Cell and Body: Individuals in Stem Cell Biology

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Abstract
Cells are often considered biological individuals. In particular, cells of multicellular organisms (the paradigmatic biological individuals) satisfy at least some major criteria for biological individuality. This essay considers the biological individuality of stem cells: undifferentiated cells that self-renew and give rise to differentiated cells. I argue that stem cells are not biological individuals in the same way as cells of multicellular organisms, but at least some stem cells are biological individuals in the way of multicellular organisms. In this argument, the general definition of 'stem cell' is conceived as an abstract model. This approach sheds light on central concepts and practices of stem cell biology, as well as the relation between cellular and organismal individuality. The stem cell case also exhibits an unexpected parallel with physics: specifically, Bohr's view of complementarity.

1. Introduction

Organisms like us are considered paradigmatic biological individuals. Such entities share a number of properties that furnish commonsense criteria for biological individuality: spatio-temporal continuity, physical boundaries, participation in biologically-significant causal processes (e.g., reproduction, evolution, and development), physiological integration, and functional autonomy.[1] One question that has concerned many philosophers is whether biological individuality extends to other levels of biological organization: genes, cells, groups, species, *etc*. Of these, the cell has arguably the strongest claim to biological individuality by "organism-

[1] For philosophically-inflected discussions of these various criteria, see Wilson and Barker 2013, Pradeu 2012 (228-232).

centred" criteria (Wilson and Barker 2013). The idea that cells are "autonomous living units" or "elementary organisms," while the organism is a "society of cells" or "cell state," dates back to mid-nineteenth-century formulations of cell theory (Conklin 1939, Hall 1969, Virchow 1859 [1958]; for recent discussions see Nicholson 2010, Reynolds 2007). The opposing view is that cells are subordinate to the organism, harmoniously organized by a top-down division of labor. Tension between these two perspectives contributes to several long-running debates about biological individuality and agency. In engaging these debates, philosophers of biology have investigated the cell-organism relation primarily in terms of evolution by natural selection. This essay takes a different perspective, focusing on cell development. The following sections examine the question: are stem cells biological individuals?

The question may seem merely a special case of a more general (and philosophically significant) question about cells. But that impression is misleading. Questions about stem cells go to the core of contemporary ideas about biological individuality. This can be brought out by considering a simple argument:

(1) Cells of multicellular organisms are biological individuals.

(2) Stem cells are cells of multicellular organisms.

(3) Therefore, stem cells are biological individuals.

At first glance, the conclusion follows deductively, by inference from the general to the particular. Premise (1) asserts that cells that make up the body of a multicellular organism (neurons, skin cells, muscle cells, and so on) qualify as biological individuals. Premise (2) asserts that stem cells are among these cells. Given the two premises, (3) follows ineluctably. The conclusion appears so obvious as to require no further defense: if stem cells are a kind of cell, and cells are biological individuals, then stem cells must be biological individuals. But the

argument's simplicity is deceptive. I will argue, in what follows, that stem cells are *not* biological individuals *in the same way* as more familiar cell types that make up the body of a multicellular organism. The simple argument for (3) therefore fails due to equivocation.

This approach targets a particular interpretation of premise (2). On another interpretation, this premise is correct: stem cells are cellular entities, and they are parts of (or derived from) multicellular organisms. But premise (1) asserts more than this. The way in which stem cells are 'cells of multicellular organisms' does not coincide with the 'cells of multicellular organisms' that are plausibly taken to be biological individuals according to familiar criteria. This equivocation between premises (1) and (2) blocks the conclusion that stem cells are biological individuals. Another approach would be to reject premise (1). However, there is considerable, though not decisive, support for the thesis that cells of multicellular organisms are biological individuals (Section 2). So we should provisionally accept premise (1).

The charge of equivocation rests on two contrasts between stem cells and cells of multicellular organisms in the sense of premise (1). These two contrasts are demonstrated in Sections 3 and 4. First, the stem cell concept is not a purely cellular notion, but involves the organismal level as well (Section 3). So the question of biological individuality for stem cells is more complicated than for cells that are parts of multicellular organisms but can be conceived as purely cellular entities. The second contrast is that the stem cell concept (understood as an abstract model) cannot be experimentally shown to correspond to single cells in biological systems. That is, stem cells cannot be experimentally individuated at the single-cell level, as cells of multicellular organisms can (Section 4). More precisely, while specialized mature cells of a multicellular organism can be defined and experimentally characterized as individual cells,

stem cells cannot. Though it sounds paradoxical, stem cell are not cells – at least, not in the same way as cells that are plausibly taken to be biological individuals.

One might accept both contrasts and yet reject premise (1). On such a view, cells of multicellular organisms and stem cells both fail to qualify as biological individuals, although for different reasons. From a developmental perspective, however, it is more interesting to consider stem cells as 'transformative entities,' mediating between two different levels of biological individuality: cell and organism. Section 5 sketches one idea along these lines, which offers independent support for conclusion (3). I argue that at least some stem cells can be considered biological individuals, by analogy to multicellular organisms. These stem cells are *model organisms* for investigating mammalian development. Finally, the evidential challenges of stem cell research reveal a surprising parallel with physics: uncertainty relations in the context of Bohr's account of complementarity (Section 6).

2. Cells as biological individuals

This section argues that there is strong support for the thesis that cells of multicellular organisms are biological individuals, clarifying the 'baseline' with which stem cells will be contrasted in later sections. The basic tenets of cell theory offer a starting point:[2]

(i) Every organism begins as a single cell, which, in multicellular organisms, gives rise to all the body's cells.

(ii) Cells reproduce by binary division.[3]

[2] Historically, principles of cell theory have been expressed in many different ways (see references in §1). Statements (i)-(iv) express views that are very widely-accepted in life sciences today. They do not capture tenets of cell theory across all relevant historical contexts.

[3] There are two modes of cell division: mitosis and meiosis. In mitosis, the genome replicates once before the cell divides. In meiosis, the genome replicates once, but two rounds of cell

(iii) The life of a cell begins with a division event and ends with either a second division event yielding two offspring, or cell death (and no offspring).

(iv) Generations of cells linked by reproductive division form a lineage.[4]

Together, these four tenets establish cells as plausible candidates for biological individuality. Tenet (i) relates cells to organisms, the paradigmatic biological individuals. Every organism begins as a single cell; most continue that way. Single-cell organisms such as bacteria and yeast "dominate life on this planet" numerically, historically, in biodiversity, and in biomass (O'Malley and Dupré 2007, and references therein). So in the majority of cases, questions of cell and organismal individuality coincide.[5]

Single-cell organisms satisfy important criteria for biological individuality. Individuals in general are thought to "have three-dimensional spatial boundaries, endure for some period of time, are composed of physical matter, bear properties, and participate in processes and events" (Wilson and Barker 2013). Single-cell organisms evidently satisfy these general criteria. They are physically contiguous, with clear spatial and temporal boundaries. Indeed, the physical boundary of semi-permeable membrane separating a cell from its external environment is among the clearest in all of biology. Single-cell organisms also bear a number of properties, including size, shape, and morphology, and function as agents within an environment. Beyond the general case, further criteria for biological individuality are diverse and somewhat contentious.[6] Among

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division follow, yielding four offspring cells with half the complement of DNA. Because stem cell and somatic cell phenomena involve mitosis, the term "cell division" is used throughout this essay to refer to that mode only.

[4] The term "cell lineage," in what follows, refers to a biological entity composed of successive cell generations, organized by reproductive relations.

[5] This coincidence cuts both ways; O'Malley and Dupré (2007) discuss interactive and multi-cellular aspects of micro-organisms, which have traditionally been underemphasized. These insights do not affect the arguments in this essay.

[6] See Chapters *** of this volume for further discussion.

the most familiar and widely-accepted are participation in biologically-significant causal processes (notably reproduction and evolution), physiological integration, and functional autonomy. Single-cell organisms clearly satisfy these criteria. Tenets (ii) and (iii) state the basics of cell reproduction, a process that yields cell lineages consisting of generations of parents and offspring (tenet iv). As members of potentially ongoing reproductive lineages, single-cell organisms are "evolutionary individuals" (Hull 1992, Godfrey-Smith 2009).[7] Moreover, 'the cell' is an exemplar of physical and physiological individuality for the biological world. Cellular metabolism, including respiration, energy storage and excretion, comprises a self-regulating system that unifies heterogeneous components into a "one discrete and cohesive entity" (Pradeu 2012, 227). Overall, single-cell organisms qualify as biological individuals, according to most criteria.[8]

For cells of multicellular organisms, matters are less clear-cut. Because multicellular organisms are paradigmatic biological individuals, it is tempting to suppose that their parts are not. Yet a strong case can be made for the biological individuality of these cells, if the term

[7] More precisely, single-cell organisms qualify as evolutionary individuals if we assume they are grouped into populations that vary with respect to properties that influence reproductive success (*e.g.*, rate of cell division). This assumption is satisfied in many (if not all) cases, as cell reproduction leads to formation of 'clonal' populations of cells, which can then diversify. More nuanced differences among philosophical accounts of evolutionary individuals can be set aside for the purpose of this paper. Thanks to T. Pradeu for pushing me to clarify this point.

[8] Another familiar criterion for biological individuality is genetic homogeneity; *i.e.*, that biological individuals are distinguished from one another by a unique chromosomal DNA sequence (Wilson 1999). Because single-cell organisms of the same lineage often have nearly-identical DNA sequences ('clones'), they do not satisfy this genetic criterion. However, due to recent discoveries of microbial diversity in functioning human organisms (and those of many other species), the genetic criterion is itself subject to question, and currently de-emphasized by many theorists (see Wilson and Barker 2013, and references therein). An updated version, tying individuality to genetic regulatory states rather than DNA sequence, does apply to single-cell organisms (Klipp et al 2009, Chapter 6). A full discussion of this issue is beyond the scope of this essay. Here I claim only that failure to satisfy the genetic criterion as traditionally conceived does not disqualify single-cell organisms as biological individuals (and, similarly, cells of multicellular organisms).

'part' is understood in a minimal, commonsense way. To say that cells are parts of a multicellular organism, in this sense, is just to say that a 'cell-level inventory' of an organism would result in a list of cells that make up that organism. Such an inventory is not a philosophical fantasy, but grounded on more than a century of biological practice yielding robust and detailed characterizations of cells extracted from multicellular organisms. Properties of these cells do not vary continuously, but cluster into discrete groups sharing a suite of structural and functional traits. These groupings are referred to as 'cell types.' Major cell types such as neurons, muscle, and blood cells exhibit considerable robustness across organismal taxa, and can be clearly distinguished from one another by microscopic examination, biochemical tests, molecular techniques, or some combination thereof. Characteristic features of cell types are typically stable across cell generations – liver cells divide to produce more liver cells, fibroblasts beget more fibroblasts, and so on.

Cells of multicellular organisms can be experimentally individuated and characterized independently of their organismal context. Because of this, many of the points made above about single-cell organisms apply to them as well. This is not to say that experimental practices are somehow constitutive of these cells' biological individuality. Rather, experimental practices of cell biology reveal facts about cells of multicellular organisms (*e.g.*, clear physical boundaries, heritable properties) from which a strong case for their biological individuality can be built. Cells of multicellular organisms evidently satisfy minimal criteria for individuality: they are physical entities with clear spatio-temporal boundaries, which bear properties and are involved in processes. In addition, tenets (i)-(iv) apply, just as for free-living single-celled organisms. Cells of multicellular organisms also satisfy physiological and evolutionary criteria, each having a metabolism, heritable structural and functional traits that vary in response to mutation, as well as

clear criteria for reproductive success. Though most cells of a multicellular organism are 'evolutionary dead ends' in the long run, this does not preclude their being units of selection within an organism's lifetime.[9]

The primary objection to premise (1) is that cells of a multicellular organism fail to satisfy a criterion of "minimal functional autonomy" (Wilson and Barker 2013). Unlike single-celled organisms, so the objection goes, cells that make up the body of a multicellular organism lack the ability to 'act for themselves.' Instead, they are controlled 'from the top down' by the integrated system that defines the organism as a biological individual. This view is the oppositional counterpart of the 'bottom-up' view that cells are individuals that make an organism. So we are back to the intuition that cells of multicellular organisms cannot be individuals because they are parts of individuals, but with the added assumption that the term 'part' implies lack of autonomy. I think we should not accept that assumption. There is currently no consensus, among biologists or philosophers, about how control is transmitted across levels of biological organization, or in what direction. Furthermore, it is not obvious that biological individuality must be a zero-sum game; that is, that an organism's functional autonomy must be at the expense of that of the cells that compose it. Though these considerations do not decisively rebut the 'functional autonomy' objection, it would be premature to rule out biological individuality for cells of multicellular organisms on its grounds. At the very least, we can conclude that cells of multicellular organisms that can be isolated and characterized (the specialized neurons, muscle cells, blood cells, and so on) satisfy some important criteria for biological individuality. Premise (1) above therefore has strong support.

[9] In fact, certain cells of the immune system participate in selective processes as a prerequisite for their specialized defensive functions (Paul 2003). Evolutionary theories of cancer posit a pathological counterpart to these processes (Nowak 2006).

3. Defining stem cells

I now turn to the stem cell case. The term 'stem cell' itself seems to imply that stem cells must be a kind of cell, and thus fall under the scope of the arguments above. However, close examination of the concept reveals two important contrasts. In this section, I show that the stem cell concept cannot be understood solely in terms of single cells isolated from their context. In the next section, I argue that stem cells cannot be experimentally individuated at the single-cell level. The overall result is that stem cells can neither be defined in terms of nor justifiably identified with individual cells. In both respects, they contrast with cells of multicellular organisms, which can be experimentally identified and characterized as single cells, analogous to free-living single-cell organisms (Section 2). Therefore, stem cells are not individual cells in the way as the specialized cells of multicellular organisms. The argument for this conclusion rests on careful analysis of the stem cell concept.

Stem cells are defined as undifferentiated cells that self-renew and give rise to differentiated cells (e.g., Ramelho-Santos and Willenbring 2007, 35; Melton and Cowan 2009, xxiv). This general definition suggests that a stem cell is a kind of cell. But in fact there are many kinds of stem cell: adult, embryonic, pluripotent, multipotent, induced, mesenchymal, neural, hematopoietic (blood-forming), embryonal carcinoma, *etc*. Any general definition must cover this bewildering variety of cases. I have elsewhere defended a modeling approach to this problem (Fagan 2013a). The basic idea is to conceive the general definition of 'stem cell' as an abstract conceptual model. Here I briefly sketch the main points and results of this analysis. The model represents the two defining capacities of stem cells: self-renewal and differentiation. Both are reproductive processes that involve comparison across cell generations. Self-renewal is cell

reproduction in which parent and offspring resemble one another, while differentiation is the process by which parts of a developing organism acquire diverse, specialized traits over time. So the two processes are complementary. It is important to note that stem cells are defined as being *capable* of participating in both. Whether those defining capacities are realized depends on environmental context (see Section 4). The stem cell concept can be represented as a model combining these two reproductive processes, with tenets (ii)-(iv) serving as background assumptions.

A 'minimal model' of a stem cell consists of one cell undergoing a division event, such that one offspring cell is similar to the parent, while the other is more specialized (Figure 1). This model consists of three objects ('cells') standing in two reproductive relations, which define a structure: cell lineage L. L is not simply a genealogical structure, but includes cross-generation comparisons. However, no two cells are similar or different in *every* respect. The comparative concepts of self-renewal and differentiation are only scientifically useful if they are understood as relative to a set of variable characters. So the model requires a set of characters C, specific values of which can be assigned to objects in the model. Characters of interest for stem cell biology are attributes of individual cells, such as size, shape, or concentration of a particular molecule. The minimal model of Figure 1 is also restricted to a single cell division event and two cell generations. Generalizing to allow for any number of cell division 'cycles' introduces a further variable (n). When estimates of cell division rate are available, this number can be converted to an interval of calendar time, which in practice range from hours to decades. These three variables (L, C, and n) suffice to define self-renewal: parent and offspring cells in lineage L resemble one another with respect to some character set C for time interval (number of cell cycles) n.

[FIGURE 1]

Differentiation is more complicated, because not all changes across cell generations count as differentiation, but only those in the 'direction' of increased specialization.[10] So an additional parameter needs to be included, alongside cell lineage, set of characters for parent-offspring comparison, and time interval. This additional parameter refers to the end of development: 'mature' cells that have completed the process. A cell *specializes* over some time interval just in case its character values are more similar to those of mature cells at the end of that interval than the beginning. The characters relevant for differentiation are those of a mature cell type of interest (character-set M). Mature cells have specialized morphology, functions, and molecular traits (an array of values for M), and therefore correspond very closely to cells of multicellular organisms as discussed above. But they have a developmental aspect, which is not presupposed by the cell types discussed in Section 2. A mature cell has reached the endpoint of development; the process is complete. We do not have a cell-level concept of complete development. Characters of mature cells (M) are defined in terms of their location and functional role within a fully-developed organism; *i.e.*, an organism that has reached reproductive maturity. Insofar as the character set and range of alternative values for M (and C) is selected with reference to a fully-developed organism, the latter concept is implicated in the process of differentiation. In this sense, the stem cell concept presupposes that of an organism.

[10] Another aspect of differentiation is *diversification*. A population of cells *diversifies* over some time interval relative to a set of characters, if and only if variation in the values of those characters increases over that interval. For simplicity, I bracket this population-level aspect of differentiation here.

Putting all this together, this general stem cell model consists of three or more objects ('cells') with characters C or M, values of which vary over some time interval n, organized by two reproductive relations (self-renewal and differentiation) into cell lineage L (Figure 2). A stem cell is defined by its position as the unique stem of L, with maximal self-renewal and differentiation potential relative to C, M, and n. These reproductive relations that define L are in turn defined in terms of comparisons of character value across cell generations. The comparisons implicate not only individual cells, but also multiple cell generations arranged in a lineage, as well as the whole organism and its specialized cellular constituents. The general stem cell concept, therefore, cannot be understood solely in terms of individual cells. Cell lineage relations and whole organisms are also implicated. So the stem cell concept, unlike that of cells of multicellular organisms, is not 'purely cellular.'

[FIGURE 2]

4. Experimental uncertainty

The above argument shows that stem cells are not defined as single cells; the concept is more complicated. However, if that concept were shown to apply to a single cell, then an argument like that of Section 2 could be made for stem cells. That is, experimental individuation of stem cells could show that objects in the stem cell model correspond to single biological cells, and therefore have clear physical boundaries, physiological integration, *etc*.[11] If the same or similar points that support the claim of biological individuality for cells of multicellular organisms could

[11] The term 'correspondence' is used loosely, to refer to a 'mapping' relation from components of the model to real biological systems. The points in this essay are independent of any technical account of the correspondence relation.

be made for stem cells, the simple argument in Section 1 would not be equivocal. So the next task is to consider how the abstract stem cell model relates to the biological world. As the previous section shows, a cell can qualify as a stem cell only relative to a lineage, set of characters, and time interval of interest. These parameters are specified by experiments that aim to isolate and characterize stem cells. The stem cell concept applies to different entities, depending on how the model's parameters are specified. So application of the concept is experiment-relative.

However, experiments that aim to isolate and characterize stem cells share a basic pattern: cells are first removed from an organismal source (which establishes the cell lineage of interest), then placed in a context where their character values can be measured, and finally moved to another context to measure self-renewal and differentiation (Figure 3). Experiments with this design specify parameters the abstract model leaves open, either as part of materials and methods, or measured results. For example, for human embryonic stem cells (hESC), the source organism is an early human embryo and the specific site of extraction is the 'inner cell mass.' Character values of interest include 'undifferentiated morphology' and a long list of molecular traits shared with embryonic or cancer cells.[12] The duration of interest is ≥50 cell divisions, with no upper limit.[13] Subtle changes in cell culture environment can (in principle) induce differentiation of hESC to any of the mature cell types that make up an adult human body: pluripotency.

[12] Characters: chromosome number and appearance, telomerase activity, cell surface molecules. Character values: rapid division in culture, homogeneous appearance, lack of specialized traits, flat round shape, large nuclei surrounded by correlatively thin cytoplasm, and prominent nucleoli.

[13] This is why ESC are described as 'immortal.'

[FIGURE 3]

Diverse experimental methods give rise to the current variety of stem cells: adult, embryonic, induced, pluripotent, multipotent, blood, neural, muscle, and many more. Pluralism about stem cells is compounded by the diversity of representational assumptions that are in principle available to link the model to biological objects: single cells, cell populations, or cell types. Cells in the model could conceivably correspond to any of these, and possibly other biological entities as well. But the representational assumption of concern here is the simplest and most intuitive: that objects in the model correspond to single cells in the biological world. Granting that the latter are biological individuals (as argued in Section 2), if this representational assumption holds, then the stem cell model picks out biological individuals. But that consequence is blocked by a severe evidential constraint. The stem cell model interpreted via the representational assumption that its objects correspond to single cells cannot be supported by experimental evidence. Hypotheses of the form 'Cell A is a stem cell' face intractable evidential problems.

As noted above, stem cell experiments involve two sets of measurements, both of which provide data about characters of single cells. *But no single cell persists through both sets of measurements*. Because cells reproduce by division, descendents and ancestors do not co-exist. A single stem cell, therefore, can only be identified retrospectively. Stem cells are defined as being capable of both self-renewal and differentiation. To experimentally establish a candidate stem cell's differentiation potential, that cell must be placed in an environment conducive to differentiation, and character-values of progeny that indicate differentiation measured later. To establish self-renewal, a candidate stem cell must be maintained in an environment that blocks

differentiation, and its progeny after n cell cycles measured for character-values that indicate similarity to the original. It is not possible to perform *both* experiments on a single cell.

Even if this could be done, self-renewal on its own presents a problem. This process is defined as production of offspring cells with the same traits and capacities as the parent. An offspring cell with the same capacities as a stem cell parent has the same potential for differentiation and for self-renewal. To establish that it has the same self-renewal capacity as the parent, the offspring cell must be maintained in the same environment as the dividing parent, and *its* offspring measured. Whether the offspring cell has the same self-renewal capacity as its parent depends on the reproductive capacities of the 'second-generation' cell. And so on. Establishing that a single cell is capable of self-renewal is infinitely-deferred. Nor can a single cell's differentiation potential be definitively established. Experiments can show what happens when a cell is placed in a particular environment, but not what would have happened if that cell had been placed in a different environment. If we have pure populations of identical stem cells, we can learn what happens to a stem cell from that population in a range of environments. But the antecedent assumes what these experiments are designed to discover: cells that share features had by all and only the stem cells of a particular lineage. If we cannot demonstrate that a single cell is a stem cell, how can we assume a population of identical ones? Even if that assumption were justified, there is no basis for generalizing claims about a cell's differentation potential beyond the range of environments used in experiments. Inferences about stem cell capacities are therefore relative to the environments examined – *i.e.*, to an experimental method, with a specific organismal source, characters measured, and manipulations of cell environment.

It follows that attribution of stem cell capacities to any individual cell is necessarily uncertain. No matter what technological advances are made in tracking and measuring single

cells, experiments cannot decisively resolve stem cell capacities at the single-cell level. This 'uncertainty principle' is an unavoidable evidential constraint for stem cell biology (see Section 6). To measure both self-renewal and differentiation potential for a single cell, and to elicit the full range of a cell's potential, multiple 'copies' of that cell are needed - a homogeneous (with respect to characters of interest) *population* of candidate stem cells.[14] So a strictly 'single-cell' stem cell model cannot be experimentally confirmed. In other words, no 'direct mapping' between objects in the stem cell model and individual biological cells can be supported by experimental evidence. To summarize the results of this and the previous section: stem cells, as currently defined and experimentally identified, do not correspond to single cells of multicellular organisms. Therefore, I conclude that stem cells are not biological individuals in the same way as cells of multicellular organisms.

5. Model organisms, model individuals

A further conclusion would be that stem cells are not biological individuals at all. But this judgment would be hasty. The above arguments suggest that stem cells mediate between different modes of biological individuality: cell and organism. Their mediating role offers a fresh developmental perspective on the cell-organism relation. The conceptual challenge of understanding this relation is a fundamental problem in biology. Paul Weiss (1940) presents the challenge incisively:

[14] This is why a thoroughgoing focus on cell populations cannot get around this evidential problem. Evidence for population-level models of stem cells also depends on the assumption of a homogeneous 'founder' stem cell population. For details, see Fagan (2013a).

At the end of development we are confronted with a unitary organized system, called an "organism," which, at the same time, is a collective of cells. At the beginning of development we find just one primordial cell – the egg… Now, there arises a dilemma. Either the egg already possesses supra-cellular organization of the same order as the later body – then it is not just another cell, but an uncellulated organism; or it is merely a cell like others…[and] development would create organization of a higher order (1940, 37-38).

Weiss' own solution is to acknowledge that the cell has "a dual character," as both an active biological individual and passive component regulated by top-down organismic control. A more sophisticated understanding of development as a collection of interrelated mechanisms, some operating from the 'bottom-up,' others 'top-down,' he argues, would dissolve the dilemma. Stem cells, I propose, are a crucial part of such an understanding of development. Moreover, in their mediating role between cell and organism, stem cells can exhibit biological individuality in a way analogous to a multicellular organism. I here argue that cultured pluripotent stem cells qualify as biological individuals in this way. We can see this, and account for contrasts between cultured stem cells and multicellular organisms, by recognizing the former as *model organisms* for investigating organismal development.

As described above (Section 3), stem cell experiments produce new biological entities, each derived from a particular organismal source and individuated by a set of measured character values. Cultured pluripotent stem cells are one such entity. They are produced by removing cells from an organismal source and placing them in artificial culture, with chemical factors added to prevent differentiation. Cells that rapidly divide under these conditions form colonies, which are selected and put into new cultures (Figure 4). This "passaging," repeated every few

weeks, maintains a continuously-growing lineage of undifferentiated cells. So self-renewal is imposed by the experimental design. Differentiation potential is demonstrated by moving small "clumps" of cells from a culture to a new environment that includes chemical factors that induce differentiation into a certain cell type: cardiac muscle, neurons, bone marrow, *etc*. Continuously-growing lines of undifferentiated cells that can differentiate under appropriate culture conditions qualify as stem cell lines. Because cells derived from very early (~5d) embryos tend to show greater self-renewal and differentiation potential than cells from older organisms, the paradigmatic pluripotent stem cell lines are embryonic stem cells.[15] Cultured pluripotent stem cells are entities that exist between "passages" from one culture to another.

[FIGURE 4]

Cultured pluripotent stem cells exhibit many of the individuating features of organisms. They have clear spatial boundaries in three dimensions, determined by the size of cell culture flasks: a transparent artificial 'body.' Cultured pluripotent stem cells also endure over time; indeed, pluripotent stem cell lines have (apparently) unlimited lifespans. They have molecular, cellular, morphological, and physiological properties (see note 12) and participate in causal processes of self-renewal, experimental manipulation, and – under suitable conditions - cell development. So cultured pluripotent stem cells satisfy minimal criteria for individuality. They are also evolutionary individuals, subject to artificial as well as natural selection. Cycles of

[15] Pluripotent stem cell lines can also be produced from partly- or fully-differentiated cells extracted from adult organisms, which are then altered by manipulating genes, culture conditions, or both (yielding induced pluripotent stem cells; iPSC).

passaging, which select for cells "with a uniform undifferentiated morphology," impose a version of the germ/soma distinction (Figure 4; Thomson et al 1998, 1147, n6).[16]

However, cultured pluripotent stem cells differ from multicellular organisms in two key respects: (i) they exhibit much simpler organization and (ii) they are artificially produced. I discuss each in turn, and argue that these contrasts do not warrant rejecting the thesis that cultured stem cells are biological individuals. First, unlike organisms, cultured pluripotent stem cells exhibit minimal physiological organization and functional integration. But in appropriate environmental conditions, they can and do transform into mature tissues and organs – indeed, a whole organism, at least in principle.[17] Even in artificial culture that encourages self-renewal, pluripotent stem cells are liable to differentiate spontaneously – particularly when they contact or "overgrow" one another. Such spontaneous differentiation is far less elaborate and organized than unmanipulated embryonic development in mammals. So pluripotent stem cell cultures could perhaps be considered 'minimal' or 'borderline' organisms.[18] They embody early mammalian development in drastically simplified form: a layer of undifferentiated, dividing cells, selected so as to minimize variation. Under certain environmental conditions, they exhibit cell differentiation processes in a very simple and manipulable way. I return to this point below.

A second contrast is that cultured stem cells, unlike naturally-occurring organisms, do not form lineages of "transparent bodies" on their own, but only as a result of experimental

[16] The selection process used in passaging resembles that of cell-level selection in many plants: many cells could give rise to a new organism, but few actually do.
[17] This has been demonstrated for mice, but (for ethical reasons) not in humans.
[18] It is interesting to consider whether cultured stem cells with more limited differentiation potential, or cell lines that are not identified as stem cells (such as cancer cell lines), also qualify as model organisms. Though I do not rule this out, I think a stronger case can be made for cultured pluripotent stem cell lines as 'minimally developing multicellular organisms' than other kinds of cultured cell. So my claims here are restricted to cultured pluripotent stem cells, leaving the question open for other cases. Thanks to Lucie Laplane for pushing me to clarify this point.

intervention. So they do not reproduce "autonomously," in the way of biological organisms. This is obviously an important difference, but it does not, I think, rule out cultured stem cells as biological individuals *tout court*. Obviously, cultured stem cells are artificial products. Though derived from organisms, pluripotent stem cells have no direct organismal counterparts. But artificiality per se does not entail lack of individuality. Many biological fields concentrate research efforts on model organisms: inbred fruitflies, mice, yeast, and zebrafish. Though not identical to their naturally-occurring counterparts, these entities are clearly organisms, as their moniker implies. Many cannot survive outside a laboratory environment. For example, certain mouse strains, widely used in biomedicine, lack an immune system and must be maintained in a sterile room. But such mice are clearly organisms, and therefore biological individuals. Therefore engineered obligate inhabitants of a laboratory can qualify as biological individuals. Cultured pluripotent stem cells, I suggest, are a new kind of model organism, derived from parts of an organism rather than a whole.

Cultured pluripotent stem cells are evidently not biological entities *found in nature*. It follows that they are not biological entities that have evolved by natural selection, strictly speaking. So one might object that they do not qualify as evolutionary individuals, being insignificant from a broad evolutionary standpoint. However, such a standpoint is not the only one relevant for questions of biological individuality.[19] In stem cell research, the developmental perspective takes priority. By taking this perspective and its epistemic goals seriously, we can better understand the biological individuality of stem cells. The 'modeling' role clearly applies

[19] It is not even the only standpoint relevant for evolution, as research in experimental evolution makes use of highly artificial bacterial populations. Thanks to Thomas Pradeu for raising this point. It is also worth noting that cultured pluripotent stem cells may count as "borderline Darwinian populations" on Godfrey-Smith's evolutionary account, exhibiting high B-, intermediate G-, and low I-values (2009, this volume).

here. Cultured pluripotent stem cells are laboratory artifacts created to study mechanisms of organismal development. By studying them, scientists hope to understand and manipulate developmental pathways in more paradigmatic biological individuals (like ourselves). The notion of a 'model organism' accounts for the contrasts between cultured pluripotent stem cells and multicellular organisms. Indeed, this distinctive modeling relation exactly captures the relation between these two biological entities.

Classic model organisms (e.g., *E. coli*, *C. elegans*, and *D. melanogaster*) are constructed to be tractable for laboratory use and to exhibit phenomena of interest in a way accessible to controlled manipulation. Model organisms for studying development, in particular, share several characteristic features: small body size, large offspring number, rapid growth rate, robust processes of reproduction and development, tractability, simplicity, and accessibility to observation and measurement (Bolker 1995, Robert 2004, see also Fagan 2013a Ch7). Cultured pluripotent stem cells exhibit all these features. Their body size, though flexible in principle, is in practice constrained by dimensions of cell culture dishes and flasks. Rapid, continuous growth is ensured, since cultured cells are selected for frequent division. Passaging occurs about once a fortnight, ensuring a rapid reproductive rate. And cultured pluripotent stem cells make cellular developmental processes visible and accessible – in striking contrast with normal mammalian development. Tractability is at present more goal than reality, as researchers work to streamline and standardize stem cell culture conditions. However, when any new stem cell line is established, the next tasks are to reduce variation among cells and manipulate culture conditions to yield reproducible experimental results. These efforts resemble earlier standardization practices for classic model organisms such as inbred mice and fruitfly strains. Cultured pluripotent stem cells also play the dual epistemic role of a model organism, being

objects of study in their own right as well as tools for representing target phenomena. Whereas classic model organisms are altered to be tractable representatives of a wide variety of unmanipulated organisms, cultured pluripotent stem cells are created to be tractable representatives of a wide variety of developing multicellular organisms. So they are model organisms in a double sense – artificial biological entities designed to exhibit features of developing multicellular organisms. Their intermediate character, between cell and organism, is crucial to their scientific role.

6. Complementarity

The preceding discussion reveals an epistemic aspect to core questions about biological individuality. Focusing on the epistemic issue of uncertainty (Section 4) also leads to a surprising cross-disciplinary parallel. To briefly recap: we cannot directly measure a single cell's capacity for self-renewal or differentiation, either separately or together. The analogy with uncertainty relations in quantum physics has not gone unremarked (*e.g.*, Nadir 2006, Fagan 2013b). Heisenberg's uncertainty principle states that certain pairs of quantities cannot be simultaneously known with precision. The most familiar instance is position and momentum: we cannot simultaneously attribute a well-defined position and momentum to a single particle.[20] This reciprocal uncertainty has a theoretical basis, entailed by probabilistic principles of quantum mechanics involving non-commuting operators (see, *e.g.*, Omnés 1999). But quantum uncertainty can also be understood in terms of mutually exclusive experimental arrangements: one can either measure a particle's direction of motion and so derive its position at a given time, or measure its wave characteristics and from these calculate its momentum. In the former, the

[20] Another familiar example is description of light as *at once* an electromagnetic wave and a particle.

particle's wave aspect is ignored (preventing calculation of its energy and momentum), while in the latter the wave's probabilistic 'spread' ensures that its direction of motion cannot be known with precision. Uncertainty relations thus quantify the "reciprocal limitations" on what we can measure for a given physical system (Saunders 2005).

Obviously, the objects of study and properties of interest for stem cell research differ from those of quantum physics. Instead of a single particle with physical properties at a given time, stem cell experiments focus on a single cell with developmental properties relative to a lineage extended in time. However, mutually exclusive experimental measurements are implicated in both cases. In stem cell biology, experimental conditions for measuring self-renewal (even provisionally) rule out the possibility of measuring differentiation potential, and vice versa. And similarly for differentiation potential; experimental conditions for measuring whether a cell can give rise to, say, heart muscle, rule out the possibility of measuring that cell's ability to give to rise blood, bone, skin, neurons, *etc*. Stem cell capacities are constrained by uncertainty relations grounded in the limitations of experimental measurement. What is the significance of the analogy to uncertainty in quantum physics? A full examination of this question, and its bearing on issues of realism and individuality, is beyond the scope of this essay. A few preliminary points, however, seem clear.

First, there is a significant disanalogy between stem cell and quantum physical uncertainty. The former is not entailed by formal theory and involves no challenge to the ordinary ('classical') causal interpretation of experiments and their results, while the latter is emblematic of the challenge of articulating an intelligible interpretation of formal quantum theory. Stem cell experiments and their outcomes can be described in ordinary causal terms; the challenge is establishing evidential relations between these descriptions and hypotheses about

single stem cells. Uncertainty in quantum physics challenges our ordinary causal understanding of physical events. Moreover, because stem cell biology lacks a formal, predictive theory, the analogy engages only the 'operational' side of quantum physics. In consequence, much philosophical work on uncertainty in quantum physics does not extrapolate to the stem cell case.

However, the analogy cuts deeper if we consider quantum-mechanical uncertainty in the context of Bohr's principle of complementarity (1949).[21] This is because Bohr speculated that complementarity might apply to fundamental issues in biology, and was committed both to retaining classical concepts and to at least a modest operationalism in defining concepts for scientific use (Omnés 1999, Saunders 2005). So Bohr's account of quantum uncertainty highlights the role of experiments, strengthening the analogy with stem cell uncertainty. His operationalism restricted well-defined concepts to aspects of the world that can be directly measured by experiment. For cases involving uncertainty, then, the question of interpretation amounts to choice of experimental method. This is the core idea of complementarity: the experiments we choose to do determine which interpretation is correct for a given case. This prevents any formal contradiction from arising between wave and particle interpretations, for example; each applies to different experimental arrangements. So we can describe experiments and their outcomes in a way that respects quantum uncertainty relations, without at once employing incompatible notions to describe physical reality. But the price of such consistency is that experimental arrangements are implicated in the correct application of interpretive models of physical systems. In this sense, the experiment is involved in the phenomenon under investigation.

[21] This insight is due to Simon Saunders (pers. comm., May 2012).

Similarly, experimental contexts and measurements are implicated in characterization of stem cells (see Sections 3-4 above). So Bohr's "quantum postulate," that there is no absolute or fundamental separation between the object of inquiry and experimental context, applies to stem cells as well. Both physical and stem cell phenomena are "contextual" in the sense that the behavior of objects of inquiry cannot be sharply separated from interaction with measuring instruments ("the agencies of observation") that define the conditions under which phenomena appear. But this contextuality, or relativity to experiment, raises different challenges in the two cases, which accordingly require different solutions. In the quantum physics case, the challenge is to avoid contradictory descriptions of the objects of inquiry. Bohr's principle of complementarity offers a solution: "… evidence obtained under different experimental conditions cannot be comprehended within a single picture, but must be regarded as complementary, in the sense that only the totality of the phenomena exhausts the possible information about the objects" (Bohr 1949, 209). Contradiction is avoided by separating experimental contexts that mutually exclude the operational definition of paired concepts such as position and momentum, particle and wave.

In the stem cell case, the challenge is not theoretical consistency but operationalization. A stem cell's defining capacities require multiple experimental contexts to demonstrate. So instead of separating experimental contexts to avoid contradiction, application of the stem cell concept requires a kind of unification across experimental contexts; inclusivity rather than exclusivity, so to speak. Multiple 'copies' of a cell are needed to measure the extent to which that cell has both capacities and the full range of its developmental potential. So evidence of stem cell capacities depends on the assumption that the population of candidate stem cells is homogeneous, relative to the characters and environments of interest. An experiment

demonstrating stem cell capacities involves a set of systematically-varied experiments using 'replicates' of the cell under investigation. So resolution (or rather, management) of uncertainty in the stem cell case takes a different form than in Bohr's quantum physics.

Finally, there is a sense in which Bohr's principle of complementarity holds for stem cell biology, though its scientific significance is not as he predicted. Saunders (2005) argues that Bohr's principle is best understood as a conjecture about different branches of science, proposing a "novel explanatory framework …[that] applied in principle to any empirical domain in which concepts could only be applied under mutually exclusive experimental conditions" (435). Stem cell biology is such an empirical domain, and substantive definition of its core concept requires integrating mutually exclusive experimental conditions. But Bohr saw this integration as the task of new non-classical laws, which the principle of complementarity provides theoretical space to discover (within a classical framework), but does not articulate. Stem cell biologists, however, do not seek laws and show scant interest in securing theoretical grounds for them. Instead, integration across experimental contexts is accomplished by provisional empirical assumptions (homogeneous cell populations with respect to characters of interest) and technical innovation. So Bohr's conjecture is both vindicated and subverted, in the case of stem cell biology.

7. Conclusion

A number of philosophers and scientists have argued that cells of multicellular organisms are biological individuals, or, more cautiously, that they exhibit some important characteristics of biological individuality. This essay extends the question to stem cells: undifferentiated cells that self-renew and give rise to differentiated cells. Their general definition suggests that a stem cell is a specialized type of cell, like neurons, red blood cells, muscle cells, *etc*. If cells that compose

multicellular organisms are biological individuals, then it follows that stem cells are biological individuals. However, counterintuitive as it may seem, we should resist this conclusion. I have argued that stem cells are not biological individuals in the way of cells of multicellular organisms. My argument uses a modeling approach, explicating the general working definition of 'stem cell' as a simple abstract model: a structure of objects and relations. The modeling approach sheds light on the central concepts and practices of stem cell biology, as well as the relation between cell and organismal individuality. This approach reveals two contrasts between stem cells and cells of multicellular organisms. First, the stem cell concept is more complex than that of a single cell bearing marks of biological individuality. Cell lineage relations, cell populations, and whole organisms are also implicated. Second, stem cell experiments cannot provide evidence that stem cells as defined in the model correspond to single cells in biological systems. So stem cells as currently defined and experimentally identified do not correspond to single cells. These contrasts show that stem cells are not biological individuals in the same way as cells of multicellular organisms (assuming that the latter are individuals).

This conclusion may sound paradoxical: a stem cell is not a cell. In fact, some scientists have argued for exactly this conclusion, proposing instead the notion of "stemness," a state defined by wide developmental potential, which individual cells may enter or leave (e.g., Zipori 2004, Lander 2009).[22] The abstract stem cell model proposed above is compatible with the 'stemness' view (Fagan 2013a). But we can learn more, I suggest, by pressing further on the question of stem cell individuality. At least some stem cells are, arguably, biological individuals in the way of multicellular organisms. More precisely, cultured pluripotent stem cells are analogous to multicellular organisms; the paradigmatic biological individuals. The key to

[22] For philosophical treatments of this issue, see Leychkis et al 2009, Laplane 2013.

understanding the biological individuality of stem cells is the concept of a model organism. Cultured pluripotent stem cells are designed to display paradigmatic features of biological individuality in simple, accessible, and exaggerated ways. In this sense, at least some stem cells are constructed to be *model individuals*. The stem cell case also exhibits a surprising parallel with physics. Bohr's principle of complementarity offers an intriguing perspective on the uncertainty inherent in measurements of individual stem cells.

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FIGURE 1 Minimal stem cell model – asymmetric cell division.

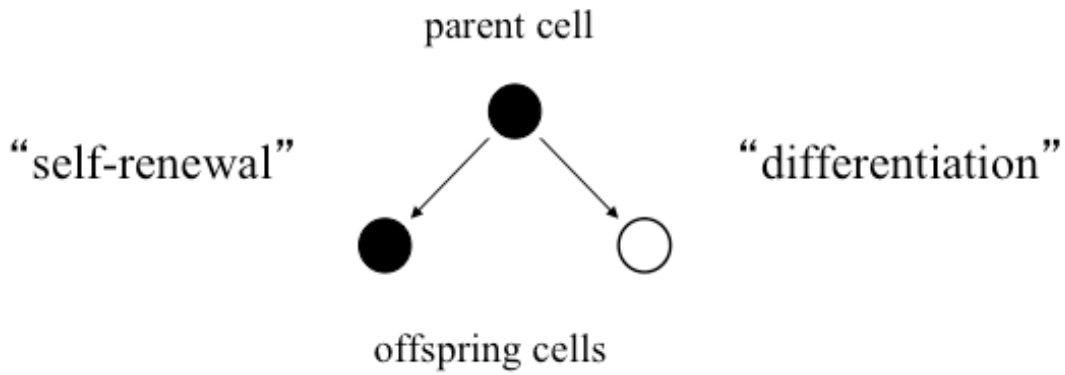

FIGURE 2 Abstract stem cell model – the general concept.

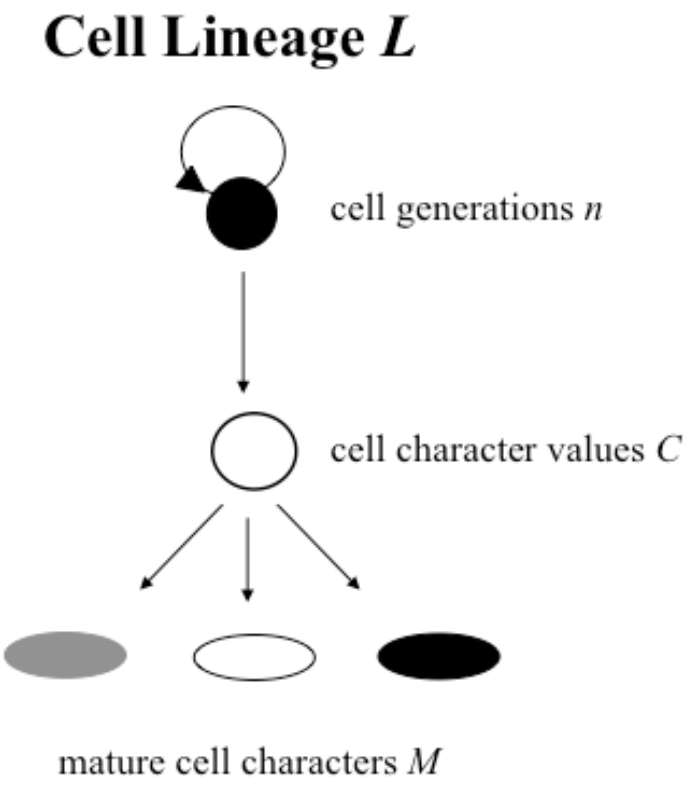

FIGURE 3 Stem cell experiments – general design.

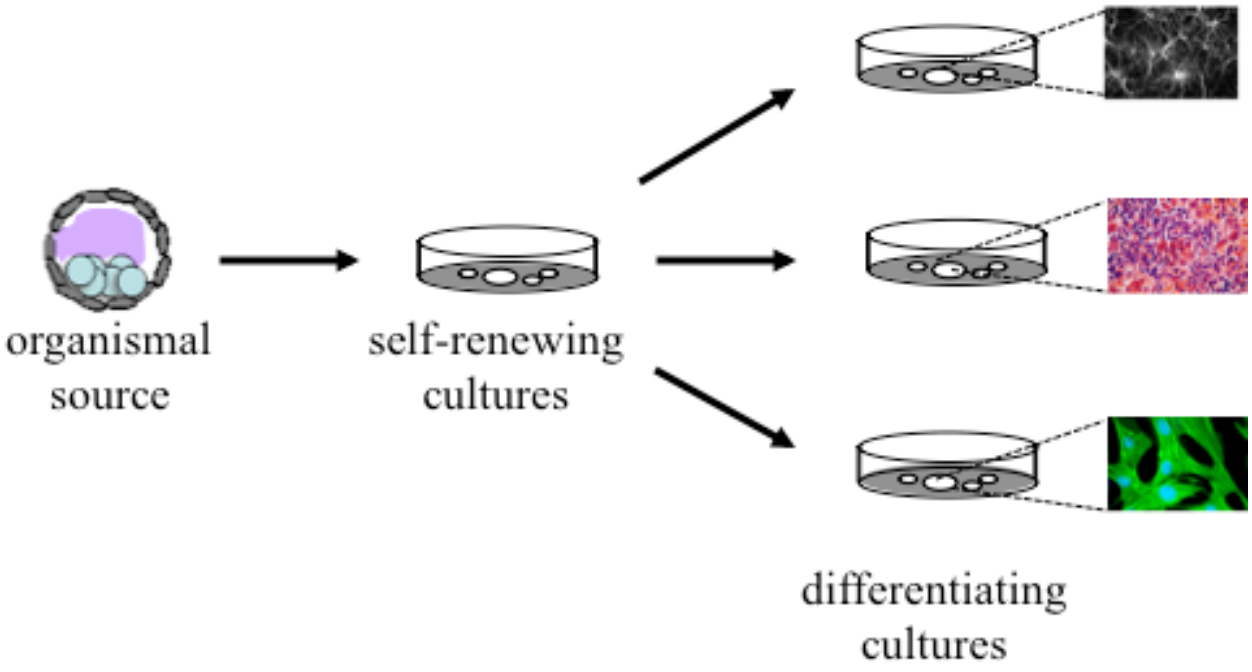

FIGURE 4 Cultured stem cells and stem cell line.