Leukemia (2002) 16, 542–548
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REVIEW

Toward a new paradigm of cell plasticity

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SPOTLIGHT

The standard paradigm of embryologic development and adult tissue reconstitution posits unidirectional, hierarchical lineages. The presumed mechanisms underlying these differentiative pathways are gene restrictions, such as methylation and heterochromatin formation, which are commonly described as irreversible. However, recent discoveries regarding multi-organ stem cells demonstrate that 'true plasticity' exists, with cells of one organ turning into cells of other organs, including differentiative transformations that cross barriers between tissues derived from different primitive germ layers. These findings, along with earlier experiments into heterokaryon formation and longstanding recognition of reactive and neoplastic lesions in humans and animals, suggest that lineage pathways are not, in fact, unidirectional. Moreover, physiologic mechanisms of reversal of gene restrictions have been recognized. Therefore, in response to these observations, we suggest a new paradigm of cell plasticity, elucidating three guiding principles of 'genomic completeness', 'uncertainty of cell characterization', and 'stochastic nature of cell origins and fates'. These principles imply a change in the way data can be interpreted and could alter subsequent hypothesis formation. This new paradigm will hopefully lead us forward to a more flexible and creative exploration of the potential of adult vertebrate cells.
Leukemia (2002) **16,** 542–548. DOI: 10.1038/sj/leu/2402445
Keywords: plasticity; stem cells; differentiation

Introduction

Over generations, progressively more precise and detailed observations of cells and tissues have led to our current concepts of cell division, differentiation, and the growth of organisms. These concepts have become the doctrine by which we understand the body, construct hypotheses for further investigation, and plan therapeutic interventions for disease. However, recent discoveries of cellular events heretofore believed unlikely or impossible require alterations in those firmly established principles. This review will discuss some of the core principles regarding plasticity of cell differentiative potential and suggest revisions to accommodate the newest relevant findings.

Standard paradigm of cell differentiative potential

Cell differentiation describes the development of new morphologic and functional characteristics. Differentiation occurs in mammals as part of embryologic development and continues into adulthood as normal cell turnover or as repair following injury. These processes are considered to be orderly and directional.

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Received 3 July 2001; accepted 26 October 2001

The stepwise progression of embryologic development has been elucidated in a number of ways. Early on, simple dissection and observation of embryonic and fetal tissues identified specific anatomic regions of the embryo that gave rise to specific organs at later stages. Three layers of cells were identified in the embryo, from which mature adult organs arise: endoderm, mesoderm and ectoderm. Through complex interactions between adjacent cells, ectoderm gives rise to the nervous system and skin; mesoderm to organs and tissues of the integument, skeletal muscle compartments, and kidney; and endoderm to most of the visceral organs.

The ability to stain or label early subpopulations of the embryo and to follow them through subsequent developmental events led to the concept that cells, at certain points of development, become increasingly restricted in the types of tissue to which they can give rise. Experiments involving transplantation of cells from one location to another in embryos have shown that at certain stages of development some cells become 'committed', maintaining their original differentiation pathway even in a changed microenvironment. Such observations led to the concept of 'cell lineages', in which cellular differentiation in normal tissue growth and turnover proceeds in an orderly and directed fashion, with incrementally augmented commitment and restriction. These studies eventually gave rise to the concepts of 'stem cells' and 'progenitor cells'. In particular, stem cells are capable both of self-renewal and of division and differentiation into progenitor cells, which are more restricted in their lineage potential. The stem cell is thus a self-renewing reservoir of new stem cells, of progenitor cells, and of mature cells.

Postulated mechanisms of lineage restriction

As cells differentiate, there is an orchestrated silencing of some genes and activation of others. Several mechanisms to accomplish this gene activation have been hypothesized, some of which have been confirmed. However, the orchestration of these events is not yet well understood. Experimental data suggest that pluripotent stem/progenitor cells are 'primed' to differentiate down several different lineages by low level transcription of genes that are characteristic of multiple lineages.[1,2] For example, using RT-PCR it has been demonstrated that both erythroid and myeloid gene expression programs can be initiated by the same progenitor cell prior to exclusive commitment toward the myeloid or erythroid lineage.[1] A corollary to this model of lineage specification in which unilineage commitment is 'prefaced' by a promiscuous phase of multilineage gene expression, is that once lineage commitment has been achieved, the genes expressed in the lineage pathways not taken are permanently silenced.

Silencing is thought to be irreversible due to formation of heterochromatin that is accompanied by, and in part depen-

dent upon, histone deacetylation, DNA methylation, and matrix attachment regions.[3–7] Heterochromatin is that DNA which remains condensed throughout the cell cycle. Heterochromatin formation, characterized by protein-rich highly condensed chromosomal regions in DNA, can lead to genetic silencing in all eukaryotes.[8] In general, hypermethylation of cytosine residues lying just 5′ of guanine residues (CpGs) is associated with gene silencing and hypomethylation of these sites is associated with gene expression.[8] This is not true for all genes; of the actively expressed genes in a eukaryotic cell, 50–60% have unmethylated CpG islands directly upstream.[9] The methylation that occurs during gene activation is regulated in part by nuclear proteins that bind to matrix attachment regions (MARs). MARs fold the chromatin into topologically independent loop domains by mediating points of attachment of the genomic DNA to the nuclear matrix.[7]

Another mechanism by which cells regulate chromatin structure is post-translational modification of histones by acetylation. Deacetylation of histones is associated with dense heterochromatin formation, and histone acetylation, which causes loss of histone binding, is associated with gene activation.[5] Within cells, the activities of histone acetylases and histone deacetylases are modulated in a gene-specific manner.[10]

Thus the current paradigm may be summed up as follows: in the developing embryo and in normal maintenance and repair of adult tissues, cells arise and differentiate in a unidirectional fashion. A selected population of stem or progenitor cells has a limited range of possible fates. Conversely, a given population of differentiated cells has a limited number of possible progenitors (Figure 1, Table 1).

Table 1 Current and new principles of cell differentiative potential

Current paradigm
I. Lineage pathways are unidirectional and narrowly restricted due to irreversible inactivation of genes that are required for alternate pathway selection.
II. Experimental characterization and manipulation of cell differentiation closely reflect physiologic lineage pathways.
III. There is differentiative restriction of both progenitors and progeny of any specific cell.

New paradigm
I. Genomic completeness: any cell which contains the entire genome, without transpositions, multiplications or deletions, has the potential to display features of any cell type of the organism from which it was derived.
II. Uncertainty of cell characterization: any attempt to observe a cell alters the state of that cell at the time of characterization and potentially alters the likelihood of subsequent differentiation events.
III. Stochastic nature of differentiation and lineage development: description of the possible progenitors or progeny of a cell must include the conditions of observation and manipulation in the system being observed and must be expressed in a stochastic, ie probability-based manner.

Challenges for the current paradigm

Observations of pathological changes that can occur in tissues have long indicated that some of the normal developmental lineage progressions may not be rigidly maintained. Two classes of lineage ‘jumping’ are routinely recognized in human tissues obtained for pathologic analysis. The first is usually conceived of as an adaptive process, known as ‘metaplasia’. The second is ‘dysplasia’, which usually implies emergence of a pre-malignant or malignant neoplastic clone.

Metaplasia is classically considered to be a reversible change in which adult cells of one type become adult cells of another type, although the change is restricted to cell types thought to derive from the same germ cell layer. Thus, epithelia of one type become epithelia of another type, or mesenchymal cells of one type become mesenchymal cells of another type. These changes often appear adaptive; the new cell type being better suited to an altered environment or chronic injury. For example, intestinal metaplasia of the usual esophageal squamous epithelium in reflux esophagitis is better able to withstand the chronic toxic insult created by the reflux (Figure 2). Less clearly adaptive is the change of skeletal muscle fibers into cartilage or bone in response to severe injury, known as osseous metaplasia.

In dysplasia, malignant cells display features of multiple lineages, which have been thought to be irreversibly denied to

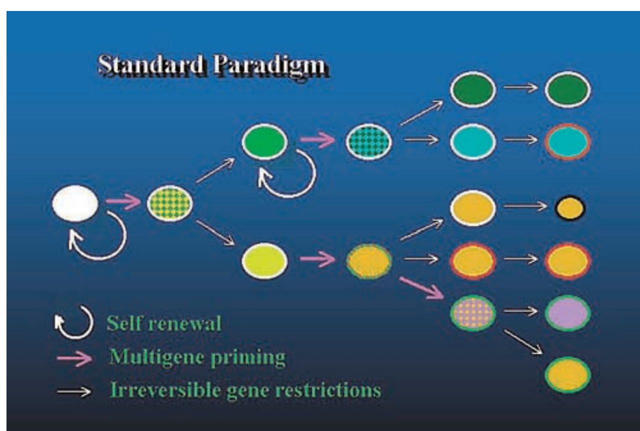

Figure 1 In the standard paradigm, cell lineages arise from self-renewing stem cells, which give rise to committed progenitor cells, which then give rise to more differentiated cells with an ever more restricted range of plasticity. Lineages have been thought to be hierarchical and unidirectional, based on the concept that many gene restrictive events are irreversible.

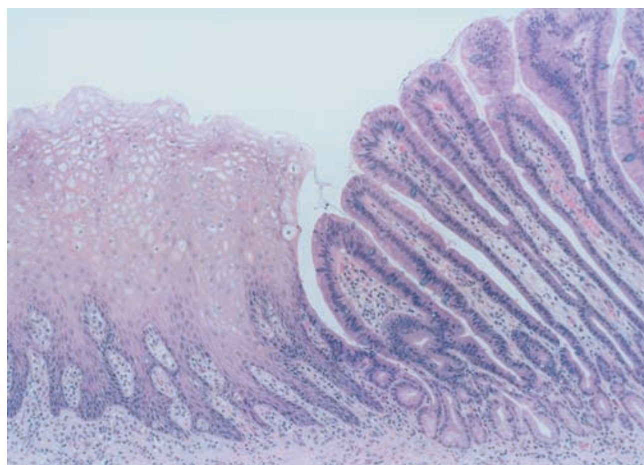

Figure 2 Barrett’s metaplasia of the esophagus. Normal squamous epithelium of the esophagus (left) gives way to intestinal type epithelium (right) in the setting of on-going reflux esophagitis. (H&E, ×40).

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the mature cells from which the neoplasm appears to derive. These exceptions to the developmental hierarchy and unidirectionality have been reconciled by postulating that the increasing number of genetic mutations required for neoplastic transformation 'unlocks' previously restricted genes. Such changes have been variously called 'de-', 're-' or 'transdifferentiation'.[11] Alternatively it has been postulated that the malignant clones develop from a multipotent precursor cell with an altered differentiation pattern.

Thus, both metaplasia and dysplasia can give rise to apparent breaches of lineage restriction, indicative of a change in gene expression patterns. These exceptions to lineage restricted pathways have been tolerated from a theoretical perspective, and not viewed as paradigm altering, for at least two reasons. The first non-scientific reason is that there is increasing 'lineage restriction' between specialists. Recent generations of anatomic pathologists and cell biologists are often located in separate realms of academic centers and do not always have the opportunity to interact scientifically. Anatomic pathologists observing these pathologic changes view them as almost mundane, and are generally not the same scholars who are actively engaged in the elucidation of cell differentiation mechanisms. Likewise, basic scientists are most familiar with experimental models that are most often selected for their specificity rather than for their exceptional nature.

The second reason is that metaplasia and dysplasia are states of chronic disease or injury that affect the cell and its genomes. Thus, exceptions to gene restriction can be attributed to abnormalities of the microenvironment. This presents a theoretical dilemma. If these cells with altered differentiation arise from a mature 'terminally' differentiated cell, then altered differentiation would require release of gene-restricting biomolecular events, such as demethylation and opening of heterochromatin.

Alternatively, the paradigm can be preserved completely intact by hypothesizing that metaplastic or neoplastic changes occur in ever more primitive facultative stem or progenitor cells. So, for example, squamous metaplasia of bronchial epithelium would represent a new squamous line of differentiation from a progenitor cell that has preserved that potential in a latent form. Similarly, dual lineage expression in a tumor such as in carcinosarcomas (containing epithelial, ie endodermal, and stromal, ie mesodermal, elements, Figure 3) would be a result of malignant transformation of a bipotent stem or progenitor cell rather than reactivation of previously silenced genes.[12]

The shifting paradigm

Beyond these routinely observed pathologic entities, an increasingly rapid-fire sequence of papers has challenged the most fundamental lineage restrictions in the course of restitution of entirely normal tissues. Pereira *et al*[13] demonstrated that bone marrow-derived stromal cells, after enrichment, passage through culture and injection into recipient animals, can produce stromal cells of bone, cartilage and lung. From a theoretical point of view, these findings are not necessarily surprising because the 'stromal' cell phenotype was maintained. It was the multiorgan, rather than marrow-specific engraftment that was unexpected, since the paradigm preserving, pluripotent stem cells have implicitly been presumed to derive from within the affected organ.

This report was followed by that of Ferrari *et al*,[14] demonstrating that bone marrow cells can differentiate into mature

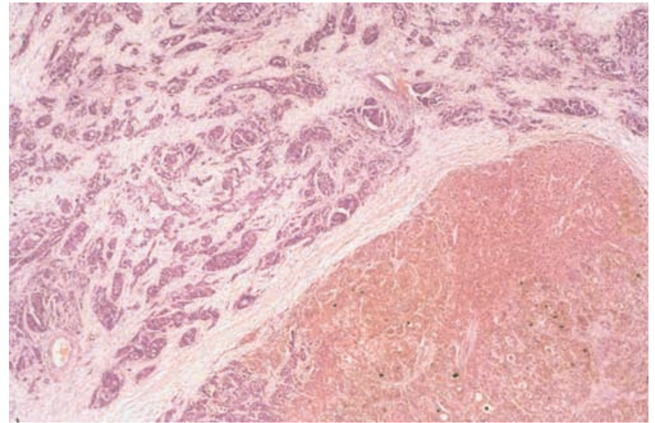

Figure 3 Mixed hepatocellular cholangiocarcinoma. A solitary 3 cm tumor from a patient transplanted hepatitis C cirrhosis displays two widely divergent phenotypes: cholangiocarcinoma (upper left) and bile producing hepatocellular carcinoma (lower right). In the absence of any other neoplastic lesions in this thoroughly examined explant specimen, the tumor represents bidirectional differentiation from a single malignant clone. (H&E, ×4).

skeletal muscle fibers *in vivo*. Although these data demonstrate mesodermal-to-mesodermal differentiation pathways, these pathways had not been suspected to exist and crossed organ boundaries previously thought to be inviolate. Next, reports of surprising cell plasticity were published involving neural stem cells serving as a source of hematopoietic cells,[15] bone marrow cells engrafting as various types of neural cells,[16–18] and bone marrow cells engrafting as liver cells.[19–23]

These experiments in rodents and, in the case of the bone marrow-to-liver engraftment, in humans, showed cells leaping over the barriers between mesoderm, ectoderm and endoderm. Moreover, our own work in collaboration with the laboratory of Saul Sharkis has demonstrated that a single, bone marrow-homing cell, capable of long-term hematopoietic engraftment, also contributes to cells of endodermal and ectodermal lineages: epithelia of liver, lung, skin, esophagus, stomach, small intestine, and large intestine.[24] Workshops, meetings and colloquia have subsequently proliferated in order for investigators to discuss and debate these surprising phenomena. While some may still doubt these studies, many more researchers have come to accept the largely reproducible findings.

The emblematic approach to preserving the current cell differentiative paradigm while reconciling these data is to postulate the presence of still more primitive stem cell populations which now, additionally, must have the ability to circulate from organ to organ. Moreover, in light of recent data of single cell transplantation of bone marrow cells giving rise to diverse organs of all three embryonic layers, one must posit a nearly totipotent stem cell residing permanently in the bone marrow. It remains to be shown whether this adult cell has the same differentiation potential of an embryonic stem cell.

Such highly plastic cells in adults are required conceptually in order to maintain the unidirectional lineage restriction paradigm. However, this approach is perhaps too restrictive for interpretation of new data and formation of new hypotheses. It may also be unnecessary: the molecular modifications leading to gene restriction (eg DNA methylation, attachment to the nuclear matrix, and histone acetylation) can be reversed experimentally and this reversal also occurs in nature.

Methylated regions of the DNA are demethylated during

embryogenesis[25] and also in adult tissues.[26] Although no specific demethylase enzyme has yet been isolated, demethylation is known to occur in two different ways. Passive demethylation occurs when the newly synthesized DNA is not remethylated during DNA replication. In contrast, 'active' demethylation occurs when a methylated cytosine residue is replaced by a nonmethylated one. Demethylation is a highly controlled event during cell differentiation and active demethylase activity has been identified in the nuclear extracts of cells in the early embryo and cells undergoing myogenesis. For example, during skeletal myogenesis, demethylation activates the 258 bp distal enhancer of the myoD gene in an active, regulated process.[27] *In vitro*, gene activation secondary to demethylation occurs in the presence of the drug 5-azacytidine.[28,29] An example of demethylation in response to regulated activity of MAR binding proteins occurs during T lymphocyte maturation. SATB1 appears to orchestrate the temporal and spatial expression of several genes during T cell development.[30]

For regulation of histone acetylation, many different transcription factors have been identified that form a complex with the acetylases or deacetylases to regulate their function.[31] Research involving heterokaryons, non-dividing fusion products of vertebrate cells of different strains, has shown that theoretically 'terminal' differentiation states can be modified by changing the cytoplasmic environment of the determined nucleus.[32–34] In fact, even X chromosome inactivation, perhaps the ultimate gene restriction, has been reversed experimentally in cloned female animals obtained from a single adult somatic cell.[35]

In summary, two independent series of discoveries impact on the working paradigm describing cell plasticity and lineage pathways. The first, and most recent, is based on demonstration that most of the commonly demonstrated hierarchical and unidirectional lineage systems represent only some of the lineage pathways possible for embryonic and adult cells. Other pathways exist which may reverse or jump between these lineages. These others can be identified experimentally and at least some also participate in physiological processes. The second series of discoveries concerns the mechanistic underpinning of these flexible differentiation pathways: the molecular processes that can reverse gene restrictions.

Reversal of gene restrictions, then, which are not controversial, have been under study for years in contrast to the recent plasticity discoveries. However, they have not led us to change the paradigms regarding cell differentiation pathways. Linking these two fields can perhaps lead us forward to a more flexible and creative exploration of the potential of adult vertebrate cells. A new paradigm – or perhaps more precisely – a modified paradigm is necessary.

Expanded paradigm of cell differentiation

We have proposed three new principles to guide our understanding of vertebrate cell differentiation and lineage progression (Table 1).[36]

First principle: genomic completeness

Lacking mechanisms of irreversible gene restriction by any portion of the genome, any cell containing the entire genome, without transpositions, multiplications or deletions, has the potential to display features of any cell type of the organism

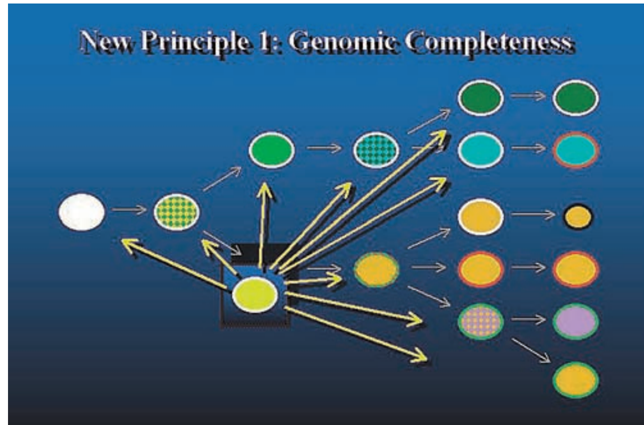

Figure 4 In the absence of irreversible gene restrictions, a cell with the entire genome intact can potentially become any cell type. The *in vivo* physiologic range may be more restrictive than this, given that cells will find themselves in a limited range of microenvironments. However, when a cell is lifted out of its normal environment and manipulated in culture, as diagrammed, there may be no inherent limits on what phenotypes may be displayed by the cell and its progeny.

from which it was derived. Some of these transformations may be of physiologic importance while others, arguably the greater proportion, may not. However, in *ex vivo* or *in vivo* experimental settings, cells of any differentiation type with completely intact genomes can conceivably be coaxed to become any other cell type by appropriate manipulations of the cell or its environment.[32–34,37,38] Such manipulations may include, but are not limited to mechanical, chemical or electrical procedures, exposure to biological modifiers (eg cytokines, chemokines, hormones, etc.), or exposure to a different environment via transplantation of cells from one location to another *in vivo* or *in vitro*. (Figure 4).

Second principle: uncertainty of cell characterization

To borrow a theoretical construct from one branch of scientific investigation (in this case physics) and apply it to another (ie cell biology), must be done with great caution. However, one can make an analogy to Heisenberg's uncertainty principle from quantum mechanics that dictates that one can never know with complete certainty both the location and momentum of an observed particle. Observations to determine one aspect necessarily alter the other and, moreover, the more completely one can characterize one aspect of the observed particle the more incomplete will be knowledge of the other. Similar conceptualization can be (and in many cases has already been) applied to cell differentiation states during lineage development.

Cell differentiation is recognized as the combination of traits reflecting specific patterns of gene expression, modulated by interactions between nuclear and cytoplasmic elements. Cells are exquisitely dynamic, reactive systems and the microenvironment is a key determinant of cell differentiation. To borrow a phrase from population geneticist/critic Richard Lewontin, 'the internal and the external codetermine' the cell.[39]

Any attempt to fully characterize a cell, beginning with the simplest isolation procedure, necessarily alters the environment of that cell. Therefore, any such attempt will potentially

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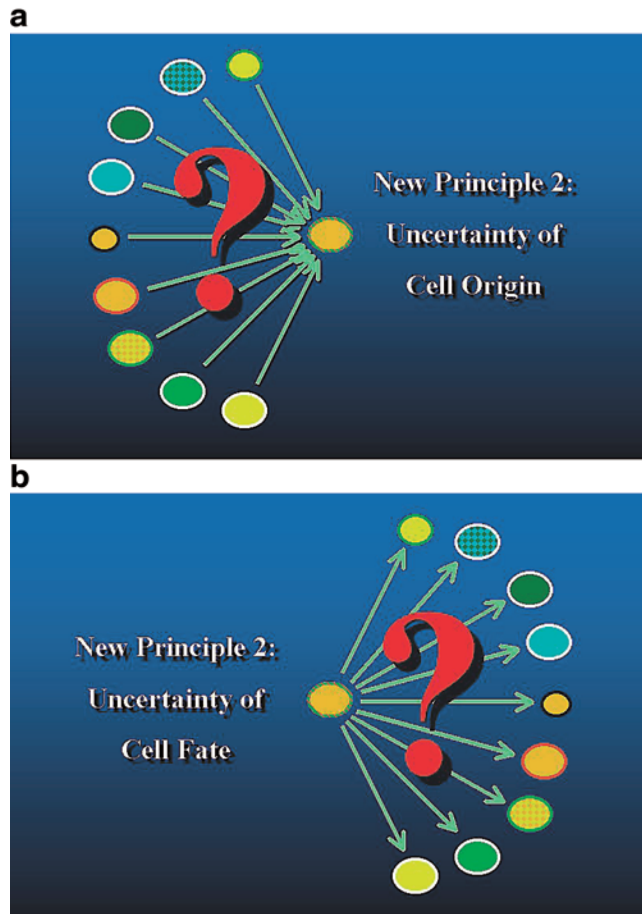

Figure 5 (a) Uncertainty of cell origin. With multiple possible precursors of a given cell, complete characterization of the origin of that cell is not possible. (b) Uncertainty of cell fate. With multiple possible progeny of a given cell, complete characterization of the differentiation fate of that cell is not possible. Moreover, any attempt to observe a cell alters the state of that cell at the time of characterization and potentially alters the likelihood of subsequent differentiation events.

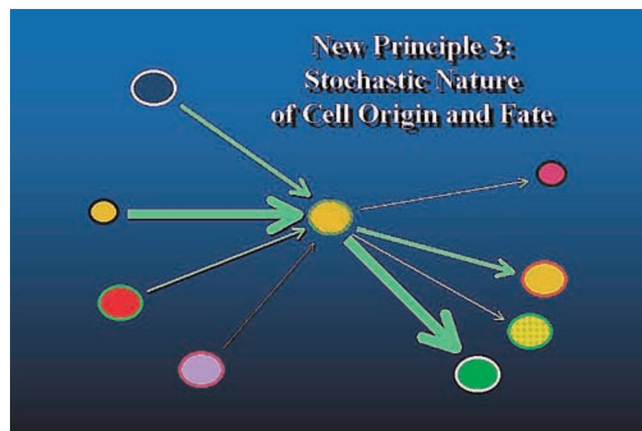

Figure 6 In light of the uncertainty of origin or differentiation fate of a given cell, all descriptions of cell lineages must be expressed in a stochastic, ie probability-based, manner. The probabilities depend on the status of the microenvironment, whether it is normal or diseased, *in vivo*, or how it is manipulated *in vitro*.

alter the gene expression profile of that cell in subtle or substantial ways, potentially changing its differentiation status and/or its lineage fate. Venopuncture to isolate circulating blood cells, for example, subjects cells to the least disturbance as they are already suspended in a fluid environment. Yet, even with this minimal disturbance, these cells are subject to unusually severe turbulence and exposure to a metal surface, both of which conditions are profound changes in microenvironment. More severe manipulations such as tissue resection and disaggregation can profoundly influence patterns of gene expression because they break cell–cell interactions and remove the cells from their normal microenvironments. Thus, any attempt to observe a cell alters the state of that cell at the time of characterization and potentially alters the likelihood of subsequent differentiation events (Figure 5).

Third principle: stochastic nature of differentiation and lineage development

Definitions of lineage systems are bi-directional, ie scientists attempt to identify the phenotypes of the precursor cells that give rise to a specific cell subpopulation and, similarly, which cell types can arise from a given cell. Since, according to the first principle of 'genomic completeness', any cell may have the capacity to give rise to any other cell, it becomes clear that there are multiple, perhaps unlimited possible precursor cell phenotypes of any given cell. Thus, description of the possible progenitors of a cell must include the conditions of observation and manipulation in the system being observed and must be expressed in a stochastic, ie probability-based manner. Likewise, since, according to the second principle of 'uncertainty', every observation may alter the differentiation capacity of a cell at the time of observation and therefore its subsequent fate, precise description of subsequent lineage capabilities of a cell must also include the conditions of observation and manipulation and must be expressed in a stochastic manner (Figure 6).

Consider hepatocyte regeneration: some adult hepatocytes will have derived from other hepatocytes,[40] some will have derived from bipotent intraorgan stem cells in the canals of Hering,[41] and others will have derived from circulating, bone marrow-derived cells.[19–21] The relative proportion of hepatocytes deriving from any one of these sources will depend on whether the tissue has been exposed to stressful conditions causing intrahepatic oval cell damage or death. Similarly, if a population of cells is isolated and induced to produce a second population of cells experimentally, the results must be described and interpreted in a probabilistic fashion and will vary depending on the experimental system of manipulation used, which must be included in any description of results. No isolated cell population is ever wholly uniform, thus results will always be variable and require stochastic expression. Attempts to limit this uncertainty of outcome by using only one cell, does not evade the problem: the single cell was inevitably selected from a population of cells that is incompletely characterized. Thus, variability of outcome is unavoidable and outcomes must be stochastically described.

Implications of the expanded paradigm

These principles imply a change in the way data can be interpreted and could alter subsequent hypotheses developed based on these data. Central to the change is acknowl-

edgement that cells are dynamic entities that can respond dramatically to environmental change or manipulation. Terms such as 'stem cell', 'epithelial cell', 'stromal cell' and 'mesodermal cell' represent categories, not a cell's inherent, fixed nature. We must avoid using these terms to imply limitations on the cell. They merely represent functional or morphological states of cells at the time of observation.

There is much debate regarding the questions of cell origin and of the specific phenotype of stem and/or progenitor cells. These difficulties arise when seemingly contradictory data support different conclusions. By broadening the paradigm, these difficulties can be alleviated. For example, several research groups have demonstrated that $CD34^+$ lin^- cells, $thy1^+$ cells, $C\text{-}kit^+$ $Sca1^+$ lin^- cells and mesenchymal stromal cells can all give rise to hepatocyte and/or cholangiocyte lineages. One might explain such variable results on the basis of contaminants or incomplete marker studies. However, such results may simply reflect different examples of what might take place in physiological circumstances and can take place experimentally.

Similarly, the traditional approach to culture, which has largely aimed at maintaining cell characteristics by mimicking features of the original microenvironment, not surprisingly leads to a self-reinforcing dogma of cell rigidity. But exposing cells to radically different microenvironments may yield surprising plasticity. There may be no limits beyond our own patience and ingenuity for transforming one cell type into another, assuming it has all the genetic information required for the change.

Some of the terminology used to describe stem and progenitor cell differentiation can now be seen to be relatively narrow or imprecise. For example, 'de-differentiation' implies that differentiation is unidirectional and linear, while it may simply represent different patterns of gene expression. 'Stem cells' are often thought of or described as being undifferentiated or 'primitive', which is an imprecise characterization. There are many types of stem cells including hematopoietic stem cells, hepatic stem cells, embryonic stem cells, etc. Each of these cells has the ability to self-renew as well as to differentiate down specific lineages. Thus, the term stem cell should be used to refer to a specific self-renewing cell and the type of stem cell must be defined based on the context in which it is used.

Conclusion

These new principles may prove to be either disheartening or encouraging to researchers. On the one hand, the recognition of the inherent uncertainty of cell characterization can be frustrating because the absolute fate of a given cell can only be known for certain within specific contexts. On the other hand, acceptance of the need to work with probability-based processes and the unlimited potential of the genome to reconfigure and express alternate phenotypes means that innovative methods for manipulation of cell differentiation are astonishingly open ended.

Ultimately, the paradigm shift we are suggesting is an expansion of existing beliefs and doctrines. It seems that our understanding of cell differentiative capacity and of lineage pathways has been largely determined by the nature of questions asked and experiments performed. Regarding the recent cell plasticity discoveries, an oft-asked question has been: why have these processes not been discovered before? The answer may, in part lie with availability of research techniques to discover them. But the answer also relates to the simple fact that no one had looked before.

As Nobel Laureate Barbara McClintock stated:[42] 'There's no such thing as a central dogma into which everything will fit . . . any mechanism you can think of you will find – even if it is the most bizarre kind of thinking. Anything . . . So if the material tells you, "It may be this", allow that. Don't turn it aside and call it an exception, an aberration, a contaminant . . . That's what's happened all the way along with so many good clues.' As was true in her field of genetics, so it is true in this rapidly changing field. It seems that wherever people have looked, they have found evidence of what their creative imaginations have envisioned. Thus we offer this new paradigm as a set of guidelines for further investigations, hopefully to open new possibilities beyond the restrictions created by dogma.

Acknowledgements

This material was first presented at a 'Focused Workshop on Stem Cell Plasticity', held in Santa Barbara CA, May 3–6, 2001, sponsored by The Leukemia and Lymphoma Society (White Plains, NY) with the help of the Great Basin Medical Research Foundation (Reno, NV). The proceedings of that meeting were published in Blood, Cells, Molecules, and Diseases, May–June 2001. This article represents the expanded form of that original presentation. The authors would like to thank Drs Rebecca Wells, Paula Vertino, Cindy Loomis, A Brian West, Rachel Brody and Vittorio Defendi for their helpful contributions, including thoughtful discussions and reviews of the manuscript. We are grateful for their receptivity to new ideas, patient teaching, and creative imaginations.

References

1 Hu M, Krause D, Greaves M, Sharkis S, Dexter M, Heyworth C, Enver T. Multilineage gene expression precedes commitment in the hemopoietic system. *Genes Dev* 1997; **11**: 774–785.
2 Billia F, Barbara M, McEwen J, Trevisan M, Iscove NN. Resolution of pluripotential intermediates in murine hematopoietic differentiation by global complementary DNA amplification from single cells: confirmation of assignments by expression profiling of cytokine receptor transcripts. *Blood* 2001; **97**: 2257–2268.
3 Tsukiyama T, Wu C. Chromatin remodeling and transcription. *Curr Opin Genet Dev* 1997; **7**: 182–191.
4 Walia H, Chen HY, Sun JM, Holth LT, Davie JR. Histone acetylation is required to maintain the unfolded nucleosome structure associated with transcribing DNA. *J Biol Chem* 1998; **273**: 14516–14522.
5 Cheung WL, Briggs SD, Allis CD. Acetylation and chromosomal functions. *Curr Opin Cell Biol* 2000; **12**: 326–333.
6 Ivanchenko M, Avramova Z. Interaction of MAR – sequences with nuclear matrix proteins. *J Cell Biochem* 1992; **50**: 190–200.
7 Cockerill PN, Garrard WT. Chromosomal loop anchorage sites appear to be evolutionarily conserved. *FEBS Lett* 1986; **204**: 5–7.
8 Klein CB, Costa M. DNA methylation, heterochromatin and epigenetic carcinogens. *Mutat Res* 1997; **386**: 163–180.
9 Cross SH, Bird AP. CpG islands and genes. *Curr Opin Genet Dev* 1995; **5**: 309–314.
10 Kouzarides T. Acetylation: a regulatory modification to rival phosphorylation? *EMBO J* 2000; **19**: 1176–1179.
11 Pitot HC. *Convergence Hypothesis of Greenstein: Fundamentals of Oncology*. Marcel Decker: New York 1986, pp 326–328.
12 Thompson L, Chang B, Barsky SH. Monoclonal origins of malignant mixed tumors (carcinosarcomas). Evidence for a divergent histogenesis. *Am J Surg Pathol* 1996; **20**: 277–285.
13 Pereira R, Halford K, O'Hara M, Leeper D, Sokolov B, Pollard M,

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Bagasra O, Prockop D. Cultured adherent cells from marrow can serve as long-lasting precursor cells for bone, cartilage, and lung in irradiated mice. *Proc Natl Acad Sci USA* 1995; **92**: 4857–4861.
14 Ferrari G, Cusella-DeAngelis G, Coletta M, Paolucci E, Stornaiuolo A, Cossu G, Mavilio F. Muscle regeneration by bone marrow-derived myogenic progenitors. *Science* 1998; **279**: 1528–1530.
15 Bjornson CR, Rietze RL, Reynolds BA, Magli MC, Vescovi AL. Turning brain into blood: a hematopoietic fate adopted by adult neural stem cells *in vivo* (see comments). *Science* 1999; **283**: 534–537.
16 Eglitis MA, Mezey E. Hematopoietic cells differentiate into both microglia and macroglia in the brains of adult mice. *Proc Natl Acad Sci USA* 1997; **94**: 4080–4085.
17 Brazelton TR, Rossi FM, Keshet GI, Blau HM. From marrow to brain: expression of neuronal phenotypes in adult mice. *Science* 2000; **290**: 1775–1779.
18 Mezey E, Chandross KJ, Harta G, Maki RA, McKercher SR. Turning blood into brain: cells bearing neuronal antigens generated *in vivo* from bone marrow. *Science* 2000; **290**: 1779–1782.
19 Theise ND, Badve S, Saxena R, Henegariu O, Sell S, Crawford JM, Krause DS. Derivation of hepatocytes from bone marrow cells in mice after radiation-induced myeloablation. *Hepatology* 2000; **31**: 235–240.
20 Theise ND, Nimmakayalu M, Gardner R, Illei PB, Morgan G, Teperman L, Henegariu O, Krause DS. Liver from bone marrow in humans. *Hepatology* 2000; **32**: 11–16.
21 Petersen BE, Bowen WC, Patrene KD, Mars WM, Sullivan AK, Murase N, Boggs SS, Greenberger JS, Goff JP. Bone marrow as a potential source of hepatic oval cells. *Science* 1999; **284**: 1168–1170.
22 Lagasse E, Connors H, Al-Dhalimy M, Reitsma M, Dohse M, Osborne L, Wang X, Finegold M, Weissman IL, Grompe M. purified hematopoietic stem cells can differentiate into hepatocytes *in vivo*. *Nat Med* 2000; **6**: 1229–1234.
23 Alison MR, Poulsom R, Jeffery R, Dhillon AP, Quaglia A, Jacob J, Novelli M, Prentice G, Williamson J, Wright NA. Hepatocytes from non-hepatic adult stem cells. *Nature* 2000; **406**: 257.
24 Krause DS, Theise ND, Collector MI, Henegariu O, Hwang S, Gardner R, Neutzel S, Sharkis SJ. Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell* 2001; **105**: 369–377.
25 Thompson EM. Chromatin structure and gene expression in the preimplantation mammalian embryo. *Reprod Nutr Dev* 1996; **36**: 619–635.
26 Bergman Y, Mostoslavsky R. DNA demethylation: turning genes on. *Biol Chem* 1998; **379**: 401–407.
27 Brunk BP, Goldhamer DJ, Emerson CP Jr. Regulated demethylation of the myoD distal enhancer during skeletal myogenesis. *Dev Biol* 1996; **177**: 490–503.
28 Bender CM, Gonzalgo ML, Gonzales FA, Nguyen CT, Robertson KD, Jones PA. Roles of cell division and gene transcription in the methylation of CpG islands. *Mol Cell Biol* 1999; **19**: 6690–6698.
29 Chiu CP, Blau HM. 5-Azacytidine permits gene activation in a previously noninducible cell type. *Cell* 1985; **40**: 417–424.
30 Alvarez JD, Yasui DH, Niida H, Joh T, Loh DY, Kohwi-Shigematsu T. The MAR-binding protein SATB1 orchestrates temporal and spatial expression of multiple genes during T-cell development. *Genes Dev* 2000; **14**: 521–535.
31 Redner RL, Wang J, Liu JM. Chromatin remodeling and leukemia: new therapeutic paradigms. *Blood* 1999; **94**: 417–428.
32 Blau HM, Blakely BT. Plasticity of cell fate: insights from heterokaryons. *Semin Cell Dev Biol* 1999; **10**: 267–272.
33 Blau HM, Pavlath GK, Hardeman EC, Chiu CP, Silberstein L, Webster SG, Miller SC, Webster C. Plasticity of the differentiated state. *Science* 1985; **230**: 758–766.
34 Hardeman EC, Chiu CP, Minty A, Blau HM. The pattern of actin expression in human fibroblast x mouse muscle heterokaryons suggests that human muscle regulatory factors are produced. *Cell* 1986; **47**: 123–130.
35 Eggan K, Akutsu H, Hochedlinger K, Rideout W 3rd, Yanagimachi R, Jaenisch R. X-chromosome inactivation in cloned mouse embryos. *Science* 2000; **290**: 1578–1581.
36 Theise ND, Krause DS. Suggestions for a new paradigm of cell differentiative potential. *Blood Cells Mol Dis* 2001; **27**: 625–631.
37 Clarke DL, Johansson CB, Wilbertz J, Veress B, Nilsson E, Karlstrom H, Lendahl U, Frisen J. Generalized potential of adult neural stem cells. *Science* 2000; **288**: 1660–1663.
38 Geiger H, Sick S, Bonifer C, Muller AM. Globin gene expression is reprogrammed in chimeras generated by injecting adult hematopoietic stem cells into mouse blastocysts. *Cell* 1998; **93**: 1055–1065.
39 Lewontin R. *It Ain't Necessarily So: The Dream of the Human Genome and Other Illusions*. New York Review of Books: New York 2000.
40 Sell S. Heterogeneity and plasticity of hepatocyte lineage cells. *Hepatology* 2001; **33**: 738–750.
41 Theise ND, Saxena R, Portmann BC, Thung SN, Yee H, Chiriboga L, Kumar A, Crawford JM. The canals of Hering and hepatic stem cells in humans. *Hepatology* 1999; **30**: 1425–1433.
42 Keller EF. *Feeling for the Organism. The Life and Work of Barbara McClintock*. WH Freeman & Co: San Francisco, 1983.