown.

1.5. Evolutionary drivers of robust cell fate decisions

Studies of developmental robustness are often traced back to C.H. Waddingtons claim that there is scarcely a mutant that is comparable in constancy with the wild type [69]. His comment reflects the consistency of adult phenotypes amidst modest levels of genetic and environmental variation, which gives way to variation when severe perturbations are introduced [70–73]. Robustness has since been attributed to individual genes and their products [74–76], as well as the systems-level architectures that they comprise [14, 65–67, 76–78]. A handful of conserved regulatory motifs, and the strategies they implement, are now understood to pervade most developmental processes [58, 59, 79, 80]. Robustness has thus come to be accepted as a fundamental organizational principle underlying the evolution of all biological systems [81, 82]. That is, selection for genotypes that confer robustness is widely assumed to shape the evolution of gene regulatory network topologies.

The specific evolutionary drivers for increased robustness of GRNs remain unclear [83]. Citing phenomenological examples, Waddington attributed robustness to evolutionary

selection for optimal traits. More recently, Siegal et al. argued that developmental robustness can arise without stabilizing selection for any particular phenotype [84]. They used a gene interaction network model to demonstrate that robustness is an emergent property of complex networks subject only to selection on the basis of their own functional stability. The same authors used a an *in silico* evolution approach to show that most genes in developmental networks buffer phenotypic variation, indicating that robustness may be an emergent byproduct of GRN architecture [85]. Computational studies have also questioned the notion that global GRN properties are a consequence of adaptive evolution. Rather, local properties of genetic circuits could drive the emergence of macroscopic features, such as those that ensure robust development [86, 87].

When a gene or its products are absent or fail to perform their functions, the simplest contingency is to have others ready to compensate. It consequently seems intuitive that cells could incorporate functional redundancy to guarantee reliable cell fate decisions [79, 88]. One computational study showed that genetic redundancy is particularly evolutionarily stable in developmental systems. By simulating functionally overlapping pairs of genes, the authors demonstrated that increasing the probability with which each gene independently fails to carry out its function places increasing selection pressure upon the pair as a whole [89]. They reasoned that the resultant increase in evolutionary stability explains the prevalence of redundancy in developmental systems, where failure rates are high and erroneous cell fate decisions yield deleterious phenotypes. Their suggestion is consistent with extensive documentation of functional redundancy in developmental systems [81].

Redundancy could arise through gene duplication or convergent evolution. A. Wagner argued against the former by leveraging gene sequence and expression data in yeast to show

that the phenotypic severity of loss-of-function mutations does not correlate with the availability of alternate functionally equivalent genes. He concluded that functional redundancy is not the primary source of robustness against genetic variation, instead favoring epistatic interactions between otherwise unrelated gene products [90]. Combined, these studies suggest the prevalence of functional redundancy in GRNs may be attributed to as-of-yet unknown forces.

References

[1] Jorge V. Beira and Renato Paro. The legacy of Drosophila imaginal discs, sep 2016. ISSN 14320886. URL http://link.springer.com/10.1007/s00412-016-0595-4.

[2] Donald F. Ready, Thomas E. Hanson, and Seymour Benzer. Development of the Drosophila retina, a neurocrystalline lattice. *Developmental Biology*, 53(2):217–240, oct 1976. ISSN 00121606. doi: 10.1016/0012-1606(76)90225-6. URL http://linkinghub.elsevier.com/retrieve/pii/0012160676902256.

[3] Andrew Tomlinson and Donald F. Ready. Neuronal differentiation in the Drosophila ommatidium. *Developmental Biology*, 120(2):366–376, apr 1987. ISSN 00121606. doi: 10.1016/0012-1606(87)90239-9. URL http://linkinghub.elsevier.com/retrieve/pii/0012160687902399.

[4] Matthew G. Voas and Ilaria Rebay. Signal integration during development: insights from the Drosophila eye. *Dev Dyn*, 229(1):162–175, jan 2004. ISSN 10588388. doi: 10.1002/dvdy.10449. URL http://doi.wiley.com/10.1002/dvdy.10449.

[5] Andrew P. Jarman, Ellsworth H. Grell, Larry Ackerman, Lily Y. Jan, and Yuh Nung Jan. atonal is the proneural gene for Drosophila photoreceptors. *Nature*, 369(6479):398–400, 1994. URL http://www.nature.com/nature/journal/v369/n6479/pdf/369398a0.pdf.

[6] Benjamin Jay Frankfort and Graeme Mardon. R8 development in the Drosophila eye: a paradigm for neural selection and differentiation. *Development (Cambridge, England)*, 129(6):1295–306, 2002. ISSN 0950-1991. URL https://www.semanticscholar.org/paper/R8-development-in-the-Drosophila-eye{%}3A-a-paradigm-Frankfort-Mardon/fac459f976737f3273b1733809e231aaf0c8d799http://www.ncbi.nlm.nih.gov/pubmed/11880339.

[7] Nicholas E. Baker and Sung-Yun Yu. Proneural function of neurogenic genes in the developing Drosophila eye. *Current Biology*, 7:122–132, feb 1997. ISSN 09609822. doi: 10.1016/S0960-9822(06)00056-X. URL https://www.sciencedirect.com/science/article/pii/S096098220600056X.

[8] F. Hsiung. Retinal development in Drosophila: specifying the first neuron. *Human Molecular Genetics*, 11(10):1207–1214, may 2002. ISSN 14602083. doi: 10.1093/hmg/11.10.1207. URL https://academic.oup.com/hmg/article-lookup/doi/10.1093/hmg/11.10.1207.

[9] Nicholas E Baker, Marek Mlodzik, and Gerald M Rubin. Spacing differentiation in the developing Drosophila eye: A fibrinogen-related lateral inhibitor encoded by scabrous. *Science*, 250(4986):1370–1377, dec 1990. ISSN 00368075. doi: 10.1126/science.2175046. URL `http://www.ncbi.nlm.nih.gov/pubmed/2175046`.

[10] Avishai Gavish, Arkadi Shwartz, Abraham Weizman, Eyal Schejter, Ben-Zion Shilo, and Naama Barkai. Periodic patterning of the Drosophila eye is stabilized by the diffusible activator Scabrous. *Nature Communications*, 7:10461, 2016. ISSN 2041-1723. doi: 10.1038/ncomms10461. URL `https://www.nature.com/articles/ncomms10461.pdfhttp://www.nature.com/doifinder/10.1038/ncomms10461`.

[11] Alain Ghysen and Christine DamblyChaudière. The specification of sensory neuron identity in Drosophila, may 1993. ISSN 15211878. URL `http://doi.wiley.com/10.1002/bies.950150502`.

[12] P Simpson. Notch signalling in development: on equivalence groups and asymmetric developmental potential. *Current opinion in genetics & development*, 7(4):537–42, aug 1997. ISSN 0959-437X. URL `http://www.ncbi.nlm.nih.gov/pubmed/9309187`.

[13] Arjun Raj, Scott A. Rifkin, Erik Andersen, and Alexander van Oudenaarden. Variability in gene expression underlies incomplete penetrance. *Nature*, 463(7283):913–918, 2010. ISSN 1476-4687. doi: 10.1038/nature08781. URL `https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2836165/pdf/nihms167246.pdf`.

[14] Malte Paulsen, Stefan Legewie, Roland Eils, Emil Karaulanov, and Christof Niehrs. Negative feedback in the bone morphogenetic protein 4 (BMP4) synexpression group governs its dynamic signaling range and canalizes development. *Proceedings of the National Academy of Sciences*, 108(25):10202–10207, jun 2011. ISSN 0027-8424. doi: 10.1073/pnas.1100179108. URL `http://www.ncbi.nlm.nih.gov/pubmed/21633009http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3121836http://www.pnas.org/cgi/doi/10.1073/pnas.1100179108`.

[15] Alejandro Burga, M Olivia Casanueva, and Ben Lehner. Predicting mutation outcome from early stochastic variation in genetic interaction partners. *Nature*, 480(7376):250–253, 2011. ISSN 0028-0836. doi: 10.1038/nature10665. URL `https://pdfs.semanticscholar.org/6b1d/9bd605bed0890a2f54a0f3e683236156a624.pdfhttp://www.nature.com/doifinder/10.1038/nature10665`.

[16] Alejandro Colman-Lerner, Andrew Gordon, Eduard Serra, Tina Chin, Orna Resnekov, Drew Endy, C. Gustavo Pesce, and Roger Brent. Regulated cell-to-cell variation in a cell-fate decision system. *Nature*, 437(7059):699–706, 2005. ISSN 0028-0836. doi: 10.1038/nature03998. URL `http://brentlab.fredhutch.org/content/dam/stripe/brent/files/48.pdfhttp://www.nature.com/doifinder/10.1038/nature03998`.

[17] Nicolás Peláez, Arnau Gavalda-Miralles, Bao Wang, Heliodoro Tejedor Navarro, Herman Gudjonson, Ilaria Rebay, Aaron R. Dinner, Aggelos K. Katsaggelos, Luís A. Nunes Amaral, and Richard W. Carthew. Dynamics and heterogeneity of a fate determinant

during transition towards cell differentiation. *eLife*, 4, nov 2015. ISSN 2050-084X. doi: 10.7554/eLife.08924. URL `http://www.ncbi.nlm.nih.gov/pubmed/26583752http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4720516`.

[18] Andrew C. Oates, Nicole Gorfinkiel, Marcos González-Gaitán, and Carl Philipp Heisenberg. Quantitative approaches in developmental biology, aug 2009. ISSN 14710056. URL `http://www.nature.com/articles/nrg2548`.

[19] Y. Lazebnik. Can a biologist fix a radio? - Or, what I learned while studying apoptosis, 2004. ISSN 00062979. URL `http://www.cell.com/cancer-cell/pdf/S1535-6108(02)00133-2.pdf`.

[20] Jorrit J Hornberg, Frank J Bruggeman, Hans V Westerhoff, and Jan Lankelma. Cancer: A Systems Biology disease. In *BioSystems*, volume 83, pages 81–90, 2006. ISBN 0303-2647 (Print)\r0303-2647 (Linking). doi: 10.1016/j.biosystems.2005.05.014. URL `http://ac.els-cdn.com/S0303264705001176/1-s2.0-S0303264705001176-main.pdf?{_}tid=1c7460a4-6972-11e7-8ef7-00000aab0f27{&}acdnat=1500132633{_}da653246f723a0377bbdd5a72e9a3262`.

[21] Ivo F. Sbalzarini. Seeing is believing: Quantifying is convincing: Computational image analysis in biology. *Advances in Anatomy, Embryology and Cell Biology*, 219:1–39, 2016. ISSN 03015556. doi: 10.1007/978-3-319-28549-8_1. URL `http://link.springer.com/10.1007/978-3-319-28549-8{_}1`.

[22] Christopher L. Frick, Clare Yarka, Harry Nunns, and Lea Goentoro. Sensing relative signal in the Tgf-β/Smad pathway. *Proceedings of the National Academy of Sciences*, 114(14):E2975–E2982, mar 2017. ISSN 0027-8424. doi: 10.1073/pnas.1611428114. URL `http://www.ncbi.nlm.nih.gov/pubmed/28320972http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC5389321http://www.pnas.org/lookup/doi/10.1073/pnas.1611428114`.

[23] Mariela D. Petkova, Gašper Tkačik, William Bialek, Eric F. Wieschaus, and Thomas Gregor. Optimal Decoding of Cellular Identities in a Genetic Network. *Cell*, 176(4): 844–855.e15, feb 2019. ISSN 10974172. doi: 10.1016/j.cell.2019.01.007. URL `https://linkinghub.elsevier.com/retrieve/pii/S0092867419300406`.

[24] Samuel C. Wolff, Kasia M. Kedziora, Raluca Dumitru, Cierra D. Dungee, Tarek M. Zikry, Adriana S. Beltran, Rachel A. Haggerty, JrGang Cheng, Margaret A. Redick, and Jeremy E. Purvis. Inheritance of OCT4 predetermines fate choice in human embryonic stem cells. *Molecular Systems Biology*, 14(9):e8140, jan 2018. doi: 10.1101/137299. URL `https://www.biorxiv.org/content/early/2018/01/26/137299`.

[25] Nima Aghaeepour, Greg Finak, Holger Hoos, Tim R Mosmann, Ryan Brinkman, Raphael Gottardo, Richard H Scheuermann, David Dougall, Alireza Hadj Khodabakhshi, Phillip Mah, Gerlinde Obermoser, Josef Spidlen, Ian Taylor, Sherry A. Wuensch, Jonathan Bramson, Connie Eaves, Andrew P. Weng, Edgardo S. Fortuno, Kevin Ho, Tobias R. Kollmann, Wade Rogers, Stephen De Rosa, Bakul Dalai, Ariful

Azad, Alex Pothen, Aaron Brandes, Hannes Bretschneider, Robert Bruggner, Rachel Finck, Robin Jia, Noah Zimmerman, Michael Linderman, David Dill, Gary Nolan, Cliburn Chan, Faysal El Khettabi, Kieran O'Neill, Maria Chikina, Yongchao Ge, Stuart Sealfon, István Sugár, Arvind Gupta, Parisa Shooshtari, Habil Zare, Philip L. De Jager, Mike Jiang, Jens Keilwagen, Jose M. Maisog, George Luta, Andrea A. Barbo, Peter Májek, Jozef Vilček, Tapio Manninen, Heikki Huttunen, Pekka Ruusuvuori, Matti Nykter, Geoffrey J. McLachlan, Kui Wang, Iftekhar Naim, Gaurav Sharma, Radina Nikolic, Saumyadipta Pyne, Yu Qian, Peng Qiu, John Quinn, Andrew Roth, Pablo Meyer, Gustavo Stolovitzky, Julio Saez-Rodriguez, Raquel Norel, Madhuchhanda Bhattacharjee, Michael Biehl, Philipp Bucher, Kerstin Bunte, Barbara Di Camillo, Francesco Sambo, Tiziana Sanavia, Emanuele Trifoglio, Gianna Toffolo, S. D. Slavica Dimitrieva, Rene Dreos, Giovanna Ambrosini, Jan Grau, Ivo Grosse, Stefan Posch, Nicolas Guex, Miron Kursa, Witold Rudnicki, Bo Liu, Mark Maienschein-Cline, Petra Schneider, Michael Seifert, Marc Strickert, and Jose M.G. Vilar. Critical assessment of automated flow cytometry data analysis techniques. *Nature Methods*, 10(3):228–238, mar 2013. ISSN 15487091. doi: 10.1038/NMETH.2365. URL `http://www.nature.com/articles/nmeth.2365`.

[26] Xiaoyi Chen, Milena Hasan, Valentina Libri, Alejandra Urrutia, Benoît Beitz, Vincent Rouilly, Darragh Duffy, Étienne Patin, Bernard Chalmond, Lars Rogge, Lluis Quintana-Murci, Matthew L. Albert, and Benno Schwikowski. Automated flow cytometric analysis across large numbers of samples and cell types. *Clinical Immunology*, 157(2):249–260, apr 2015. ISSN 15217035. doi: 10.1016/j.clim.2014.12.009. URL `https://www.sciencedirect.com/science/article/pii/S1521661614002873`.

[27] Saumyadipta Pyne, Lisa M Maier, T.-I Lin, Kui Wang, Elizabeth Rossin, Xinli Hu, Pablo Tamayo, David A Hafler, Philip L De Jager, Geoffrey J McLachlan, Clare Baecher-Allan, and Jill P Mesirov. Automated high-dimensional flow cytometric data analysis. *Proceedings of the National Academy of Sciences*, 106(21):8519–8524, may 2009. ISSN 0027-8424. doi: 10.1073/pnas.0903028106. URL `http://www.ncbi.nlm.nih.gov/pubmed/19443687http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC2682540`.

[28] Bradley E Bernstein, Myles Brown, David S Johnson, X Shirley Liu, Chad Nussbaum, Richard M Myers, Tao Liu, Wei Li, Clifford A Meyer, Jérôme Eeckhoute, and Yong Zhang. Model-based Analysis of ChIP-Seq (MACS). *Genome Biology*, 9(9):R137, sep 2008. ISSN 1465-6906. doi: 10.1186/gb-2008-9-9-r137. URL `http://genomebiology.biomedcentral.com/articles/10.1186/gb-2008-9-9-r137`.

[29] Jan Hellemans, Geert Mortier, Anne De Paepe, Frank Speleman, and Jo Vandesompele. qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. *Genome Biology*, 8(2), feb 2007. ISSN 1465-6914. doi: 10.1186/gb-2007-8-2-r19. URL `http://genomebiology.biomedcentral.com/articles/10.1186/gb-2007-8-2-r19`.

[30] Ben Langmead and Steven L Salzberg. Fast gapped-read alignment with Bowtie 2. *Nature methods*, 9(4):357–9, apr 2012. ISSN 1548-7105. doi: 10.1038/nmeth.1923. URL `http://www.nature.com/articles/nmeth.1923http://www.ncbi.nlm.nih.gov/pubmed/22388286{%}0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3322381`.

[31] Cole Trapnell, Lior Pachter, and Steven L. Salzberg. TopHat: Discovering splice junctions with RNA-Seq. *Bioinformatics*, 25(9):1105–1111, may 2009. ISSN 13674803. doi: 10.1093/bioinformatics/btp120. URL `https://academic.oup.com/bioinformatics/article-lookup/doi/10.1093/bioinformatics/btp120`.

[32] Sylvain V. Costes, Dirk Daelemans, Edward H. Cho, Zachary Dobbin, George Pavlakis, and Stephen Lockett. Automatic and quantitative measurement of protein-protein colocalization in live cells. *Biophysical Journal*, 86(6):3993–4003, jun 2004. ISSN 00063495. doi: 10.1529/biophysj.103.038422. URL `https://www.sciencedirect.com/science/article/pii/S0006349504744392`.

[33] Lawrence A Kelley, Stefans Mezulis, Christopher M Yates, Mark N Wass, and Michael J.E. Sternberg. The Phyre2 web portal for protein modeling, prediction and analysis. *Nature Protocols*, 10(6):845–858, jun 2015. ISSN 17502799. doi: 10.1038/nprot.2015.053. URL `http://www.nature.com/articles/nprot.2015.053`.

[34] Anne E Carpenter, Thouis R Jones, Michael R Lamprecht, Colin Clarke, In Han Kang, Ola Friman, David A Guertin, Joo Han Chang, Robert A Lindquist, Jason Moffat, Polina Golland, and David M Sabatini. CellProfiler: Image analysis software for identifying and quantifying cell phenotypes. *Genome Biology*, 7(10):R100, oct 2006. ISSN 14747596. doi: 10.1186/gb-2006-7-10-r100. URL `http://genomebiology.biomedcentral.com/articles/10.1186/gb-2006-7-10-r100`.

[35] Ahmad Paintdakhi, Bradley Parry, Manuel Campos, Irnov Irnov, Johan Elf, Ivan Surovtsev, and Christine Jacobs-Wagner. Oufti: An integrated software package for high-accuracy, high-throughput quantitative microscopy analysis. *Molecular Microbiology*, 99(4):767–777, feb 2016. ISSN 13652958. doi: 10.1111/mmi.13264. URL `http://doi.wiley.com/10.1111/mmi.13264`.

[36] Johannes Schindelin, Ignacio Arganda-Carreras, Erwin Frise, Verena Kaynig, Mark Longair, Tobias Pietzsch, Stephan Preibisch, Curtis Rueden, Stephan Saalfeld, Benjamin Schmid, Jean Yves Tinevez, Daniel James White, Volker Hartenstein, Kevin Eliceiri, Pavel Tomancak, and Albert Cardona. Fiji: An open-source platform for biological-image analysis, jul 2012. ISSN 15487091. URL `http://www.nature.com/articles/nmeth.2019`.

[37] Christoph Sommer, Christoph Straehle, Ullrich Kothe, and Fred A. Hamprecht. Ilastik: Interactive learning and segmentation toolkit. In *Proceedings - International Symposium on Biomedical Imaging*, pages 230–233. IEEE, mar 2011. ISBN 9781424441280. doi: 10.1109/ISBI.2011.5872394. URL `http://ieeexplore.ieee.org/document/5872394/`.

[38] Florian Jug, Tobias Pietzsch, Stephan Preibisch, and Pavel Tomancak. Bioimage Informatics in the context of Drosophila research. *Methods*, 68(1):60–73, jun 2014. ISSN 10959130. doi: 10.1016/j.ymeth.2014.04.004. URL `https://www.sciencedirect.com/science/article/pii/S1046202314001480`.

[39] Johannes Schindelin, Curtis T. Rueden, Mark C. Hiner, and Kevin W. Eliceiri. The ImageJ ecosystem: An open platform for biomedical image analysis, jul 2015. ISSN 10982795. URL `http://doi.wiley.com/10.1002/mrd.22489`.

[40] N. Barkai and S. Leibler. Robustness in simple biochemical networks. *Nature*, 387(6636): 913–917, jun 1997. ISSN 00280836. doi: 10.1038/43199. URL `http://www.nature.com/articles/43199`.

[41] Richard C Yu, C Gustavo Pesce, Alejandro Colman-Lerner, Larry Lok, David Pincus, Eduard Serra, Mark Holl, Kirsten Benjamin, Andrew Gordon, and Roger Brent. Negative feedback that improves information transmission in yeast signalling. *Nature*, 456(7223):755–61, 2008. ISSN 1476-4687. doi: 10.1038/nature07513. URL `https://www.nature.com/nature/journal/v456/n7223/pdf/nature07513.pdfhttp://dx.doi.org/10.1038/nature07513`.

[42] U. Alon, M. G. Surette, N. Barkai, and S. Leibler. Robustness in bacterial chemotaxis. *Nature*, 1999. ISSN 00280836. doi: 10.1038/16483.

[43] T.-M. M Yi, Yun Huang, Melvin I Simon, and John Doyle. Robust perfect adaptation in bacterial chemotaxis through integral feedback control. *Proceedings of the National Academy of Sciences*, 97(9):4649–53, 2000. ISSN 0027-8424. doi: 10.1073/pnas.97.9.4649. URL `http://www.pnas.org/content/pnas/97/9/4649.full.pdfhttp://www.pnas.org/cgi/doi/10.1073/pnas.97.9.4649http://www.pnas.org/content/97/9/4649.full.pdfhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=18287{&}tool=pmcentrez{&}rendertype=abstract`.

[44] Dale Muzzey, Carlos A. Gómez-Uribe, Jerome T Mettetal, and Alexander van Oudenaarden. A Systems-Level Analysis of Perfect Adaptation in Yeast Osmoregulation. *Cell*, 138(1):160–171, 2009. ISSN 00928674. doi: 10.1016/j.cell.2009.04.047. URL `https://ac.els-cdn.com/S009286740900508X/1-s2.0-S009286740900508X-main.pdf?{_}tid=e095f21a-73af-44bf-8998-fbeb03ab23d3{&}acdnat=1523285892{_}3269a18d028b9b3e95e15a1c9e001929https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3109981/pdf/nihms121222.pdf`.

[45] Wenzhe Ma, Ala Trusina, Hana El-Samad, Wendell A Lim, and Chao Tang. Defining Network Topologies that Can Achieve Biochemical Adaptation. *Cell*, 138(4):760–773, 2009. ISSN 00928674. doi: 10.1016/j.cell.2009.06.013. URL `https://ac.els-cdn.com/S0092867409007120/1-s2.0-S0092867409007120-main.pdf?{_}tid=257c8902-9152-4762-a24c-dec4af470d67{&}acdnat=1523897402{_}593100e7c1be810b2fe3361296d3ba06`.

[46] Neta Rahimi, Inna Averbukh, Michal Haskel-Ittah, Neta Degani, Eyal D Schejter, Naama Barkai, and Ben Zion Shilo. A WntD-Dependent Integral Feedback Loop Attenuates Variability in Drosophila Toll Signaling. *Developmental Cell*, 36(4):401–414, 2016. ISSN 18781551. doi: 10.1016/j.devcel.2016.01.023. URL `http://dx.doi.org/10.1016/j.devcel.2016.01.023`.

[47] Dirk Benzinger and Mustafa Khammash. Pulsatile inputs achieve tunable attenuation of gene expression variability and graded multi-gene regulation. *Nature Communications*, 9(1):3521, dec 2018. ISSN 2041-1723. doi: 10.1038/s41467-018-05882-2. URL `http://www.nature.com/articles/s41467-018-05882-2`.

[48] Miri Adler and Uri Alon. Fold-change detection in biological systems. *Current Opinion in Systems Biology*, 8:81–89, apr 2018. ISSN 2452-3100. doi: 10.1016/j.coisb.2017.12.005. URL `https://www.sciencedirect.com/science/article/pii/S2452310017301889https://doi.org/10.1016/j.coisb.2017.12.005`.

[49] Pencho Yordanov and Jörg Stelling. Steady-State Differential Dose Response in Biological Systems. *Biophysical Journal*, 114(3):723–736, feb 2018. ISSN 15420086. doi: 10.1016/j.bpj.2017.11.3780. URL `https://linkinghub.elsevier.com/retrieve/pii/S0006349517350427`.

[50] David K. Lubensky, Matthew W. Pennington, Boris I. Shraiman, and Nicholas E. Baker. A dynamical model of ommatidial crystal formation. *Proceedings of the National Academy of Sciences*, 108(27):11145–11150, 2011. ISSN 0027-8424. doi: 10.1073/pnas.1015302108. URL `http://www.pnas.org/content/108/27/11145.full.pdfhttp://www.pnas.org/cgi/doi/10.1073/pnas.1015302108`.

[51] Gustavo J. Melen, Sagi Levy, Naama Barkai, and Ben-Zion Shilo. Threshold responses to morphogen gradients by zero-order ultrasensitivity. *Molecular Systems Biology*, pages 1–11, 2005. ISSN 1744-4292. doi: 10.1038/msb4100036. URL `http://msb.embopress.org/content/msb/1/1/2005.0028.full.pdf`.

[52] Zhi-Chun Lai and Gerald M Rubin. Negative Control of Photoreceptor Development in Drosophila by the Product of the yan Gene, an ETS Domain Protein. *Cell*, 70(609420), 1992. URL `http://ac.els-cdn.com/009286749290430K/1-s2.0-009286749290430K-main.pdf?{_}tid=f955cd7a-1d35-11e7-9dfe-00000aab0f6b{&}acdnat=1491750511{_}22ccbdfb0875302083e33919f38dc8c0`.

[53] R. Rogge, P. J. Green, J. Urano, S. Horn-Saban, M. Mlodzik, B Z Shilo, V. Hartenstein, and U. Banerjee. The role of yan in mediating the choice between cell division and differentiation. *Development*, 121(12):3947–3958, 1995. ISSN 0950-1991.

[54] Ilaria Rebay and Gerald M. Rubin. Yan functions as a general inhibitor of differentiation and is negatively regulated by activation of the Ras1/MAPK pathway. *Cell*, 81:857–866, 1995.

[55] Thomas G. W. Graham, S. M. Ali Tabei, Aaron R. Dinner, and Ilaria Rebay. Modeling bistable cell-fate choices in the Drosophila eye: qualitative and quantitative perspectives. *Development*, 137(14):2265–78, jul 2010. ISSN 1477-9129. doi: 10.1242/dev.044826. URL `http://dev.biologists.org/content/develop/137/14/2265.full.pdfhttp://dev.biologists.org/cgi/doi/10.1242/dev.044826{%}5Cnpapers3://publication/doi/10.1242/dev.044826http://www.ncbi.nlm.nih.gov/pubmed/20570936http://www.pubmedcentral.nih.gov/articlerender.`

[56] Arkadi Shwartz, Shaul Yogev, Eyal D. Schejter, and Ben-Zion Shilo. Sequential activation of ETS proteins provides a sustained transcriptional response to EGFR signaling. *Development*, 140(13):2746–2754, 2013. ISSN 1477-9129. doi: 10.1242/dev.093138. URL `http://dev.biologists.org/content/develop/140/13/2746.full.pdfhttp://dev.biologists.org/lookup/doi/10.1242/dev.093138.`

[57] Jean-François Boisclair Lachance, Nicolás Peláez, Justin J. Cassidy, Jemma L. Webber, Ilaria Rebay, and Richard W. Carthew. A comparative study of Pointed and Yan expression reveals new complexity to the transcriptional networks downstream of receptor tyrosine kinase signaling. *Developmental Biology*, 385:263–278, jan 2014. ISSN 00121606. doi: 10.1016/j.ydbio.2013.11.002. URL `http://linkinghub.elsevier.com/retrieve/pii/S0012160613006027.`

[58] Matthew Freeman. Feedback control of intercellular signalling in development. *Nature*, 408(November):313–319, 2000. ISSN 0028-0836. doi: 10.1038/35042500. URL `https://me.ucsb.edu/{~}moehlis/APC514/2002{_}2.pdfhttp://www.ncbi.nlm.nih.gov/pubmed/11099031{%}5Cnhttp://www.nature.com/nature/journal/v408/n6810/pdf/408313a0.pdf.`

[59] Uri Alon. Network motifs: theory and experimental approaches. *Nature reviews. Genetics*, 8(6):450–61, 2007. ISSN 1471-0056. doi: 10.1038/nrg2102. URL `https://www.nature.com/nrg/journal/v8/n6/pdf/nrg2102.pdfhttp://www.ncbi.nlm.nih.gov/pubmed/17510665.`

[60] Marcelo Behar, Nan Hao, Henrik G Dohlman, and Timothy C Elston. Mathematical and computational analysis of adaptation via feedback inhibition in signal transduction pathways. *Biophysical journal*, 93(3):806–21, 2007. ISSN 0006-3495. doi: 10.1529/biophysj.107.107516. URL `http://ac.els-cdn.com/S0006349507713383/1-s2.0-S0006349507713383-main.pdf?{_}tid=227f53e0-8070-11e7-a70c-00000aab0f01{&}acdnat=1502660656{_}cca3148074153087b8a339f6366981d5http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1913166{&}tool=pmcentrez{&}rendert.`

[61] Upinder S. Bhalla, Prahlad T. Ram, and Ravi Iyengar. MAP Kinase Phosphatase As a Locus of Flexibility in a Mitogen-Activated Protein Kinase Signaling Network. *Science*, 297(5583):1018–1023, 2002. ISSN 0036-8075, 1095-9203. doi: 10.1126/science.1068873. URL `http://science.sciencemag.org/content/sci/297/5583/1018.full.pdfhttp://www.sciencemag.org/content/297/5583/1018{%}5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/12169734{%}5Cnhttp:`

`//www.sciencemag.org/content/297/5583/1018.full.pdf`.

[62] Raymond Cheong, Alex Rhee, C. J. Wang, Ilya Nemenman, and Andre Levchenko. Information Transduction Capacity of Noisy Biochemical Signaling Networks. *Science*, 334(6054):354–358, 2011. ISSN 0036-8075. doi: 10.1126/science.1204553. URL `http://science.sciencemag.org/content/sci/334/6054/354.full.pdf`.

[63] Tau-Mu Yi, Hiroaki Kitano, and Melvin I Simon. A quantitative characterization of the yeast heterotrimeric G protein cycle. *Proceedings of the National Academy of Sciences of the United States of America*, 100(19):10764–9, 2003. ISSN 0027-8424. doi: 10.1073/pnas.1834247100. URL `http://www.pnas.org/content/100/19/10764.full.pdfhttp://www.pnas.org/content/100/19/10764`.

[64] Mustafa Khammash. An engineering viewpoint on biological robustness, mar 2016. ISSN 17417007. URL `http://bmcbiol.biomedcentral.com/articles/10.1186/s12915-016-0241-xhttp://www.ncbi.nlm.nih.gov/pubmed/27007299http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4804522`.

[65] Justin J. Cassidy, Alexander J. Straughan, and Richard W. Carthew. Differential masking of natural genetic variation by miR-9a in Drosophila. *Genetics*, 202(2): 675–687, 2016. ISSN 19432631. doi: 10.1534/genetics.115.183822. URL `https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4788242/pdf/675.pdf`.

[66] Justin J Cassidy, Aashish R Jha, Diana M Posadas, Ritika Giri, Koen J T Venken, Jingran Ji, Hongmei Jiang, Hugo J Bellen, Kevin P White, and Richard W Carthew. MiR-9a minimizes the phenotypic impact of genomic diversity by buffering a transcription factor. *Cell*, 155(7):1556–1567, 2013. ISSN 10974172. doi: 10.1016/j.cell.2013.10.057. URL `https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3891883/pdf/nihms543592.pdf`.

[67] Xin Li, Justin J Cassidy, Catherine A Reinke, Stephen Fischboeck, and Richard W Carthew. A MicroRNA Imparts Robustness against Environmental Fluctuation during Development. *Cell*, 137(2):273–282, 2009. ISSN 00928674. doi: 10.1016/j.cell.2009.01.058. URL `https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2674871/pdf/nihms104381.pdf`.

[68] Margaret S. Ebert and Phillip A. Sharp. Roles for microRNAs in conferring robustness to biological processes. *Cell*, 149(3):515–524, 2012. ISSN 1097-4172 (Electronic) 0092-8674 (Linking). doi: S0092-8674(12)00464-3[pii]10.1016/j.cell.2012.04.005. URL `http://ac.els-cdn.com/S0092867412004643/1-s2.0-S0092867412004643-main.pdf?{_}tid=7f25e6fa-8cd6-11e7-b642-00000aab0f6c{&}acdnat=1504024034{_}ea251b3bd7d6068bb351a33d320fd66ahttp://ac.els-cdn.com/S0092867412004643/1-s2.0-S0092867412004643-main.pdf?{_}tid=775f4790-`.

[69] CH H Waddington. Canalization of Development and the Inheritance of Acquired Characters. *Nature*, 150(3811):563–565, 1942. ISSN 0028-0836. doi: 10.1038/150563a0. URL `https://www.nature.com/nature/journal/v150/n3811/pdf/150563a0.pdfhttp:`

//chd.ucsd.edu/{_}files/winter2009/waddington.nature.pdf.

[70] K G Bateman. The genetic assimilation of four venation phenocopies. *Journal of Genetics*, 56(3):443–474, 1959. ISSN 00221333. doi: 10.1007/BF02984796. URL https://link.springer.com/content/pdf/10.1007/BF02984796.pdf.

[71] J M Rendel. Canalisation of the scute phenotype of Drosophila. *Evolution*, 13: 425–439, 1959. doi: 10.1111/j.1558-5646.1959.tb03033.x. URL file:///Users/Sebi/Downloads/Rendel-1959-Evolution.pdf.

[72] J M Rendel, B L Sheldon, and D E Finlay. Selection for Canalization of the Scute Phenotype. II. *The American Naturalist*, 100(910):13–31, 1966. ISSN 00030147. doi: 10.2307/2459376. URL http://www.publish.csiro.au/bi/pdf/bi9600036http://www.jstor.org/stable/2459376.

[73] Willem Scharloo. Canalization: genetic and developmental aspects. *Annual Review of Ecology and Systematics*, 22(1991):65–93, 1991. ISSN 0066-4162. doi: 10.1146/annurev.es.22.110191.000433. URL http://www.annualreviews.org/doi/pdf/10.1146/annurev.es.22.110191.000433http://www.jstor.org/stable/2097255.

[74] R.B. Dun and A.S. Fraser. Selection for an Invariant Character - 'Vibrissa Number' - in the House Mouse. *Nature*, 182:1638 – 1640, 1958. URL https://www.nature.com/nature/journal/v181/n4614/pdf/1811018a0.pdf.

[75] G Gibson and David S. Hogness. Effect of Polymorphism in the Drosophila Regulatory Gene Ultrabithorax on Homeotic Stability. *Science*, 271(5246):200–203, 1996. doi: 10.1126/science.271.5246.200. URL http://science.sciencemag.org/content/271/5246/200.long.

[76] Suzanne L Rutherford and Susan Lindquist. Hsp90 as a capacitor for morphological evolution. *Nature*, 396(6709):336–342, 1998. ISSN 0028-0836. doi: 10.1038/24550. URL http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve{&}db=PubMed{&}dopt=Citation{&}list{_}uids=9845070https://www.nature.com/nature/journal/v396/n6709/pdf/396336a0.pdf.

[77] Avigdor Eldar, Ruslan Dorfman, Daniel Weiss, Hilary Ashe, Ben-Zion Shilo, and Naama Barkai. Robustness of the BMP morphogen gradient in Drosophila embryonic patterning. *Nature*, 419(September):304–308, 2002. ISSN 0028-0836. doi: 10.1038/nature01061. URL http://dx.doi.org/10.1038/nature01061.

[78] Charles M Denby, Joo Hyun Im, Richard C Yu, C. Gustavo Pesce, and Rachel B Brem. Negative feedback confers mutational robustness in yeast transcription factor regulation. *Proceedings of the National Academy of Sciences*, 109(10):3874–3878, 2012. ISSN 0027-8424, 1091-6490. doi: 10.1073/pnas.1116360109. URL http://www.pnas.org/content/109/10/3874.full.pdfhttp://www.pnas.org/content/109/10/3874.

[79] John L I V Hartman, Barbara Garvik, and Lee Hartwell. Principles for the Buffering of Genetic Variation. *Science*, 1001(2001):1–5, 2001. ISSN 00368075. doi:

10.1126/science.1056072. URL `http://science.sciencemag.org/content/sci/291/5506/1001.full.pdfpapers3://publication/doi/10.1126/science.1056072`.

[80] David C Marciano, Rhonald C Lua, Panagiotis Katsonis, Shivas R Amin, Christophe Herman, and Olivier Lichtarge. Negative Feedback in Genetic Circuits Confers Evolutionary Resilience and Capacitance. *Cell Reports*, 7(6):1789–1795, 2014. ISSN 22111247. doi: 10.1016/j.celrep.2014.05.018. URL `http://dx.doi.org/10.1016/j.celrep.2014.05.018`.

[81] Hiroaki Kitano. Biological Robustness. *Nature Reviews Genetics*, 837(November):826–837, 2004. doi: 10.1038/nrg1471. URL `https://www.nature.com/nrg/journal/v5/n11/pdf/nrg1471.pdf`.

[82] Jörg Stelling, Uwe Sauer, Zoltan Szallasi, Francis J. Doyle, and John Doyle. Robustness of cellular functions, sep 2004. ISSN 00928674. URL `https://www.sciencedirect.com/science/article/pii/S0092867404008402`.

[83] Mark L Siegal and Jun-Yi Leu. On the Nature and Evolutionary Impact of Phenotypic Robustness Mechanisms. *Annual Review of Ecology, Evolution, and Systematics*, 45:495–517, 2014. ISSN 1543-592X. doi: 10.1146/annurev-ecolsys-120213-091705. URL `http://www.annualreviews.org/doi/pdf/10.1146/annurev-ecolsys-120213-091705`.

[84] Mark L Siegal and Aviv Bergman. Waddington's canalization revisited: developmental stability and evolution. *Proceedings of the National Academy of Sciences of the United States of America*, 99:10528–10532, 2002. ISSN 00278424. doi: 10.1073/pnas.102303999. URL `http://www.pnas.org/content/99/16/10528.full.pdf`.

[85] Aviv Bergman and Mark L. Siegal. Evolutionary capacitance as a general feature of complex gene networks. *Nature*, 424(6948):549–552, 2003. ISSN 00280836. doi: 10.1038/nature01765. URL `https://www.nature.com/nature/journal/v424/n6948/pdf/nature01765.pdfhttp://www.nature.com/doifinder/10.1038/nature01765`.

[86] Michael Lynch. The evolution of genetic networks by non-adaptive processes. *Nature reviews. Genetics*, 8(10):803–13, 2007. ISSN 1471-0064. doi: 10.1038/nrg2192. URL `https://www.nature.com/nrg/journal/v8/n10/pdf/nrg2192.pdfhttp://www.ncbi.nlm.nih.gov/pubmed/17878896`.

[87] Andreas Wagner. How the global structure of protein interaction networks evolves. *Proceedings. Biological sciences / The Royal Society*, 270 (1514):457–66, 2003. ISSN 0962-8452. doi: 10.1098/rspb.2002.2269. URL `http://rspb.royalsocietypublishing.org/content/royprsb/270/1514/457.full.pdfhttp://www.ncbi.nlm.nih.gov/pubmed/12641899{%}5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC1691265`.

[88] Harley H. McAdams and Adam Arkin. It's a noisy business! Genetic regulation at the nanomolar scale, 1999. ISSN 01689525. URL `http://www.cell.com/trends/genetics/pdf/S0168-9525(98)01659-X.pdf`.

[89] M A Nowak, M C Boerlijst, J Cooke, and John Maynard Smith. Evolution of genetic redundancy. *Nature*, 388(6638):167–71, 1997. ISSN 0028-0836. doi: 10.1038/40618. URL `https://www.nature.com/nature/journal/v388/n6638/pdf/388167a0.pdfhttp://www.ncbi.nlm.nih.gov/pubmed/9217155`.

[90] Andreas Wagner. Robustness against mutations in genetic networks of yeast. *Nature genetics*, 24(4):355–61, 2000. ISSN 1061-4036. doi: 10.1038/74174. URL `http://www.nature.com.turing.library.northwestern.edu/ng/journal/v24/n4/pdf/ng0400{_}355.pdf`.