Towards predictive models of stem cell fate

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Abstract

Quantitative approaches are essential for the advancement of strategies to manipulate stem cells or their derivatives for therapeutic applications. Predictive models of stem cell systems would provide the means to pose and validate non-intuitive hypotheses and could thus serve as an important tool for discerning underlying regulatory mechanisms governing stem cell fate decisions. In this paper we review the development of computational models that attempt to describe mammalian adult and embryonic stem (ES) cell responses. Early stochastic models that relied exclusively on statistical distributions to describe the *in vitro* or *in vivo* output of stem cells are being revised to incorporate the contributions of exogenous and endogenous parameters on specific stem cell fate processes. Recent models utilize cell specific data (for example, cell-surface receptor distributions, transcription factor half-lives, cell-cycle status, etc.) to provide mechanistic descriptions that are consistent with biologically observed phenomena. Ultimately, the goal of these computational models is to, a priori, predict stem cell output given an initial set of conditions. Our efforts to develop a predictive model of ES cell fate are discussed. The quantitative studies presented in this review represent an important step in developing bioengineering approaches to characterize and predict stem cell behavior. Ongoing efforts to incorporate genetic and signaling network data into computational models should accelerate our understanding of fundamental principles governing stem cell fate decisions.

Introduction

A prerequisite for the clinical use of stem cells is a quantitative understanding of mechanisms that regulate the expansion of stem cell numbers or directs their differentiation into appropriate lineages. Systematic approaches that integrate experimental and computational methods provide a foundation for quantifying stem cell responses. As research on stem cell-based technologies moves towards clinical application, regulatory bodies will require stem cell bioprocesses that are robust and controllable. A particular challenge for the clinical use of stem cells however, is that stem cell responses to different cues often seem to be entirely random (without pattern), difficult to predict, and not amenable to control. This type of behavior has provoked hypotheses that stem cell fate decisions are determined by cell-intrinsic parameters, and may

therefore not be extrinsically controlled. However, a large body of evidence from *in vivo* and *in vitro* studies, and from multiple stem cell systems, shows that stem cell fate can be influenced by exogenous factors. These findings are important from a bioengineering perspective as they provide an opportunity to control and direct stem cell fate decisions to desired cell outcomes. Tools such as soluble factors, cell-cell interactions, and cell-extracellular matrix (ECM) interactions can be used to modulate stem cell responses. A combination of these exogenous signals affects stem cell survival, proliferation, adhesion, self-renewal or differentiation by activating networks of molecular regulators (i.e., signaling molecules, transcription factors) that ultimately determine stem cell fate. These signals may stabilize stem cell populations or elicit their differentiation into specific functional cell types. A quantitative understanding of how these

exogenous factors are incorporated should facilitate the development of bioprocesses to predictably control stem cell responses, thereby allowing us to meet the minimal regulatory criteria for the clinical use of stem cells.

A large amount of data on stem cell responses to exogenous factors already exists; this data can be quantitatively analyzed to develop computational models that describe existing data or predict stem cell behavior under different conditions. Computational models typically fall into two categories: those that support arguments that cell fate decisions are stochastic (i.e., occur randomly due to cell-autonomous properties), and those that cite correlations between exogenous cues and cell fate specifications. A new category that has recently emerged combines elements of both cell autonomous and non-autonomous processes. A summary of the different computational modeling approaches in hematopoietic stem cells (HSC), intestinal crypt stem cells, neural stem cells (NSC) and embryonic stem (ES) cells are presented in this review (Table 1). These models have used simplified parametric representations of actual phenomenon to mathematically calculate or describe stem cell processes. Our analysis of the work suggests that stochastic models are not sufficiently complex, on their own, to comprehensively address stem cell fate determination processes. Analytical approaches that combine elements of extrinsic regulation with cell-intrinsic properties are best able to account for the observed data. These models have postulated different mechanisms, for example, feedback regulation, location of cells in a niche, types and levels of soluble factors, etc., to explain stem cell behavior. The theoretical frameworks thus developed can be tested against existing data and extended to predict and evaluate new hypotheses. Our ES cell-based computational model, for example, correlated existing data on ES cell behavior under different doses of leukemia inhibitor factor (LIF) to predict the output of cultures given an initial set of conditions (Viswanathan et al., submitted (a)). While the mathematical representation of stem cell behavior varies from system to system, a common theme in all the studies is the attempt to elucidate mechanisms for disparate phenomenon. Mathematical approaches have long played an important role in other sciences, and are now taking on an increasingly important role in biology.

Single cell vs. population models

Computational models that account for stem cell fate typically obtain parameters by fitting models to experimental data that are based on net changes in the composition of cell populations. That is, most models are based on average responses of a cell population rather than individual cell responses to a given exogenous stimuli. Additionally, because functional assays typically identify stem or progenitor cells retroactively (based on the progeny they generate), most computational models are based on data that do not continuously track individual cell responses *in situ*, and thus are limited in their ability to characterize heterogeneity (with respect to cell-cycle status, receptor distribution, level of critical molecular regulators, etc.) in cell populations. Since the diversity in the cell population is unaccounted for, population responses often exhibit large variability (especially when small numbers of input cells are used) and thus can appear to be governed by random or 'unseen' mechanisms. The resulting population-based computational models have corresponding random elements, and are useful in describing, but not in predicting experimental observations. Most models discussed in this paper are population models that describe bulk responses of stem cells. Very few attempts have been made to develop models detailing variability between individual cells mainly because the tools to measure such variability are not widely available. Additionally, models that specifically link cell behavior to extrinsic or cell-intrinsic regulators are difficult to evaluate because there are so many interrelated parameters affecting cell fate that it is impossible or simply impractical to follow them all in detail (Novak and Stewart, 1991). However, there are some notable exceptions, and in this paper we discuss two such single cell-based models that describe cell responses in the HSC and ES cell systems. As tools for the systematic incorporation of data into mathematical descriptions develop, it is expected that this detailed approach will become more feasible.

Hematopoietic stem cells

Hematopoiesis is the process by which blood cells ($\sim 10^{10}$ red blood cells hr^{-1} and 10^9 white blood cells hr^{-1}) are continuously produced. Hematopoiesis must therefore be coordinated by an array of regulatory mechanisms that maintain the balanced production

Table 1. Computational models describing stem cell behavior in different systems

Model type	Model description	Stem cell	Reference
Single cell models	Track single cell fates as a function of different exogenous stimuli	HSC, ES, Intestinal stem cells	Nordon et al., 1997, 1999; Oostendorp et al., 2000; Zhang et al., 2001
Population-based stochastic models	Uses statistical probability distributions to describe stem cell fate	HSC	Siminovitch, 1963; Till, 1964; Humphries et al., 1981; Nakahata et al., 1982; Kurnit et al., 1985
Population-based progressive stochastic models	Soluble factors selectively support progenitor cell survival/proliferation	HSC	Mayani et al., 1993; Ogawa, 1993, 1999;
Population-based deterministic models	Stem cell fate is determined by microenvironmental stimuli	HSC, NCSC, CNS, ES	Curry and Trentin, 1967; Trentin, 1978; Van Zant and Goldwasser, 1979; Metcalf and Nicola, 1984; Metcalf, 1988
Population-based instructive models	Soluble factors instructively specify stem cell fate	HSC, NCSC, CNS, ES	Zandstra et al., 1997; Metcalf, 1998b; Ramsfjell et al., 1999
Population-based combination models	Combines statistical probability distributions describing individual cell heterogeneity with extrinsic regulation that can bias the probability functions	HSC, Intestinal crypt stem cells, oligodendrocyte progenitors	Blackett, 1987; Solberg, 1990; Gordon and Blackett, 1994 Agur et al., 2002
Population-based branching process models	Uses a branching process to describe diversification of cell fates with cell division	Intestinal crypt stem cells and oligodendrocyte progenitors	Yakovlev et al., 1998a, b; Loeffler and Grossmann, 1991; Loeffler et al., 1993, 1997; Bjerknes, 1994; Boucher et al., 1999
Chemical equilibrium dynamics model	Oscillations in metabolite, chemical and signaling equilibria direct stem cell fate decisions	HSC and stem cell systems in general	Furusawa and Kaneko, 1998; 2000a, b, 2001, 2002
Activation energy model	Movement of stem cells from areas of high free energy to low free energy or vice versa is representative of stem cell fate decisions	Stem cell systems in general	Waddington and Robertson, 1966
Boolean network model	Coordinated activation/inhibition of specific combinations of genes regulate stem cell fate decisions	HSC	Preisler and Kauffman, 1999
Kinetic models	Use kinetic growth parameters, exogenous stimuli to predict stem cell behavior in culture systems	HSC, ES	Ching-An Peng, 1996; Audet et al., 2002

of HSC, committed progenitors and mature cells for the lifetime of the host. For this reason and because HSC were the first stem cell system to be quantitatively characterized (Siminovitch, 1963; Till, 1964), HSC have been the system of choice to investigate mechanisms that regulate stem cell fate decisions, and have thus served as an experimental and computational model for other stem cells.

Self-renewal vs. differentiation models

Quantitative approaches have been developed to model early decisions of stem cells to remain a stem cell (i.e., self-renew) or exit the stem cell compartment (i.e., differentiate). This approach does not take lineage specification decisions into account, although differentiation to a default lineage is possible within this model. The decision to self-renew or differentiate can be modeled as a random stochastic process or as a specific deterministic process.

Stochastic self-renewal vs. differentiation models

Most models of hematopoietic stem cell self-renewal vs. differentiation responses have been predominantly stochastic and population-based in nature (Siminovitch, 1963; Till, 1964; Humphries et al., 1981; Nakahata et al., 1982; Kurnit et al., 1985). Stochastic descriptions have been used to account for the random variability encountered in the number, size and type of cells derived in clonal colony-forming assays; this variability was apparent even between paired daughter cells cultured under seemingly identical conditions (Suda et al., 1983, 1984a,b). These results led to the argument, originally characterized as ‘HER’ or ‘hematopoiesis engendered randomly’, that the decision of a stem cell to self-renew or differentiate is stochastic (Till, 1964; Ogawa, 1983). The original model was based on observations that the numbers of colony-forming unit-spleen (CFU-S) cells varied from one spleen colony to another in irradiated recipients and could be described using statistical distributions. According to this model, a cell at the time of division can choose between two fates: the formation of a secondary spleen colony (i.e., self-renewal) or ‘birth’, or the production of a cell with no colony-forming ability (i.e., differentiation) or ‘death’. The choice between the two fates was assumed to be random and governed by fixed probabilities, obtained by fitting CFU-S frequency data to Gamma distributions (Till, 1964). Stochastic models have also been used to explain the high re-plating efficiency of hematopoietic

progenitors (i.e., progenitor self-renewal) (Nakahata et al., 1982), the frequency of CFU-GEMM (colony-forming unit-granulocyte, erythrocyte, macrophage, megakaryocyte) in total colonies (i.e., self-renewal of multipotent progenitors) (Nakahata et al., 1982), the secondary colony formation by mast cells (i.e., self-renewal of mast cells) (Pharr et al., 1985), and the variability in re-plating efficiency of T-cell colonies (i.e., self-renewal potential of T lymphocytes) (Wu, 1983).

These stochastic models typically used computer-generated random numbers to empirically fit probabilities of self-renewal, differentiation or lineage commitment to observed data enumerating frequency of mature cell types in individual colonies (Vogel et al., 1969; Korn et al., 1973; Gusella et al., 1976; Humphries et al., 1981; Nakahata et al., 1982; Leary et al., 1984; Kurnit et al., 1985; Leary et al., 1985; Nakahata et al., 1985; Pharr et al., 1985). To assess differentiation or lineage commitment, endpoint colony-forming assays were usually conducted in supportive and/or stimulatory medium. Largely because such tools were unavailable, specific contributions of growth factors to cell survival, proliferation or differentiation could not be accounted for, and thus early events mediating cell loss, survival, or proliferation were difficult to obtain (typically only mature phenotypes were enumerated). As a result, the heterogeneity in endpoint assays was thought to be reflective of the arbitrary nature of cell commitment processes. The statistical models used to describe the observed randomness in the cell responses served as the groundwork for the later development of more detailed computational models.

Deterministic self-renewal vs. differentiation models

Deterministic models such as the ‘HIM’ or ‘hematopoietic inductive microenvironment’ argued that HSC self-renewal vs. differentiation decisions are influenced by their niche microenvironment (Curry and Trentin, 1967; Trentin, 1978) or by cytokine control of cell fate decisions (Van Zant and Goldwasser, 1979; Metcalf and Nicola, 1984; Metcalf, 1988). According to such models, progenitors should consistently produce the same number and type of daughter cells provided that environmental conditions are similar. However, the observed randomness in experimental data argues against the existence of deterministic cell specification processes. The inability of Poisson distributions to fit the cumulative frequency distribution of CFU-GEMM (Humphries et al., 1981) or to fit

the distributions of B (erythropoietic bursts), GM (granulocyte macrophage) M (megakaryocyte) or EM (erythrocyte megakaryocyte) colonies in total colonies generated (Nakahata et al., 1982), is often used to reject the argument that cell fate specification processes can be predetermined. The poor fit of Poisson distributions to observed frequency data does not mean, however, that the cell fate specification is not deterministic, but rather that the process is not fixed or invariant. Variations at the level of individual cells (for example, due to unsynchronized cell cycling or due to the presence of different cell sub-populations) or microenvironmental perturbations (including autocrine secretion of soluble factors) may cause seemingly identical cells to behave in a non-identical manner. That is, individual cells may specify cell fates (in relation to their position in the developmental hierarchy) according to a Poisson process, which at a population level appears to be randomly distributed.

Recent studies have provided stronger evidence in support of a deterministic role of soluble factors and other microenvironmental influences in specifying stem cell self-renewal vs. differentiation decisions. For example, studies based on empirical models (using factorial design analysis) have linked the self-renewal of primitive and intermediate hematopoietic progenitors to distinct combinations and concentrations of hematopoietic cytokines (Zandstra et al., 1997; Audet et al., 2002; Zhang et al., 2001). These quantitative analyses are informative as they provide insights into the complexities of interacting growth factors, although they do not demonstrate the mechanisms by which cytokines promote primitive progenitor expansion. Other studies have shown that thresholds in the levels and types of soluble factor signaling might be critical determinants of self-renewal vs. differentiation decisions of primitive hematopoietic progenitors (Zandstra et al., 1997; Ramsfjell et al., 1999; Audet et al., 2001), thus suggesting that signals emerging from soluble factors might specifically activate or suppress gene programs associated with a particular cell fate.

Lineage commitment models

Increasing evidence of cytokine involvement in hematopoietic cell fate decisions have allowed investigators to include cytokine or growth factor contributions to stem cell fate. The controversy has shifted (from stochastic vs. deterministic contentions) to arguing the roles of cytokines in stem cell specification processes, and whether they are instructive or selective.

The bulk of this argument has focused on decisions of multipotent progenitors to commit to one lineage vs. another, and whether this decision is instructively or permissively modulated by soluble factors (Cross et al., 1997; Enver et al., 1998; Mayani et al., 1993; Metcalf, 1991; Morrison et al., 1997; Ogawa, 1993). In the instructive mode of cytokine regulation, cytokines specify a particular cell outcome (choice of lineage commitment). Whereas, in the selective mode of cytokine regulation, while lineage choice is predetermined by cell-intrinsic processes, a specific sub-set of cells are selectively allowed to survive and proliferate in response to cytokine stimulation. There is evidence for both selective and instructive roles of cytokine-mediated regulation of HSC lineage commitment decisions.

Selective lineage commitment models

Studies that support the selective lineage commitment hypothesis typically model cytokines as factors that selectively influence the survival of progenitor cells without influencing the decision of uncommitted progenitor cells to commit to various lineages (Mayani et al., 1993; Ogawa, 1993, 1999). These progressive stochastic models have been reinforced by observations that prevention of cell death by blocking apoptosis in hematopoietic cell lines (Nunez et al., 1990; Baffy et al., 1993; Fairbairn et al., 1993) and hematopoietic progenitors (Domen et al., 2000) yielded multilineage differentiation in the absence of growth factors. Chimeric receptor studies have also questioned the specificity of cytokine-induced signals in directing lineage commitment (Alexander et al., 1996; Smith et al., 1997). Studies that report the presence of multiple lineage-restricted genes in hematopoietic progenitors prior to commitment (Liu et al., 1994; Hu et al., 1997; Enver et al., 1998) have been used to argue for an inherently arbitrary 'mechanism' for lineage commitment. This argument is further confirmed by applying statistical methods to analyze gene expression profiles in developing osteoblasts, which suggests that diverse gene activation pathways can be employed in forming the same mature cell (the choice of the actual pathway may be stochastically selected or determined by environmental conditions (Madras et al., 2002)). However, even though multiple genes are present, it is not clear if these genes are simultaneously or sequentially transcribed, as different mRNAs would have varying half-lives. That is, it is not clear if progenitors commit to certain cell fates by random processes or if they are progressively restric-

ted along a pre-determined differentiation pathway. As differentiation along a specific pathway results in the upregulation or stabilization of a particular subset of genes (likely by a cell's interactions with the microenvironment), one could argue for deterministic control of lineage commitment. On the other hand, stabilization of genes may occur by cell-intrinsic stochastic processes or by selective action of cytokines, as specified by the progressive stochastic model. In fact, statistical analysis of gene expression in single cells reveals that fluctuations in gene expression can be due to a combination of both stochastic (microscopic events that are cell-intrinsic) and extrinsic (cellular components such as the levels of enzymes that may be extrinsically controlled) elements (Swain et al., 2002). Both the cell-intrinsic or extrinsic modes of regulation are consistent with observations that transcription factors that for example, regulate myeloerythroid commitment of hematopoietic progenitors simultaneously promote the upregulation of genes associated with a specific cell fate while downregulating an alternative fate (Orkin, 2000). Taken together, the combined evidence from studies citing non-specific roles for cytokine in lineage-commitment imply that cytokines improve cell survival and proliferation of different progenitor cells without affecting lineage commitment decisions (considered a cell-intrinsic process that is not amenable to external regulation).

Instructive lineage commitment models

Instructive models, on the other hand, counter that cytokines (and other microenvironmental cues) specifically affect the choice of lineage commitment. According to the instructive lineage specification argument, the window of opportunity during which cytokines can influence cell fate decisions might be quite narrow (Metcalf, 1998). If cytokines are administered after this period has elapsed, they may appear not to affect the lineage commitment of (already committed) progenitors, and thus appear to promote cell survival and proliferation non-specifically (as postulated by progressive stochastic models). Without knowing cell proliferation history and cell position in the developmental hierarchy, it would be difficult to conclude whether cytokine action, based solely on cell responses in an endpoint assessment is instructive (i.e., specifying lineage commitment choices) or selective (i.e., promoting cell survival and proliferation). Consequently, it is argued that experiments that do not take all factors into account cannot definitively rule out roles for cytokines in instructing lineage specification.

This argument is further strengthened by studies that demonstrate an instructive role of cytokines in stem cell lineage commitment decisions. For example, addition of thrombopoietin (Tpo) to stem cell factor (SCF)-supplemented medium increases the proportion of blast colonies that contain megakaryocyte progenitors without impacting total progenitor cell numbers (Metcalf, 1998); similarly addition of interleukin-5 (IL-5) to SCF-supplemented medium increases the frequency of eosinophil progenitors in blast colonies without affecting total progenitor numbers (Metcalf, 1998). Another striking example is given in a study that demonstrates cytokine-mediated lineage specification (lymphoid vs. myeloid lineages) upon macrophage colony-stimulating factor (M-CSF) stimulation of an IL-7-dependent pre-B-cell line overexpressing the M-CSF receptor (M-CSFR) (Borzillo, 1990). Although these studies seem to demonstrate a deterministic mode for regulation of lineage commitment events, it is not clear if a phenotype switch is sufficient evidence for differentiation commitment.

Taken together, there is evidence to support both the selective (i.e., progressive stochastic models) and instructive roles (i.e., instructive and deterministic models) of cytokine-mediated regulation of HSC fate. It is important to note that one source of underlying confusion between the stochastic and deterministic arguments can be found in the fact that they deal with entirely separate issues. The decision of stem cells to self-renew or differentiate vs. commitment of cells fated for differentiation to specific lineages are separate decisions, and either or both of these may be subject to extrinsic controls (Blackett and Gordon, 1999). In this paper, we have tried to distinguish between models describing these distinct decisions. Increasingly, we are now seeing the development of computational models that combine both stochastic and deterministic modes of regulation to provide a more comprehensive description of stem cell fate determination events that include both self-renewal/differentiation and lineage commitment decisions.

Combination models

Stochastic and deterministic combination models that are population-based

Certain studies (Blackett, 1987; Gordon and Blackett, 1994) postulate that heterogeneity in stem cell properties, for example cell-cycle entry, in an otherwise homogeneous population can in itself account

for the variability in experimental observations. According to one such model, uniform populations of stem cells with different propensities for recruitment from G_0 contribute to the observed randomness in the ability of single stem cell-derived clones to sustain hematopoiesis following bone marrow transplantation. The probability that a cell will be recruited into the cell-cycle is considered to be stochastic and cell-intrinsic, and can be represented by a statistical distribution. However, this probability can be biased by microenvironmental stimuli, for example, greater recruitment occurs after irradiation than during normal hematopoiesis (Till, 1964) or after treatment with particular cytokines (for example, Miller, 1997). Another example of a combination model that reconciles extrinsic influences (e.g., cell-cell interactions) of cell fate regulation with intrinsic mechanisms (i.e., an internal clock that determines the onset of differentiation as a function of stem cell numbers in the neighbourhood) shows that the onset of differentiation in bone marrow-derived stem cells is influenced by microenvironmental cues (Agur et al., 2002). This model combines exogenous parameters such as the geometry and location of stem vs. differentiated cells (within the bone marrow) and the time for cell differentiation and proliferation, with stochastic probabilities representing internal clock counters to determine bone marrow differentiation. Both these combination models address self-renewal vs. differentiation events. A third combination model, on the other hand, addresses lineage commitment events by using growth factor-biased stochastic processes to simulate HSC progression along the megakaryocytic lineage (Solberg, 1990).

A common theme to these combination models is that the observed randomness in cellular responses is explained by fundamental heterogeneity between individual cells, present in all cell populations. That is, the randomness is not caused by a specific biological property unique to a certain class of stem or progenitor cells, but is due to the inherent differences between any two cells, even in a seemingly homogenous population. It is postulated that the molecular basis for inherent stochasticity in any given cell population may be due to the unequal portioning of receptors, signaling molecules and/or gene regulators (Paulus et al., 2001). The existence of these differences between individual cells prejudices their subsequent interactions with their immediate environment, and results in altered cell fates, suggesting

that stem cell fate regulation processes can be both intrinsically and extrinsically modulated.

Stochastic and deterministic combination models that are single cell-based

Models that correlate differences between individual cells (arising due to stochastic events) to their responsiveness to extrinsic stimuli are able to combine both intrinsic and extrinsic elements of cell regulation at a single cell level. One such model connects differences in individual cell proliferative properties to their self-renewal responsiveness to a cocktail to exogenous stimuli by a cocktail of growth factors (Zhang et al., 2001). The authors employed an integrated experimental and computational approach based on a high-resolution cell-division tracking method (Nordon et al., 1997, 1999; Oostendorp et al., 2000; Zhang et al., 2001) to link changes in the proliferative and apoptotic status of the population as a consequence of altered responsiveness of single cells (i.e., variation in cell-cycling rate, cell-cycle recruitment, lag period, etc.). The model was therefore a useful tool in deducing the effects of extrinsic stimuli (i.e., type and concentration of soluble factors) because it accounted for heterogeneity in responsiveness of individual cells. Another single cell-based model was recently developed to represent *in vivo* and *in vitro* HSC lineage commitment data (Roeder and Loeffler, 2002) by simulating variations in two cell properties (i.e., the attachment affinity of a cell to a particular niche and its cycling status). This model was proposed to illustrate the concept of stem cell 'plasticity'; cell fate decisions were not restricted to particular cell properties, but rather were considered to be the sole function of affinity attachment and cycling status. A useful extension of the model would be to detail variations in stem cell fate as a function of changes in microenvironmental stimuli (which would consequently cause changes in cell properties).

Neural stem cells

NSCs found in the central nervous system (CNS) or in the neural crest (NC) are defined as self-renewing cells that can give rise to different types of glia and neurons. CNS stem cells can give rise to neurons, astrocytes and oligodendrocytes, while neural crest stem cells (NCSC) give rise to neurons and glia of the peripheral nervous system as well as connective cell types like smooth muscle cells (rev. in Morrison, 2001).

A

B

$$\frac{dS_n}{dt} = 2\mu_S [P_{p(n-1)} S_{n-1}] - \mu_S [P_{p(n)} S_n] - \alpha_S [Q_{p(n)} S_n] - d_S [R_{p(n)} S_n]$$

$\frac{\text{stem cell \#}}{\text{time}}$ = proliferation of stem cells - differentiation of stem cells - death/apoptosis of stem cells

Figure 1. Biological vs. computational representation of stem cell responses. (A) Schematic of stem cell responses to exogenous factors over one cell division. (B) Mathematical representation of (a) as cells move from the $n - 1$ th cell division to the n th division. Rates of stem and differentiated cell proliferation are denoted as μ_S and μ_D respectively. Rates of stem and differentiated cell death are denoted as d_S and d_D , respectively. Rate of stem cell differentiation is denoted as α_S . Probabilities of stem cell self-renewal, stem cell differentiation and stem (or differentiated) cell death in the n th division is given as $P_{p(n)}$, $Q_{p(n)}$ and $R_{p(n)}$, respectively.

Because NSC undergo self-renewal and differentiation in culture, the differentiation and proliferation of individual stem cell colonies can be monitored simultaneously under different exogenous conditions, and questions regarding the modes of cytokine regulation (i.e., selection vs. instruction) of NCS lineage commitment decisions to glial or neuronal lineages can be addressed.

Lineage commitment studies that illustrate instructive modes of cytokine regulation

The decision of CNS or NCSC to commit to glial vs. neuronal fates appears to be complexly regulated by multiple exogenous factors including bone morphogenetic proteins (BMPs), neuregulin and transforming growth factor ($TGF\beta$), ciliary neurotrophic factor (CNTF) and thyroid hormone (T3) (Shah, 1994; Shah et al., 1996; Gross, 1996; Johe, 1996; Li, 1998). In CNS stem cells, neuronal differentiation precedes glial differentiation, and the mechanism regulating the timing of these events is thought to be cell-intrinsic (Qian, 2000), although interactions with microenvironment cannot be ruled out (Morrison, 2000). The initial differentiation event to neural lineages is regulated by soluble factors (for example, BMPs), which induce basic helix-loop-helix transcription (bHLH) factors such as Neurogenin and Mash-1 to drive neurogenesis by specifically activating the expression of a cascade of neuronal genes (Lo, 1998; Ma, 1998). These proneural transcription factors not only actively promote neurogenesis, they simultaneously inhibit gliogenesis (Nieto, 2001; Sun, 2001; Tomita, 2000). It thus appears that soluble factors in the CNS exercise an instructive mode of regulation in lineage determination decisions. Similarly, the secondary glial differentiation event (i.e., requiring a switch from neurogenesis to gliogenesis) also appears to be instructively regulated by exogenous Notch signaling (Furukawa, 2000; Gaiano, 2000; Hojo, 2000; Morrison et al., 2000; Tanigaki, 2001). Notch signaling also appears to dually instruct gliogenesis and inhibit neurogenesis, although these actions may be context-specific. Taken together it would appear that lineage specification choices between glial and neuronal lineages are jointly regulated by cell-extrinsic and cell-intrinsic parameters in an instructive process that results in the specification of one lineage over the other.

Self-renewal vs. differentiation models

Clonal studies of oligodendrocyte (myelin-forming cells of the CNS derived from rat optic nerves) differentiation from dividing oligodendrocyte type-2A astrocyte (O-2A) progenitors in response to exogenous stimuli have also been conducted in detail (Ibarrola et al., 1996). These studies provide an opportunity to quantitatively analyze mechanisms regulating O-2A progenitor self-renewal vs. differentiation responses.

Differentiation into oligodendrocytes from clonal O-2A cultures appears to be synchronized (suggestive of an intrinsic biological clock), although the proportion of oligodendrocytes generated varies from clone to clone and is sensitive to environmental signals. A branching stochastic model was evoked to explain the process of oligodendrocyte differentiation from single cells (Yakovlev et al., 1998a,b; Boucher et al., 1999). This approach mechanistically demonstrated that oligodendrocyte differentiation is neither wholly symmetric nor asymmetric but includes both possibilities. That is, progenitors undergo symmetric self-renewing divisions for a number of mitotic divisions (which varies from clone to clone and is represented by random distribution) until a critical division number is reached; at this point progenitors are competent to differentiate. The probability of self-renewal, p , is fixed at 1 for the length of the latency period, after which the probability is estimated by a statistical distribution. Importantly, this statistical probability of self-renewal vs. differentiation is sensitive to external influences including signaling from T3, CNTF, neurotrophin-3 and fibroblast growth factor-2 (FGF-2), once the critical division number is attained. This combined model provides a good fit to the mean progenitor and oligodendrocyte distribution, but not to the variance. The model fit was improved by incorporating cell-cycle history (Boucher et al., 1999), partial likelihood functions based on a random walk model (Von Collani et al., 1999) and computer-intensive stochastic optimization methods (Zorin et al., 2000), all of which better accounted for the heterogeneity in critical division number and clone size.

A recent version of the model proposes that stochastic probability of self-renewal decreases gradually (with cell division) rather than as a threshold decrease. Importantly, this model also maintains that environmental factors influence the stochastic probability of cell differentiation (Boucher et al., 2001). A recent finding that oligodendrocyte precursor cells (cultured under serum-free conditions in the absence

of T3) can be maintained indefinitely in culture (Tang et al., 2001), may impact model predictions. It would be interesting to see if the model can account for this phenomenon (under the specified culture conditions) or if model parameters would have to be revised to account for the new paradigm. Notably, this model, like HSC models, represents a working hypothesis of stem cell fate regulation that can be constantly revised and updated to account for new experimental evidence.

Intestinal crypt stem cells

Another interesting system, which undergoes constant regeneration and has provided a model for stem cell behavior, is the intestinal crypt. Maintenance of the crypt is dependent on a stem cell population (Withers and Elkind, 1968, 1970); as stem cells divide they populate the crypt, which grows until it reaches some critical size, and then divides to form two smaller crypts (Bjerknes, 1986).

Lineage commitment model: Pedigree model

Spatial arrangements and migration of cells within the cylindrical intestinal crypts have been modeled as a stochastic process dependent on cellular pedigree i.e., whether primitive stem cells, intermediate transit proliferating cells or mature cells (Loeffler et al., 1986). The base of the cylindrical crypt is occupied by non-dividing Paneth cells followed by stem cells, transit amplifying cells and then differentiated globular or columnar epithelial cells. Cells migrate vertically and become progressively more restricted until terminal differentiation. The probability of differentiation appears statistically determined.

Self-renewal vs. differentiation model

A discrete Galton-Watson branching process (Harris, 1963) was used to describe the behavior of stem cells within the crypt; each cell division representing a node in the branch. At the time of cell division, a stem cell produces two, one or no daughter stem cells each with a fixed, randomly-determined probability (p , r , and q , respectively, such that $p + q + r = 1$) (Loeffler and Grossmann, 1991) that are not dependent on other assumptions. Model parameters including cell cycling time, stochastic probabilities for symmetric and asymmetric self-renewal/differentiation, the threshold number of stem cells (the number at which the crypt splits into two), and the segregation of stem cells into

two daughter crypts on fission are empirically determined by fitting the steady state model to experimental data. Since there are no markers to enumerate putative intestinal stem cells, somatic mutations were introduced by nitroethylurea (NEU) treatment (Winton and Ponder, 1990), and time of crypt conversion to monoclonality (i.e., all cells were mutated and derived from a single stem cell clone) was measured to enumerate stem cell numbers.

The model was based on the assumption that stem cells are equivalent (i.e., a homogeneous population) and have independent probabilities governing production of daughter stem cells. The model explains stem cell organization in intestinal crypts as a continuous process of birth and extinction and predicts experimental size distributions but only when the probability of asymmetric cell division is fixed at 0.8, i.e., the cells divide asymmetrically 80% of the time (Loeffler et al., 1993). A major limitation of this approach was the need to assume asymmetric division 80% of the time; a constraint that contrasted with data obtained from the mutation experiments (Winton and Ponder, 1990). The problem lay in the assumption that probabilities of cell fate decisions were immutable and randomly determined; if probability values were allowed to vary with extrinsic cues, the model would better fit the data (Bjerknes, 1994). Accordingly, a revised model incorporating an autoregulative element that allowed probability values to be determined by numbers of stem cells present in the crypt (i.e., due to feedback mechanisms representing cell-cell, cell-ECM and soluble factor interactions) was developed (Loeffler et al., 1997). Probability values and stem cell cycling time were expressed as functions of existing numbers of stem cells. The revised model was better able to predict the numbers and population sizes of the crypts without having to resort to artificial constraints in the model. The incorporation of feedback mechanisms representing extrinsic cues complements the original stochastic model and suggests that the stem cell population of intestinal crypts are best described when both stochastic and deterministic modes of regulation are combined.

Embryonic stem cells

Murine ES cells, derived from day 3.5 blastocysts (Evans and Kaufman, 1981; Martin, 1981) represent a pluripotent cell population that can be maintained in an undifferentiated state for prolonged periods of

time by soluble factors from the gp130 family (Smith et al., 1988; Williams et al., 1988). Murine ES cells have several advantages as a model system to investigate cytokine-mediated stem cell fate regulation over HSC, intestinal crypt stem cells, or CNS progenitors. ES cells are pluripotent and therefore decisions to self-renew or differentiate to multiple lineages can be modeled. ES cells are an experimentally accessible system, i.e., they are readily available and form relatively homogenous cultures of undifferentiated cells *in vitro*. In contrast, adult stem cells are typically rare populations, which are difficult to isolate, purify and propagate. Heterogeneity in initiating populations of adult stem cells confounds cell fate analyses, and makes it difficult to discern regulatory mechanisms. Moreover, ES cells respond to single cytokines from the interleukin-6 (IL-6) family in a predictable manner, and their responses can be quantified by a variety of functional, phenotypic and molecular assays (Viswanathan et al., 2002; Zandstra et al., 2000), some of which correlate with their functional ability to give rise to chimeras when re-introduced into the mouse blastocyst (Bradley et al., 1984). Importantly, decisions made by ES cells to self-renew, differentiate, die, proliferate, adhere, migrate, etc. are influenced by many of the same soluble factors (for example, by LIF, BMPs or by FGF family members), cell-cell and cell-ECM interactions that also play important roles in adult tissue-specific stem cells. This implies that at least some determinants of stem cell fate are conserved between the adult and embryonic systems. Additionally, ES cells are non-transformed cell lines that remain responsive to regulatory cues that govern normal mammalian development, as evidenced by their ability to participate in normal embryogenesis following re-introduction into developing blastocysts. Overall, the relative ease with which ES cells can be cultured and characterized makes them a powerful system in which to conduct stem cell fate investigations.

Self-renewal vs. differentiation models

Deterministic model that is single cell-based

Recognizing the importance of a deterministic model that correlates fundamental heterogeneity between individual cells to their behavior, we developed a single cell-based model that allowed us to discern regulatory mechanisms governing ES cell fate. Our model was based on the premise that levels of ligand-receptor signaling complexes formed (on individual cells) are critical to the activation of gene programs associated

Figure 2. Schematic representation of ligand-receptor signaling threshold (LIST) model-mediated determination of stem cell fate (Viswanathan et al., 2002). (A) Individual stem cell fate (i.e., symmetric differentiation, asymmetric self-renewal or symmetric self-renewal) is determined by levels of ligand-receptor signaling complex numbers. Cells with a large number of signaling complex numbers undergo symmetric self-renewal; cells with an intermediate number of signaling complexes undergo asymmetric self-renewal, while cells with a small number of signaling complexes undergo symmetric differentiation. Stem cells are depicted as gray cells, while differentiated cells are depicted as white cells. (B) Population dynamics are determined by experimentally measured rates of stem and differentiated cell division, differentiation and death. Rates governing stem and differentiated cell division, μ_S and μ_D , respectively, and stem cell differentiation, α_S , are shown. Population composition in the 'nth' cell division is determined by the population probability of self-renewal ($P_{p(n)}$), which is determined as a function of ligand-receptor signaling complex numbers as previously described (Viswanathan et al., 2002).

with specific cell fate outcomes (Zandstra et al., 2000). We demonstrated a direct correlation between the magnitudes of ligand-receptor signaling complexes formed and the probability of ES cell self-renewal (Figure 2A). Specifically, quantitative differences in ligand-receptor binding properties and stoichiometries translated into distinct cell responses (Viswanathan et al., 2002). The calculated signaling complexes were compared with experimentally determined complexes for self-renewal; if the calculated complexes exceeded the threshold, the cell self-renewed, otherwise the cell differentiated. A second threshold for differentiation was also introduced, which allowed cells to divide asymmetrically. The ES cell model combined extrinsic regulation with statistical distributions of cell-intrinsic (i.e., receptor numbers which could not be directly measured) properties to generate a model of individual cell fate determination. While this initial model predicted cell fate decisions at an individual cell level for a single cell division, it was limited because it did not take variations in population dynamics due to cell proliferation, differentiation or apoptosis into account.

Combining individual cell responses to predict population outcomes

A model incorporating individual stem cell fate decisions to predict cell population outcomes as a function of exogenous stimulation has the potential to identify parameters that are relevant to the design and optimization of stem cell bioreactor systems. With this in mind, the single cell-based ligand-receptor signaling threshold (LIST) model was extended to include kinetics of cell survival, proliferation and differentiation over multiple divisions (Viswanathan et al., submitted (a), Figure 2B). Using a transgenic ES cell line that expressed green fluorescent protein (GFP) when cells were undifferentiated (and lost expression as cells differentiated), we simultaneously tracked undifferentiated ES and differentiated cell responses to various exogenous stimuli (Viswanathan et al., submitted (b)), and incorporated these measurements in our LIST model. We showed that the decisions of individual cells to die vs. survive, to self-renew vs. differentiate, and the rates at which cells grew, died or differentiated are modulated by levels of ligand-receptor signaling complexes. The model also included a lag-term (based on measured rates of transcription factor decay) to explain the experimentally observed delay in differentiation upon LIF withdrawal in adherent cultures (Viswanathan et al., 2002). Cells undergo symmetric self-renewal divisions during this

latency period, a concept similar to the delay incorporated in models describing differentiation competency of oligodendrocyte precursors.

The model thus developed predicts the population composition and dynamics of our experimental system, in which ES cells were maintained at different concentrations of LIF (under serum-free conditions), over multiple cell divisions. Model parameters were varied ± 10 -fold to assess their relative importance on stem or differentiated cell generation, and to make predictions that were not limited to the experimental system at hand. The resulting simulations identified parameters that could be engineered to effectively modulate stem cells or their derivatives. For example, one such finding showed that at low levels of LIF receptor-occupancy, the generation of ES cell-derived progeny was more sensitive to parameters that affected their growth rates than their rate of differentiation from the stem cell pool. This suggested that an effective strategy to maintain the differentiated progeny was to promote the proliferation of already differentiated cells rather than to induce increased differentiation. Model predictions were experimentally verified by the addition of mitogenic factors, like FGF-4, which increased differentiated cell expansion by promoting their proliferation (Viswanathan et al., submitted (b)). Model simulations also assessed stem or differentiated cell numbers under other simulated conditions that altered the rates of differentiation, proliferation or apoptosis, the half-lives of transcription factors, or the sensitivities to exogenous stimuli, etc, and thus was a useful tool in assessing and predicting the importance of various biological parameters on stem cell behavior. For instance, model simulations confirm experimental observations that show LIF to be more effective than equimolar concentrations (>50 pM) of Hyper-IL-6 (HIL6, a fusion protein of IL-6 and sIL-6R (Fischer et al., 1997)) in maintaining undifferentiated ES cells (Viswanathan et al., 2002; Viswanathan et al., submitted (b)). Importantly, these model simulations could be re-created experimentally to validate simulated conditions.

Another advantage to our model simulations was that they allowed us to ask and answer questions that would be difficult to answer by traditional experimental investigations. For example, our approach allowed us to effectively de-couple the individual contributions of cell kinetics from the (ligand-receptor-mediated) probability functions governing cell fate; our findings show that probability functions were more important than growth parameters in modulating dif-

differentiated cell fate. It would be difficult to design an experiment that directly evaluates the effects of the individual parameters. However, insight gained from our analytical approach can be experimentally tested, thus validating simulated results.

Findings from our computational approach also allowed us to mechanistically re-interpret existing data. For example, model findings suggest that the net amplification of stem cells requires the simultaneous modulation of their growth, differentiation and death responses. This can be accomplished by a single factor, such as LIF in the ES cell system (Viswanathan et al., submitted (b)), or by multiple factors, such as the early acting cytokines, SCF, FL and IL-3 in the hematopoietic system (Ogawa, 1993). According to our model, threshold levels of each ligand-receptor interaction can individually or synergistically affect HSC ability to meet the survival, self-renewal or differentiation criteria, and thus affect cell fate. Unless the right combination and relative concentrations of cytokines are presented to the cell (Zandstra et al., 1997), net amplification of the stem cell population will not result. Our model computations can thus be useful in identifying the optimal level of each ligand-receptor interaction that is needed to achieve desired results.

The computational model was useful not only in describing the ES cell responses within and outside the range of experimental conditions tested, but it was also successful in making qualitative predictions for other types of stem cells, thereby helping to deduce mechanisms of stem cell fate regulation. In general, our computational approach complements and extends findings from experimental systems. However, our model, like all models, simply represents a working hypothesis of stem cell fate determination and is subject to revision, addition and improvement. To fully assess the effects of cytokine regulation on stem cell fates, it will be necessary to incorporate downstream signaling interactions, cell-cell, cell-ECM interactions and feedback regulation, all of which impinge on cell kinetics and cell fate. Our model can be further developed by incorporating a more detailed list of parameters or by choosing critical parameters that govern cell behavior (see below). Importantly, the current model allows a kinetic evaluation of culture conditions that support stem vs. differentiated cell populations, reminiscent of a previous study describing such processes in hematopoietic progenitor cell cultures (Ching-An Peng et al., 1996). This kind of analysis has important applications in the development

of bioreactor systems that can support the expansion of stem cells and their derivatives.

Incorporating signaling pathways/gene networks in stem cell fate models

We have so far reviewed stochastic, deterministic or combination modes of stem cell fate regulation in different stem cell systems in the context of self-renewal/differentiation vs. lineage commitment decisions, at both the single cell and bulk population level. In this section, we will review conceptual models that define stem or differentiated cell states based on the sum of multiple interactions between signaling pathways, soluble factors, transcription factors, genes, etc. These global regulatory models can be applied to multiple stem cell systems and represent a macroscopic view of cell fate determination processes.

Chemical equilibrium dynamics model

An example of models that present a more unified concept of stem cell fate determination include those that combine the regulation of chemical and equilibrium dynamics with decisions of stem cells to self-renew or differentiate (either in response to extrinsic cues or as stochastic processes). These models ascribe states (i.e. the composite of complex metabolic, chemical and signaling pathways) to different cells, with stem and differentiated cells occupying stable states (i.e., associated with low free energy). In a heterogeneous population, cell states may oscillate due to extrinsic perturbations caused by chemical or soluble factor fluxes, cell-cell interactions or intrinsic instability (i.e., stochastic) until they reach a stable state. A model by Furusawa and colleague (Furusawa and Kaneko, 2001) proposed that stem cells occupy stable positions as 'attractors' within culture systems and that oscillations in culture dynamics cause perturbations that remove stem cells from their stable 'basins' and push them towards various differentiated states, the pathway for commitment being determined by the particular combination of perturbations. This model accounts for negative feedback processes, for example, the creation of a large number of differentiated cells upsets system equilibrium and forces a new balanced state by eliminating differentiated cells (death) or by eliciting stem cell self-renewal.

Activation energy model

Another conceptual model describes stem cell differentiation to multiple lineages as the path taken by a ball (representing a stem cell) perched on top of a hill that can roll down multiple valleys or canals (each representing a lineage) (Waddington and Robertson, 1966). This ‘canalization’ process has been revised to include furrows within each valley, which represent low free energy states (i.e., stable regulatory/metabolic states); transition of cells from one furrow to another within a valley (i.e., normal differentiation) or across valleys (i.e., transdifferentiation) depends on the height of the furrows and valleys, and may be considered equivalent to the ‘activation energy’ needed to achieve such transitions. Differentiation down particular lineages, according to this model, may then be achieved by providing cells with the precise combination of stimuli needed to achieve the stable regulatory/metabolic state associated with that lineage pathway.

Regulatory model of gene networks

As cells transit between stem and differentiated cell compartments they activate or repress a different set of genes. One dynamic model of gene expression regulation (‘on/off’ states) uses a Boolean Network approach to account for the stochastic decisions of stem cells to remain or exit the stem cell compartment (Preisler and Kauffman, 1999). This model allows microenvironmental cues (i.e., cytokines, niche environment or physical parameters such as glucose or oxygen levels (Cross et al., 1997)) to bias the stochastic processes of gene up- or down-regulation (and/or gene interactions) as cells oscillate between different gene expression states. According to this approach, a variety of contradictory lineage specific genes can be present at low levels in stem cells. As cells differentiate into certain lineages-specific genes would be upregulated while others would be turned off, thus leading to a stable state. This model supports the presence of multiple genes seen in primitive hematopoietic progenitors (Liu et al., 1994; Hu et al., 1997; Enver et al., 1998). Importantly, according to this model cell fate specification can occur either through deterministic (i.e., a cell’s interactions with its microenvironment) or stochastic (i.e., cell-intrinsic) processes, both of which result in the upregulation and/or stabilization of certain genes. Both these modes (cell extrinsic vs. intrinsic) influence the rates, types (associated with specific cell

fates) and efficiencies of gene transcription, and thus result in divergent cell fates.

Summary and future directions

The quantitative models presented in this study provide a reasonable means of translating biological observations into fundamental, rules of interaction that can routinely be used to predict cell behavior. This kind of systematic analyses will be important to defining quantitative parameters in the design and development of bioreactor systems that can produce stem cells or their derivatives in a controlled environment (Zandstra and Nagy, 2001). It is clear from these approaches that multiple factors, be they cell-extrinsic (for example, the level or type of soluble factor stimulation, cell-cell interaction, niche microenvironment, divisional history, chemical fluxes, etc.), cell-intrinsic (for example, levels of surface receptors, levels of cyclin proteins, levels of critical transcription factors, etc.), or a combination of the two have distinct and measurable effects on stem cell fate decisions. Our model on ES cell fate for example, considered both extrinsic and intrinsic parameters, i.e., levels of cytokine stimulation, kinetic rates constants, and the half-life of a critical transcription factor (Viswanathan et al., submitted (a)). To further refine our model, we can choose between a ‘systems biology’ or a reductionist approach. With the ‘systems biology’ approach, we could make our model more comprehensive by considering additional parameters for example, the duration of cytokine stimulation, the trafficking properties of ligand-receptor signaling complexes, the levels of multiple molecular regulators, etc. Models incorporating these parameters would have to simultaneously compute stem cell behavior as a complex function of these multiple parameters (and their interactions). A version of this approach has been used by Bhalla and colleagues to model (hippocampus) neuronal cell response to glutamate via the activation of four signaling pathways: the Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII), the protein kinase A (PKA), the protein kinase C (PKC) and the mitogen-associated protein kinase (MAPK) pathways (Bhalla and Iyengar, 1999). The authors were able to identify the combined effects of multiple pathways on cell behavior, and thus elucidate mechanisms of cell regulation that would not have been possible by considering each pathway individually. Alternatively, a more reductionist approach could be undertaken wherein one or

more cell fate-limiting parameters are identified and continuously monitored. With this approach, the computational model would be narrowly focused on a few selected parameters that are most critical to stem cell fate determination. However, it would be necessary to justify the selection of the model parameters and to demonstrate how these parameters can be extrinsically controlled. While the two methods might offer different insights into stem cell regulation, both would provide quantitative analyses of stem cell fate determination processes, an important step in the development of stem cell bioprocesses. In the long term, the success of stem cell-based technologies will depend, to a large extent, on the ability to identify and control microenvironmental conditions that specify either the expansion of stem cells or direct differentiation to appropriate lineages.

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