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Postmodern Biology: (Adult) (Stem) Cells Are Plastic, Stochastic, Complex, and Uncertain

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Abstract This chapter will discuss recent findings regarding cell plasticity and stem cell behavior, focusing on ways in which experimental design, observer interference, and inherent stochasticity and complexity are serving to create a new, postmodern biology. The chapter will summarize: (a) the four recognized pathways whereby cell plasticity occurs physiologically; (b) recent findings regarding unexpected epigenetic reversibility of gene restrictions that provide the mechanistic core of plasticity; (c) current evidence for the stochastic nature of gene expression and, therefore, of cell fate decisions. It will be noted that stochastic, however, does not imply completely random; rather, constrained randomness, intermediate between rigid determinism and complete disorder is what is usually seen experimentally. Possible sources of such constrained disorder, from a biomolecular point of view, will be discussed. The chapter will conclude with discussions of how these findings contribute to a Complexity Theory formulation of the body as self-organizing emergence of interacting biomolecules and the implications of such concepts for design and interpretation of experimental results (i.e., a cellular version of Heisenbergian uncertainty).

Keywords Stem cells · Plasticity · Stochasticity · Complexity · Uncertainty principle

1 Introduction

Nearly every field of academic studies has been made slippery for traditionalists by introduction of "postmodern" analyses that highlight the observer dependence and uncertainty of nearly any investigated phenomenon. Biology, however, in its glory with the modern successes of molecular and cell biology has remained relatively unchallenged in this regard.

However, studies of adult stem cell plasticity in combination with contemporaneous findings from other fields, demand reconsideration of long-held dogmas. Up for revision are doctrines about reversibility of gene restrictions, the role of stochasticity in cell fate decisions, and the ability of cell biologic experiments, in vivo and ex vivo, to accurately reflect physiologic phenomena (Theise 2002; Theise and Krause 2001, 2002). Biology begins to get more slippery in a postmodernist (parenthetical) sort of way.

We begin with the fact that the increasing intricacy of adult stem cell plasticity's phenomenology and of our gradually expanding appreciation of its underlying mechanisms requires a clarification of language. This is where postmodern approaches encroach upon orthodox certainties. A revisionist approach to language is often the first postmodern shot across the bow.

A full discussion of the terminology issues is beyond the scope of this chapter; however, for clarity of discourse, two labels, usually linked in a single, politically potent phrase, require extrication from each other. These are the terms "stem cells" and "plasticity." While they are related in some situations, they are not to be treated like conjoined twins.

As Helen Blau has eloquently discussed, stem cells are not cellular "entities," but, rather, cells that perform certain stem cell "functions" (Blau et al. 2001). The classical definition of a stem cell is still useful: a stem cell has capacities for self-renewal and for asymmetric division leading to generation of other differentiated cell types (either with each cell division or, in aggregate, when populations are studied over time). Whether a stem cell is always a stem cell, however, and whether non-stem cells can ever be recruited or induced to behave like stem cells will be one of the topics addressed in this review. This is the point of one set of parentheses in the title of this chapter: while the ideas to be discussed arose in the context of stem cell research, we now understand that they are not restricted to stem cells, per se.

Meanwhile, the word "plasticity" has been used to describe differentiative events (or capacities), which are unexpected according to accepted standard definitions of various cell lineages. Thus, hematopoietic stem cells give rise to the full array of hematopoietic lineages, but this is not generally referred to as plasticity; rather, plasticity is invoked when an unexpected differentiation event is revealed. Examples from 1999, when *Science* declared "Stem cell plasticity" to be the Breakthrough of the Year, included marrow-derived cells becoming skeletal muscle and liver and neural stem cells giving rise to

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hematopoiesis (Bjornson et al. 1999; Ferrari et al. 1998; Petersen et al. 1999). The term "plasticity" is used in these contexts because cells are unexpectedly crossing organ boundaries or even embryonic lineage boundaries.

One must question, though, whether the experimental confirmation of plasticity events then eliminates the need for the word: if a differentiation pathway is considered normative, i.e., unsurprising, is it still plasticity? Or must the notion of plasticity be invoked when hypothetical embryonic trilaminar lineage boundaries are breached? Or must we consider something in between, such as when organ boundaries are breached? And is it only stem cells that can be plastic? Or is plasticity something that might be demonstrated by a wide variety (or perhaps all) cells, differentiated or otherwise?

These questions are up for discussion and will be among the topics of this review. To address them we will proceed through consideration of five experimental and/or conceptual sub-topics:

1. We will briefly summarize the four recognized pathways whereby cell plasticity occurs physiologically (for our purposes, in this review, "plasticity" will imply a more generalized sense of "differentiative potential"). The experimental data demonstrating these pathways, though somewhat scattershot and mechanistically undefined, point to the need for alternate models for the nature and behavior of cells.
2. Recent findings regarding unexpected epigenetic reversibility of gene restrictions, the underlying molecular events at the core of plasticity, will be updated for the reader. These more systematically acquired data provide the mechanistic core of plasticity.
3. Current evidence for the stochastic nature of gene expression and, therefore, of cell fate decisions will be considered. It will be noted that stochastic, however, does not imply completely random; rather, constrained randomness, intermediate between rigid determinism and complete disorder is what is usually seen experimentally. Possible sources of such constrained disorder, from a biomolecular point of view, will be discussed.
4. Taking these data together, we will then extend our prior discussion of how a complex systems analysis may be applied to cell behavior. We have argued previously that cells, in vivo, behave as interacting agents giving rise to emergent self-organization of cell lineages, tissues, organs, and bodies. Here we will consider that they are actually located within a hierarchy of complex systems and that they, themselves, are emergent phenomena self-organizing from the biomolecules which they comprise.
5. The implications of such concepts for design and interpretation of experimental results (i.e., a cellular version of Heisenbergian uncertainty) will complete the review.

All of these diverse strands of thought have been considered in prior papers, by us and by other investigators, but it is perhaps time to weave them together,

to better imagine the emerging tapestry that may well turn out to be a new biology for this new millennium.

2
Pathways of Plasticity

Physiologic, in vivo expressions of differentiative potential can be seen in four flexibly employed processes or pathways (Theise and Wilmut 2003). First, of course, is the classic hierarchical, unidirectional concept of lineage commitment. In embryonic/fetal development, this begins with an embryonic stem cell or with a fetal stem cell. In adults, one is speaking of normative, tissue maintenance or repair after injury. In all these cases, one begins with a multi- or totipotent stem cell which, through asymmetric division, self renews and also gives rise to more differentiated cell types. These more differentiated daughter cells mature in an ordered, hierarchical, unidirectional fashion.

This model is certainly the dominant pathway for development and tissue maintenance, if not, perhaps the only pathway. As such, it was the most readily demonstrable experimentally. Detailed transplantation experiments established the hierarchical, unidirectional aspects. In moving cells from one part of the embryo to another, they would be influenced by the new microenvironment to change differentiative pathways until a certain temporal point in development, after which they would be "committed" and not respond to the new environmental cues. Even before the structure of DNA revealed how genetic encoding took place, these observations led to the idea of a restriction of gene expression that eventually confined a cell to a "terminally differentiated" state, from which it could not be coaxed.

The second pathway of plasticity is one of "dedifferentiation", i.e., reversion of a differentiated cell into a progenitor, often blast-like (i.e., primitive or undifferentiated) phenotype, which can then give rise to different lineages. In mammals, this has only been confidently recognized in neoplasia, in particular in malignancy, in the context of genetic mutations and other genomic derangements. While metaplastic phenomena may also include such a process, they are usually hypothesized to represent activation of an alternate pathway of a multipotent, intraorgan stem cell (Theise and Krause 2002). Examples of this would include osseous metaplasia within skeletal muscle, when mature bone forms after mechanical injury, or the squamous metaplasia of respiratory lining cells in the lungs of smokers. However, in limb-regenerating amphibians, there appear to be proteins which can induce a mature cell to reverse differentiative direction, giving rise to blasts which then give rise to the necessary mature cells in the regrowing limb (Endo et al. 2004).

The third and forth pathways are those which currently capture the most controversial attention. The third is that of cells from one lineage directly differentiating into cells of another lineage (Krause et al. 2001) (This has often

been referred to as "transdifferentiation," a term which engenders still more unnecessary debate and we now feel is best avoided.). Such direct differentiative events which jump between (dogmatically) hypothesized lineage or organ boundaries have now been convincingly demonstrated in vivo and ex vivo and are induced by local microenvironmental effects that lead to alterations in gene expression (Harris et al. 2004; Ianus et al. 2003; Ishikawa et al. 2003; Jang et al. 2004; Newsome et al. 2003).

The fourth pathway is that of cell–cell fusion, sometimes followed by nuclear–nuclear fusion. The idea of cell fusion was originally suggested as part of a critique of the findings regarding plasticity arising from direct differentiation, used to polemically dismiss those new findings as "artifact" (Newsome et al. 2003; Willenbring and Grompe 2003). However, it is now not merely a rhetorical or theoretical challenge for undermining one set of new, controversial findings, but is, itself, established as yet another alternate and surprising physiologic, in vivo process (Alvarez-Dolado et al. 2003; Willenbring et al. 2004). In this case, the plasticity of gene expression is induced not by microenvironmental effects, but by cytoplasmic and/or nuclear factors. This is directly analogous to the findings in experimental heterokaryons studied by Blau and colleagues two decades ago (Blau et al. 1983).

As Blau and investigators ultimately concluded "differentiation is an actively maintained state" (Blau et al. 1985). Examining all these pathways in aggregate, one comes to realize that plasticity is simply a comparatively macro-level change in function and phenotype arising from the micro-level alterations in gene expression. Just as the early transplantation experiments showing restriction of developmental potential over time implied molecular restriction of gene expression, these more newly described phenomena imply reversibility of these "irreversible" gene restrictions. In the earlier instance, decades had to pass before the mechanistic, molecular underpinnings could be confirmed. However, in these more recent and controversial demonstrations of plasticity, the implied reversibility of gene restrictions was already being studied, in parallel, by other investigators.

3
Gene Restrictions: Irreversible Versus Reversible

In the standard model of cell differentiation, the cell makes a series of simple (usually binary) fate decisions that are irreversible and thus restrict the cells to a particular lineage. Implicit in this model is the concept of commitment; if cell fate decisions are irreversible, once a cell has made a particular fate decision, it is committed to that lineage and cannot alter its fate. In this model of differentiation, cell fate is determined by two separate components: the external microenvironment—the extracellular signals that a cell is exposed to—and an internal cellular memory, that is, to which lineage it has already been restricted.

Classic experiments by Nicole Le Douarin and colleagues showed that during neural crest migration and differentiation cells become both committed and restricted to their lineage. Neural crest cells that are transplanted to more rostral or caudal regions of the neural tube retain their fate programming and thus must be committed to their lineage before any obvious morphological or migratory changes occur (Le Douarin and Dupin 1993).

Outside of development, the most widely studied model of differentiation is the hematopoietic system. Under the hierarchical paradigm, a hematopoietic stem cell (HSC), which is already committed to a hematopoietic fate, further differentiates into a common lymphoid progenitor (CLP) or common myeloid progenitor (CMP). These cells are committed to the lymphoid or myeloid lineages, respectively, but can differentiate into any of the cells of that lineage. These then make further cell fate decisions, which eventually result in their terminal differentiation into T cells or B cells or neutrophils or macrophages and so on. Recent experiments have actually identified cells that appear to fit all the requirements of committed CMPs and CLPs (Akashi et al. 2000; Kondo et al. 1997). When used in bone marrow transplant experiments, the putative CLPs differentiated into mature B-cells, T cells, and natural killer cells but were restricted solely to these lineages: no donor-derived myeloid cells could be found. In vitro experiments using cytokine cocktails that promote myeloid differentiation of HSCs resulted in apoptosis rather than myeloid or even lymphoid differentiation. Likewise, the putative CMP was restricted solely to the myeloid lineage and showed differentiation into all cell types in the myelo-erythroid lineage.

While lineage commitment initially restricts a cell to a particular subset of cell fate decisions, those decisions themselves—such as the decision of the HSC to differentiate into the CMP or CLP—are likely to be mostly affected by the microenvironment. Unlike lineage restriction, microenvironmental effects on cell fate are probably stochastic in nature. While extracellular signals may push a cell toward one particular fate decision or another, some cells in a population will follow the alternate path. Lineage commitment, in this traditional model, however, is theoretically complete.

3.1 Mechanisms of Lineage Restriction

The limiting of a cell to a subset of possible fates has two putative molecular mechanisms. The first, which may be thought of as passive lineage restriction, depends on the subset of proteins actually expressed in the cell. A cell cannot differentiate down a pathway if it does not contain the proteins that can respond to the intracellular and extracellular signals that are required by that pathway.

The second mechanism, which may be thought of as active lineage restriction, depends on the continued silencing of genes that are master regulators of

alternate lineages (Jaenisch and Bird 2003). In this model the cell retains a working memory of its ancestry, in the form of epigenetic (i.e., non-DNA-encoded) modifications to the genome that render particular gene sets available or less accessible (restricted) for transcription. According to this epigenetic view of differentiation, the cell makes a series of choices (some of which may have no obvious phenotypic expression and are spoken of as determination events) that lead to the eventual differentiated state. Thus, selective gene repression or derepression at an early stage in differentiation will have a wide-ranging consequence in restricting the possible fate of the cell. As usual, there is evidence that both of these mechanisms are important.

The absence of particular signaling receptors or effectors can prevent the differentiation of a cell down a particular lineage. In the hematopoietic system, the HSC makes a fate decision to differentiate into either the CMP or the CLP. IL-2 promotes differentiation down the myeloid lineage. After commitment to the myeloid lineage, the IL-2β receptor is upregulated in the CMP, whereas after lymphoid commitment, no IL-2βR protein can be detected in the CLP. Lineage restriction at early time points after commitment to the CMP or CLP is due to the absence of the IL-2β receptor. If CLPs are isolated from mice that express the human IL-2β receptor they can be induced to differentiate down the myeloid lineage by treatment with human IL-2 (Kondo et al. 2000).

This conversion of lymphoid committed progenitors to the myeloid fate comes at the expense of the lymphoid lineage and is indicative that CLPs have latent GM lineage differentiation potential that can be initiated through IL-2 signaling. It is important to note, however, that this IL-2-induced myeloid differentiation is somewhat transient, and cultured CLPs "irreversibly" commit to the lymphoid lineage after 2 days. It is likely, therefore, that this mechanism of lineage commitment is important only in the early stages of differentiation of a cell type; after this, more permanent silencing of alternate lineages occurs. Many important transcription factors and signaling molecules have different effects in different cell types—an obvious statement but one which is obviously important when talking about this type of lineage restriction. IL-2 becomes important in the end stages of T cell development. There must therefore be a mechanism to limit the transcriptional response of a cell to a particular signal.

3.2 Lineage Restriction by Chromatin Silencing

Differentiation involves the selective activation and silencing of particular gene expression programs. One potential mechanism to restrict cells to a particular lineage is to irreversibly silence the cell-type-specific genes of the alternate lineages. The classic paper by Weintraub and Groudine in 1976, showing that active genes have a different chromatin structure than silenced genes, was the first evidence that DNA accessibility was important in transcriptional regulation (Weintraub and Groudine 1976).

In mammals, there are two major mechanisms of epigenetic control; methylation of DNA at cytosine and modifications of the histone proteins (Jaenisch and Bird 2003). DNA methylation is associated with gene silencing, especially in imprinting and X-inactivation. After DNA synthesis, the daughter strand is methylated by reference to the parental strand, thus maintaining methylation patterns through mitosis. There are no known DNA demethylases and it is thought to be an extremely stable modification. Experiments using transgenes have shown that DNA methylation is stable over more than 50 divisions.

There is increasing evidence that DNA methylation can also be the cause of gene restriction during differentiation, rather than just an associated effect. Some of the first experiments used 5-aza-2′-deoxycytidine (5-Aza-dC), a nucleotide analog that inhibits DNA methyltransferases, and therefore results in hypomethylation of daughter cells, after division. For example, when fibroblasts were treated with 5-aza-dC they acquired the ability to spontaneously differentiate into a range of different mesenchymal lineages, including chondrocytes, adipocytes, and multinucleated myotubes (Taylor and Jones 1979). Such experiments show that reversal of epigenetic modifications associated with gene silencing also reverse the lineage restriction of a cell, allowing it to differentiate down several unexpected pathways.

More recently, this sort of epigenetic reprogramming has been used to increase the number of viable blastocysts in nuclear transfer experiments (Enright et al. 2003). Treatment of nuclei with combinations of Aza-dC and TSA significantly increase the number of viable blastocysts, potentially implying that removing the chromatin-linked lineage restriction of mature cells is necessary for the formation of the totipotent zygote.

3.3 Post-Translation Modification of Histones

At least 100 different post-translational modifications of the various histone proteins are now known. The types of modifications known include acetylation, methylation, phosphorylation, and ubiquitinization. All of these modifications seem to correlate with the transcriptional state of the chromatin in question: active, silenced or potentiated (Jaenisch and Bird 2003). The best-known histone modification is the acetylation of histone H4. Increased acetylation of H4 correlates with active transcription and the major repressor complexes such as the N-CoR and SMRT complexes contain a variety of histone deacetylases.

An implication of an irreversible chromatin silencing mechanism for gene restriction is that more pluripotent cells will have a chromatin structure in which the genes expressed in all future mature lineages will be in a potentiated state rather than a totally silenced state. Following on from Weintraub and Groudine's experiments, it was shown that in FDCP mix cells, an early myeloerythroid cell line, the β-globin locus is susceptible to DNAse degradation well

before significant β-globin transcription occurs, indicating some sort of gene potentiation (Jimenez et al. 1992).

In order for certain genes to be permanently epigenetically repressed, active marks such as acetylation of histone H4 must be removed. Recent experiments have shown that histone deacetylases seem to be important in cell fate and lineage restriction. Hematopoietic stem cells that are grown in the presence of the histone deacetylase inhibitor Trichostatin A (TSA) remain pluripotent and do not differentiate, even if grown in the presence of cytokines that promoted differentiation (Milhem et al. 2004). Additionally, it has been shown that histone deacetylase activity is required for differentiation in ES cells (Lee et al. 2004).

Somatic cell nuclear transfer experiments have shown that the more differentiated a cell is, the less likely it is to form a developing blastocyst. Subsequent reprogramming experiments have shown that nuclei with a more generally open chromatin structure, as evidenced by H4 acetylation and H3 Lys 4 methylation, are more likely to result in successful nuclear transfer (Santos et al. 2003).

3.4 Reversibility of Gene Restrictions

If nonlineage-restricted differentiation events truly occur in nature, then the molecular modifications leading to epigenetic gene restriction must be reversible. There is plenty of experimental evidence that all of the epigenetic means of lineage restriction we have mentioned, such as DNA methylation and histone modifications can be reversed by artificial manipulation. In addition, overexpression of single transcription factors can result in a complete change of fate. For example, overexpression of the transcription factor MyoD in fibroblasts also converts them into multinucleated myotubes (Davis et al. 1987), just as treatment with Aza-dC does. Likewise, overexpression of C/EBPα in mature B cells will directly convert them to mature macrophages, with a clear macrophage phenotype including phagocytosis (Xie et al. 2004).

Other experiments looking at reprogramming of nuclei show that epigenetic modifications are far from irreversible. ES cell cytoplasm will effectively reprogram nuclei, increasing histone acetylation and H3 Lys 4 methylation. Cloning by nuclear transfer is obviously an extreme version of such a process. Collas et al. have provided abundant evidence that nuclear and cytoplasmic extracts can reprogram nuclei, for example from a fibroblast to a T lymphocyte, demonstrating the existence of factors that can reverse epigenetic modifications, if not yet identifying them (Collas 1998, 2003).

There is also some indirect evidence that reversal of epigenetic silencing occurs during normal development. During activation of T cells, the promoter of the IL-2 genes is actively demethylated within 7 h, yet 15 h after activation only 13% of cells have entered S-phase (Bruniquel and Schwartz 2003). In this case, DNA demethylation occurs in a gene-specific, tissue-specific manner, so

obviously there is nothing fundamentally irreversible about this epigenetic modification.

Recently, two mechanisms for the demethylation of histones have been discovered. In the first, the human peptidylarginine deiminase 4 (PAD4) converts methyl-arginine residues to citrulline (Cuthbert et al. 2004; Wang et al. 2004). It specifically converts histone H3 methyl-Arg 3 and methyl-Arg 17 to citrulline. Secondly, LSD1, a nuclear homolog of amine oxidases, functions as a histone demethylase and specifically demethylates histone H3 lysine (Shi et al. 2004). Although these modifications are associated with active transcription and not gene silencing, the discovery of mechanisms for active demethylation of histones is an important step.

What does this mean for plasticity? The hierarchical, unidirectional view of differentiation is overwhelmingly true during most of development. As such, these dominant pathways were the first and easiest to elucidate (Theise 2004). Less frequent pathways, however, were often obscured by relative insensitivity of experimental techniques. New approaches, revealing the unexpected plasticity phenomena reported in recent years, as well as nuclear transfer experiments (including the heterokaryon experiments of Blau and the now-documented fusion events for repair of visceral epithelia), demonstrate that other pathways exist. These are now gradually being unveiled by epigenetic researchers.

4
Stochasticity Versus Determinism in Cell Behavior

A long-standing debate has been whether cell behavior is determined or is stochastic, i.e., best described as a statistical process incorporating some degree of random behavior. The two possibilities can be distinguished experimentally, in a reductionist approach, looking at the behavior of individual cells, or with a systems approach, looking at behavior of cells in aggregate. The latter is most often done using computational techniques to generate computer models that, with more or less fidelity, give rise to virtual biologically relevant behaviors.

The balance of the debate has been manifested in studies of the hematopoietic system. The experimental ability to isolate single hematopoietic stem cells and grow them in colony-forming units has allowed for comparison of behaviors between individual cells. While some experiments of this type have indicated a deterministic behavior, the bulk of the data suggests variability of cell differentiation upon initiation of colony formation (Ogawa 1999). For example, diverse combinations of more differentiated cells are identified in individual colonies derived from single cells (Leary et al. 1984). Analysis of colonies derived from paired progenitors also reveals variability in modes of differentiation (Leary et al. 1984; Marley et al. 2003). This work is contrasted with other efforts indicating that inductive factors in the microenvironment

(e.g., cytokines, cell-matrix adhesion) lead to constraint of differentiative capacity (Metcalf 1998).

The key word in the paragraph above is "constraint." Does constraint imply determinism? Or is it possible to have intermediate degrees of constraint, yielding behaviors that lie intermediate between complete randomness and those that are rigidly determined? This is where the mathematical modelers have been making a large contribution. Models that incorporate some degree of constrained stochasticity faithfully reflect biological behaviors and generate testable hypotheses (Agur et al. 2002; Deenick et al. 2003; Furusawa and Kaneko 2001; Loeffler and Roeder 2002, 2004; O'Neill and Schaffer 2004; Roeder and Loeffler 2002; Roeder et al. 2003). Indeed, as is so often the case in scientific debates, with data mounting on either side of the question, we find ourselves, with time, coming to an acceptable middle ground. In this particular debate, constraint of stochasticity provides the middle ground between the seemingly opposed ideas of stochasticity and determinism. We will return to this concept of constrained randomness in Sect. 5 of this chapter dealing with Complexity Theory and the implications of a systems analysis for cell behavior, below.

Meanwhile, what are possible sources for stochastic behavior in differentiation of cells? Of course, for blood, the movement of cells through the vascular tree, subject to highly stochastic influences of fluid dynamics, brings them into different microenvironments and therefore exposes them to different inductive influences. Most tissues, however, are not fluid in this manner; the cellular microenvironment in intact. Noninjured adult tissues appears rather stable, at least at the supracellular level. But if we look into the biomolecular dynamics on the scale of the cell and its compartments, we find possible sources for stochasticity.

Work from Peter Quesenberry's laboratory demonstrates that a cell's differentiation capacity is tied to its temporal position in the cell cycle (Colvin et al. 2004). With ex vivo synchronization of cell cycle, isolated hematopoietic stem cells, transplanted at different points in their transit through the first cell cycle, display different reconstituting behaviors. This work details "differentiation hotspots" in the cell cycle at which one either finds long-term reconstitution, short-term reconstitution, or lineage restricted reconstitution (e.g., erythropoiesis, leukopoiesis, lymphopoiesis). It is hypothesized that chromatin remodeling as the cycle proceeds underlies changes in gene expression, reflected for example in changes in expression of adhesion molecules and cytokine receptors. The stochasticity of entry into cell cycle is therefore tied to stochasticity of gene expression and differentiation.

Detailed studies of dynamic changes of chromosomal structure indicate entry points for randomness into gene expression and control (Carmo-Fonseca 2002; Carmo-Fonseca et al. 2002). One example: fluorescent labeling of euchromatin in the interphase nucleus reveals movement that is best modeled as a "random walk" diffusion process (Vazquez et al. 2001). This implies that interactions of genes with the important regulatory proteins in the spatially

organized nucleosome, while tightly regulated in so many ways, also have an irreducible stochastic element. Thus, stochastic sequestration of genes within sites within the three-dimensional conformational structure of the interphase chromatin results in stochastic variation of gene expression (Vermaak and Wolffe 1998).

David Hume also locates stochasticity at the level of transcription initiation, redefining transcription as a digital rather than an analog process (Hume 2000). Each one of the multiple DNA templates for many genes is either "on" or "off," depending on whether the preinitiation complex of molecules is in place to lead to transcription. But the assembly of these complexes is experimentally demonstrated to be probabilistic. Hume generalizes to the concept that mRNA production is produced in "pulses," the mean frequency of which is determined by the probability of formation of the preinitiation complex. On this basis, he argues that "it is more meaningful to talk about the probability and frequency of transcription rather than the rate" and describes a "quantal" understanding of production of mRNA and, therefore, of gene expression.

5
Complexity Theory and Emergence of Cellular Phenomena

"Complexity theory" is not actually so complex. It describes the phenomena of interacting individuals which, when they fulfill certain criteria, self-organize into emergent structures. When such emergence arises, the system is found to be adaptive, i.e., it can react and change its organization or behavior, despite alterations in the environment, thus surviving as an entity (Theise 2004).

The most commonly used example is that of the ant colony. Ants have a limited number of ways in which they interact with each other: recognition and response to nine different pheromones and to direct contact. Out of those limited interactions, with no central planning, the highly detailed organizational social structure of the ant colony emerges. If one computer models the interactions of ants on the micro scale, similarly complex virtual ant colonies arise on the macro scale without the programmers having written computer code to directly create such structure. This is what is meant by "self organization."

Any system of interacting individuals, actual or virtual, that fulfills certain criteria gives rise to complex adaptive systems. These are:

1. Large numbers of individuals (though determining how many are necessary is not yet well understood).
2. Means of recognizing effects of other individuals within the system.
3. Homeostatic, negative feedback signaling between interacting individuals.
4. Low level stochasticity (too little and the system is rigid and nonadaptive, too much and the system devolves into figurative or literal chaos). This last criterion is referred to as "quenched disorder."

We have previously described cells and cell lineages as examples of complex adaptive systems and showed how they fulfill all the criteria (Hussain and Thiese 2004; Theise 2004; Theise and d'Inverno 2004). In particular, the constrained stochasticity discussed above supports this analysis. Thus, one can expect cells to give rise to emergent self-organization and, of course, they do: from the unfolding of the embryo and fetus, with formation of all tissues and organs necessary for development, and then the adaptive stability displayed throughout postnatal life (Furusawa and Kaneko 2000, 2002). Investigators have moreover begun computer modeling cell–cell interactions and find that they can demonstrate the emergence that is seen in life, gaining insight into physiological processes governing, for example, growth and maintenance of small intestinal crypt/villous lining cells and the fluctuations of clones in leukemias (Paulus et al. 1992; Potten and Loeffler 1990; Roeder et al. 2005). Significant discussion already surrounds conceptualization of immune system diversity and response as adaptive self-organization (Brusic and Petrovsky 2003). The current leading hypothesis regarding consciousness is that it is an emergent phenomenon on the macro scale arising from interactions of neuronal networks on the micro scale (Gell-Mann 2001).

This latter concept hints at an aspect of complex systems that we will now discuss in more detail. We have already stated, on the one hand, that cell–cell interactions give rise emergently to tissues and organs. This is, of course, true for the neuronal networks of the brain and the brain as a whole. Yet, for consciousness investigators, the neuronal networks are not considered the macro-level emergence, but rather, the micro-level interacting individuals. Thus, complex systems can exist as hierarchies. The aggregate self-organization of one system can play the role of an interacting agent in a higher level system, giving rise to higher level emergence. Thus, cells give rise to the emergent phenomena of living, moving people. But people, in turn, interact and give rise emergently to the organization of social structures, such as cities, cultures, and civilizations.

Thus, having turned our attention "upwards" from cells, we may also consider turning our attention in the other direction: cells might be emergent self-organization arising from interacting individuals on a smaller, "lower" scale. What would those interacting individuals be? Biomolecules, of course. And it is clear that biomolecules certainly fulfill most of the criteria for a complex system: they occur in enormous numbers, they interact with each other following defined molecular/chemical rules, and these interactions form homeostatic feedback loops. Do they display quenched disorder?

Recent investigations of individual biomolecular "machines" indicate, surprisingly, that the answer is "yes" (Yanagida and Ishii 2003). One example, of many, comes from the work of Toshio Yanagida, of Osaka University (Kitamura and Yanagida 2003). Observing the interactions of single actin and myosin strands, fluorescently labeled and held in place for observation with "laser tweezers," he has demonstrated that the ATP does not supply the en-

ergy for bending of the myosin elbow, resulting in movement, but, instead, the movement is random and constant, in response to Brownian motion of the surrounding fluid. The energy from the release of phosphate from ATP provides the energy to constrain this random movement in a directional, physiological fashion. Similar experiments are showing the same phenomena in other interacting elements of molecular motors such as EGF and EGF-receptor binding (Ichinose et al. 2004), kinesin movement along microtubules (Nishiyama et al. 2002), MMP-1 along collagen (Saffarian et al. 2004), and appears, increasingly, to be a generalizable phenomenon (Ait-Haddou and Herzog 2003).

Thus, there is quenched disorder in the way biomolecules interact and so they, too, fulfill all the criteria of a complex system. The emergent phenomenon is the cell. Our concrete understanding of the nature of the cell and how we explore its behavior is altered by this formulation (Kurakin 2005). On the one hand, cells are indeed "things", i.e., building block-like entities that are the fundamental, indivisible subunit of the body. But, also, on the other hand, they are not things, but ephemeral, ever-changing and adapting molecular organization in space and time. Much in the way that one may consider ant colonies, bee hives, or human cities to be things with their own character and structure, but also, alternatively, as organizations of smaller things. It all depends on the scale of observation and investigation.

This formulation keeps clear several features of cells that are often forgotten when investigators are locked into particular frames of reference and of scale. These features will be the subject of the next two sections of this chapter, as we see how genomic plasticity and the complex nature of cells has implications for the debates about stochasticity vs determinism in cell behavior, as well as the impact of observation on the nature of cells.

6
Cellular Uncertainty: Analogy or Metaphor?

We have previously described cell behavior and differentiation as displaying uncertainty (Theise 2002; Theise and Krause 2001, 2002), echoing earlier statements by Potten and Loeffler (Potten and Loeffler 1990), analogous to that of Werner Heisenberg's famous description of quantum physical processes. We initially based this idea on the truism that, as Richard Lewontin has written: "the inside and the outside codetermine the cell." Paying careful attention to this concept, one may infer that to observe or otherwise interact with a cell necessarily changes the microenvironment and therefore necessarily changes the differentiation state or capacity of that cell. From simple venopuncture to more extreme acts of tissue disaggregation and culture, no scientific experiment leaves a cell unchanged.

This includes some of the most fundamental, basic approaches to cell characterization employed by contemporary cell biologists. These include antibody

binding to cell surface molecules, which we often refer to as markers, as though they were merely name tags worn by the cell for our purposes. The activities of some markers, such as CD5 and CD45, have been extensively studied (Lozano et al. 2000; Sasaki et al. 2001). It is clear that while some binding of ligand to these receptors can activate some cell processes, other forms of binding will produce alternate effects. So, before isolation with an anti-marker antibody can be assumed to be merely an isolation process, lacking influence on subsequent differentiation events, the relative inertness of the antibody binding needs to be established. If it has not been established, then the interpretation of such data must take into consideration that possibility. However, most markers are not so well characterized and most do not have such a wide array of specific antibodies available for detection. A prime example of this is CD34: it still remains unclear what this molecule actually does (Krause et al. 1996); thus we have no way to determine what the sequelae of the use of detecting antibodies might actually be.

However, whether our use of Heisenbergian uncertainty is simply a useful metaphor or is a precise analogy remains largely unconsidered. The difference lies in whether the ability to truly determine with certainty what a cell is and will do is an artifact of our current technological limitations or whether it is a fundamental aspect of the cell. As with Heisenberg's initial pronouncement, the question becomes: is it possible to create a perfect machine which would eliminate uncertainty? In the case of physics, the answer was no, uncertainty was not artifact, but a fundamental aspect of the nature of the universe. But could we perhaps develop a perfect MRI machine, for example, by which a cell could be completely characterized, in situ, and yet remain unchanged?

A potential answer to this lies in our analysis of cells as emergent phenomena arising from complex interactions of biomolecules. That analysis mandates the dual consideration, depending on the level of scale of observation, that cells exist both as defined entities (the cell and tissue level perspective) or not as defined entities (from the biomolecular perspective). Thus, as stated above, there is no "thing-ness" to a seeming object that is the emergent self-organization from lower-scale elements. They have no independent, stable existence and thus cannot be pinned down with certainty in all of their particulars at any given moment. There can be no perfect machine to accomplish the task. Thus, Cellular Uncertainty is not mere artifact of technological limitations, but is a fundamental aspect of cell nature. On some level, the cell and, therefore, the body itself, are incompletely knowable and must remain so.

7
Postmodern Biology

The implications of these ideas for those interested in biology from a theoretical or from a pragmatic, biomedical point of view, are profound. From hypothesis

formation to design of cell biologic experiments to interpretation of data, little remains unchanged. That any isolated cell population can ever be described as truly homogeneous vs heterogeneous falls by the wayside. Variations within an isolated population are often dismissed or criticized as contamination, but while contaminants need to be guarded against, there will always be a degree of inhomogeneity reflective of uncertainty and stochasticity. The rigid use of experimental data, obtained from reductionist approaches, to describe what is happening within the body is a false approach. There is no cell in the body that acts in isolation from the system; thus, cell behavior deduced from analysis of the cell in isolation is only partially informative. Biological processes arise from simultaneous interactions of all elements of the system rather than in the linear mode inherent in most twentieth century experimental design.

Practically speaking, for those interested in biomedical applications, these perhaps discouraging limitations actually open up a broad range of new possibilities. That cells with the entire genome intact can experimentally become any other cell type opens up an astonishing array of possibilities for cell-based therapies (Theise 2003). Whether cells are embryonic, fetal, or postnatal does not matter as much as how clever we are in figuring out how to manipulate them for desired ends. Also, getting at those other parentheses in our title, the issue is not really about adult stem cells except in so far as these concepts are applicable to all cells, not just those in postnatal life and not only those that display stem- ell functioning.

The engineering approach to tissue engineering appears flawed. In treating tissues as an engineering problem about the arrangement of cellular building blocks (literally), it misses the possible opportunities for cells to self-organize into useful tissues, ex vivo (Hussain and Thiese 2004). Putting them where we want them, on carefully constructed scaffolds, may not be as interesting as aggregating them in different conditions, quantities, etc., and seeing what they create on their own.

Finally, recognition that cells are merely emergent phenomena breaks the lock of traditional cell doctrine on ways to analyze and describe the body. Testable and reproducible bodily effects that have no anatomical correlate cannot be explained simply on the basis of cells, per se, and require alternate models for explanation. An example of this is the use of acupuncture to influence physiologic processes (Ma 2004). The organ-related meridians shown to be of testable import for placement of needles and success of therapy do not correspond to any structure identifiably made of cells. In the absence of such a correlate, limiting ourselves to cell doctrine limits us in our ability to understand acupuncture and more thoroughly investigate it. Again, depending on the scale of observation, the nature of the body changes. From the molecular point of view, with interacting biomolecular agents in fluid states, the body might just as readily be conceived as a fluid syncytium, cell walls simply representing semi-permeable partitioning of the fluid compartment. A complexity approach reveals that alternate models may be as valid as standard ones.

Truly, plasticity, stochasticity, uncertainty, and complexity bring biology (at last?) into the postmodern age, appropriate for a new millennium. How quickly we can manifest the unlocked potential of our bodies will depend on how quickly we can unlock the fetters of dogma. We can be assured, however, that as with everything in the postmodern era, change occurs with increasing speed. We will not have to wait as long as we waited from the shift from Newtonian mechanics to relativity (centuries), or even from relativity to string theory (decades). The shifts are happening. We must simply let our minds keep pace with them.

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