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Molecular Control of (Stem) Cell Fate

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Introduction

The notion of cell identity or phenotype has undergone a seismic shift over the past decade. Until then, cell biologists largely regarded terminally differentiated somatic (i. e. non-germ line) cells as deriving from more plastic progenitors via an essentially one-way route. Only recently was the question of reversibility of cell differentiation, a by-product of the inherent stochasticity and plasticity of cells, raised by researchers such as Roeder and Loeffler [1]. The explosion of research into stem cells over the past decade in particular has vindicated these early suggestions of mutability and plasticity of cell phenotypes. A recently as 2006, Yamanaka announced the startling discovery that somatic cells can be reprogrammed to pluripotency by a cocktail of transcription factors [2]. Subsequent research has shown that it may be possible to reprogram somatic cells of one type into those of a different type, such as reprogramming skin epithelial cells to neural cells. The idea that a cell's identity is better described as a probabilistic property than a fixed one is now becoming more widely accepted.

Although work to date on cell reprogramming and other forms of cellular transformation, such as directed differentiation of a pluripotent or multipotent progenitor to a terminally differentiated cells, has largely relied on genetic or viral modification of cells, there is a small but rapidly increasing interest in the role that specifically designed small organic molecules may have in cellular reprogramming. This chapter summarizes some of the progress towards small molecule control of cell fate, and chemically induced cell reprogramming. The ability to have fine control over cell identity and fate will clearly lead to major medical advances in tissue and organ regeneration and cancer, now increasingly thought to have aberrant stem cells as a significant cause.

I will briefly summarize the properties and potential uses of stem cells, describe the role of gene regulatory networks in controlling cell fate decision and providing the origin of cell plasticity or stochastic behaviour, discuss how aberrant stem cell programming can drive cells towards detrimental phenotypes, then summarize early progress in the use of small organic molecules to control the fate of cells and drive transitions between different cell phenotypes:

- Pluripotent to somatic
- Somatic to pluripotent
- Somatic to somatic
- Aberrant pluripotent to somatic or death

The chapter will finish with a brief perspective of the future for small molecule-induced cellular reprogramming.

Properties and Potential Uses of Stem cells

Stem cells are cells with multiple differentiation options (Fig. 1). There are essentially three types: germ line stem cells, about which nothing further will be discussed; pluripotent stem cells (of which embryonic stem cells are a subset); and adult stem cells that have an essential role in maintaining bodily tissues that wear out, are damaged, or lost.

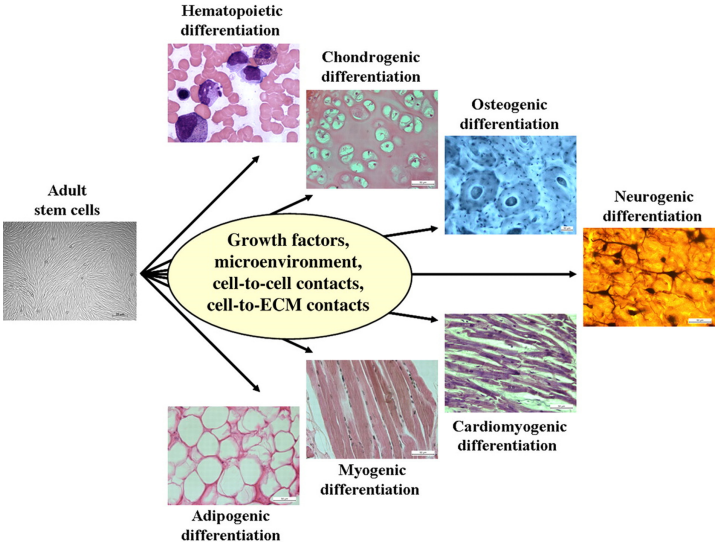


Figure 1. Multipotential capacity of embryonic stem or iPS cells to generate terminally differentiated tissues under the action of environmental factors. (Danišovič *et al.* (2012) *Exp. Biol. Med.* **237:**10 – 17)

Embryonic stem cells (ES cells) are transient pluripotent stem cells derived from the inner cell mass of the embryonic blastocyst. They possess two distinctive properties: pluripotency (can generate cells of almost any type): ability to replicate almost indefinitely.

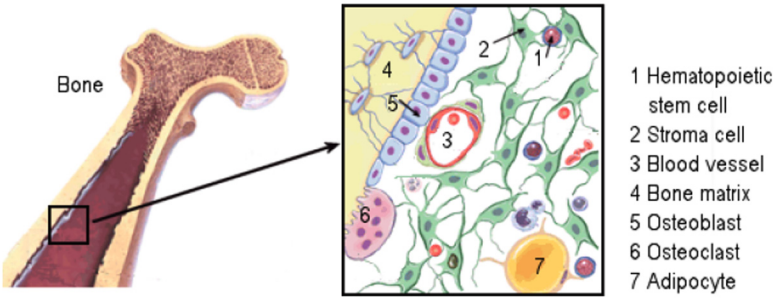


Figure 2. Illustration of HSC niche in the endosteal region of the bone marrow showing some of the niche components.

Adult stem cells are undifferentiated cells, found throughout the body, that divide to replenish dying cells and regenerate damaged tissues. They reside in very specific '*niches*' that control their fates (see figure 2 for an illustration of a haematopoietic stem cell niche). They possess two important properties: self-renewal (the ability to go through numerous cycles of cell division while still maintaining its undifferentiated state); and multipotency or multidifferentiative potential-the ability to generate progeny of specific but limited cell types.

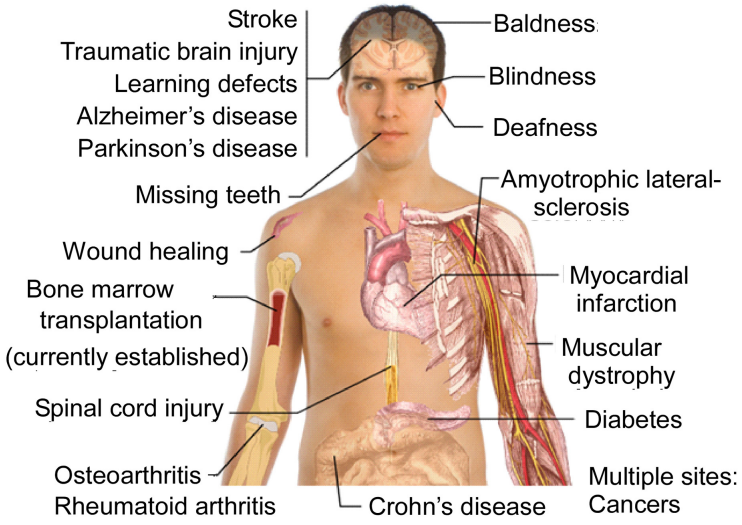


Figure 3. A summary of potential stem cell therapies for major diseases.

Because pluripotent stem cells have the potential to become many types of somatic cell types if their differentiation can be controlled, they offer incredible potential as therapies to replace worn, diseased, or damaged parts of the body. Reprogrammed cells have the additional advantage of allowing the patient's own cells to be transformed into new tissue, largely overcoming any immune rejection that allografts often encounter. Potential uses for stem cell therapies are summarized in figure 3.

Cells are Stochastic Objects, Pluripotency is a Probabilistic Property – cell plasticity

Cells and cell phenotypes were once thought to be fixed, and transitions between different cell types largely biologically or molecularly impossible because of epigenetic imprinting. Cell gene regulatory networks control phenotype, and cell state is now seen to be a potentially reversible, probabilistic state. Expressed phenotypes can be quite heterogeneous, regulatory trajectories can take multiple paths to the same endpoint, and cellular reversion or dedifferentiation is possible. Cell phenotypes are also heterogeneous, hinting at the underlying stochastic behaviour of gene expression, which is nonetheless still tightly regulated. This stochasticity is illustrated in figure 4 by the substantial fluctuations in expression of a key pluripotency transcription factor Nanog in mouse ES cells [3].

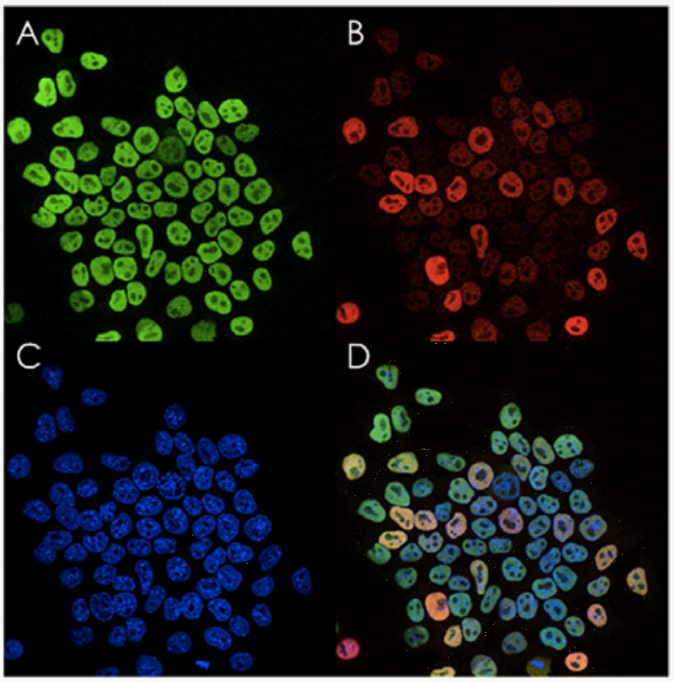


Figure 4. Immunofluorescence staining for **(A)** Oct4 and **(B)** Nanog, and **(C)** staining with DAPI; **(D)** an overlay of A-C. In mouse ES cells, Oct4 staining appears to be relatively homogeneous, whereas Nanog expression levels differ substantially within individual ES cells [3]. Roeder and Radtke (2009), *Development:* Image courtesy of Austin Smith.

Not only is expression of cell surface marker genes in stem cells stochastic and heterogeneous, but the trajectory of gene expression that is followed from one cell state to another can also be highly heterogeneous, as shown by Huang in elegant experiments summarized in figure 5 [4]. He drove neutrophils into differentiation using retinoic acid or DMSO. Both differentiation triggers resulted in the neutrophils differentiating into the same phenotypic cell but analysis of the gene expression profile of both processes showed that the trajectories were markedly different. This shows that cells can start at the same point but transit completely different regulatory gene expression programs before arriving at a common endpoint.

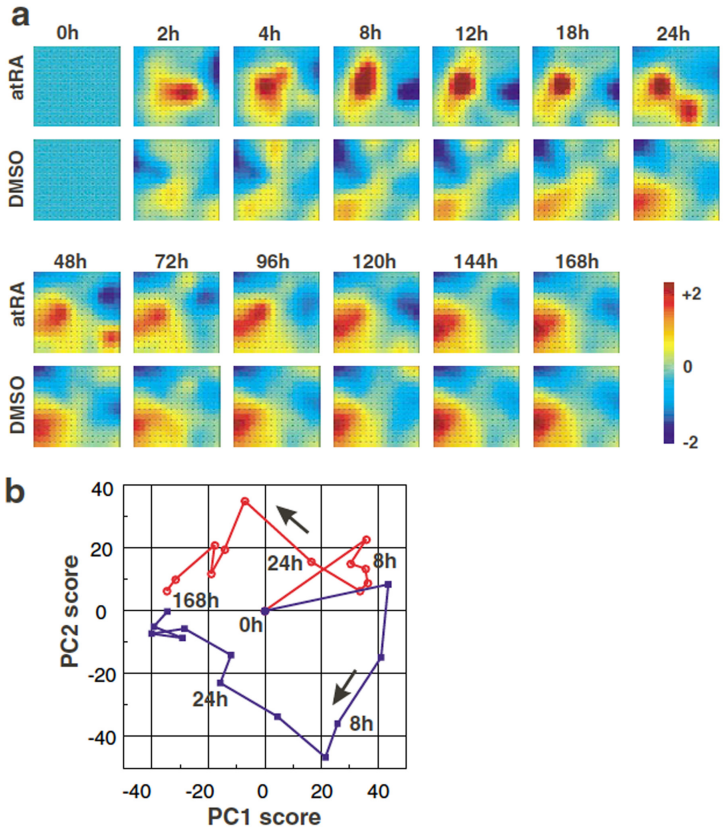


Figure 5. Comparison of the two gene expression trajectories during neutrophil differentiation. **(a)** The genes were clustered by a self-organizing map into 15 × 16 "miniclusters" with regard to their temporal profiles across both differentiation processes. Tile colors indicate the expression level of the cluster centroid; numbers on color bar: gene expression levels in SLR units. **(b)** Principal component analysis. Each point represents an individual expression profile S(t) within one of the two differentiation processes (red circles: RA; blue squares:DMSO) projected onto the first two principal components. Huang *et al.* (2005) PRL 94, 128701 [4]

These gene regulatory programs have been likened to mutable information networks where connections between nodes (genes) are made and broken depending on the presence and promoter/repressor binding of relevant transcription factors that control gene expression. Clearly, other control mechanisms involving microRNAs, changes to chromatin structure and epigenetic marks, and the presence of external cues such as growth factors, cytokines, adhesion molecules, cell-matrix and cell-cell mechanical and chemical cues can also influence the state and operation of gene regulatory networks (Fig. 6).

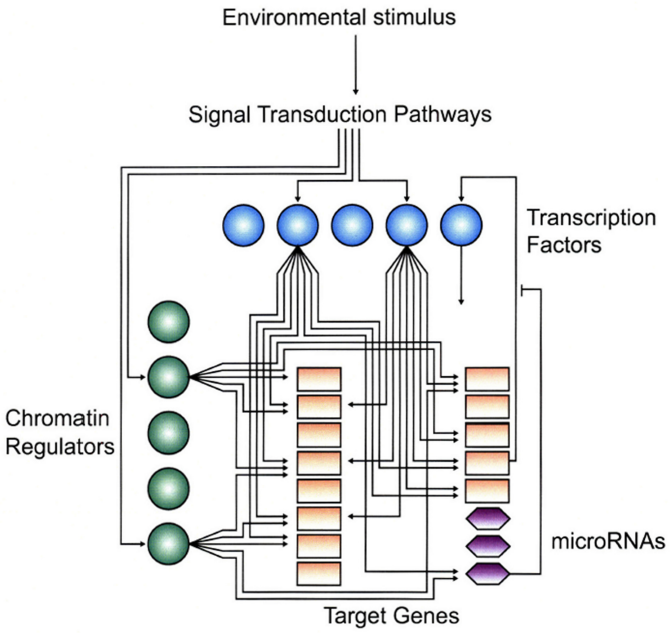


Figure 6. Representation of gene regulation as a control process. Marson Doctoral thesis MIT 2008

These networks are rich in regulatory loops, suggesting a complex system exhibiting a wide range of context-dependent dynamic behaviours. Such dynamic networks exhibit a set of stationary states called attractors (Fig. 7) that have been suggested by Kauffman to correspond to the observed number of different types of cells of the body [5]. Cell states and transitions between them can therefore be visualized as features on a gene regulatory surface or landscape, a term first coined by Waddington [6]. This simple but powerful description is relevant to the discussion of cellular reprogramming below.

Complex networks of gene interactions have a limited number of stationary attractor states. Kauffman hypothesized that these states correspond to stable cell phenotypes, and the region of states near an attractor that lead to that attractor is called the basin of attraction.

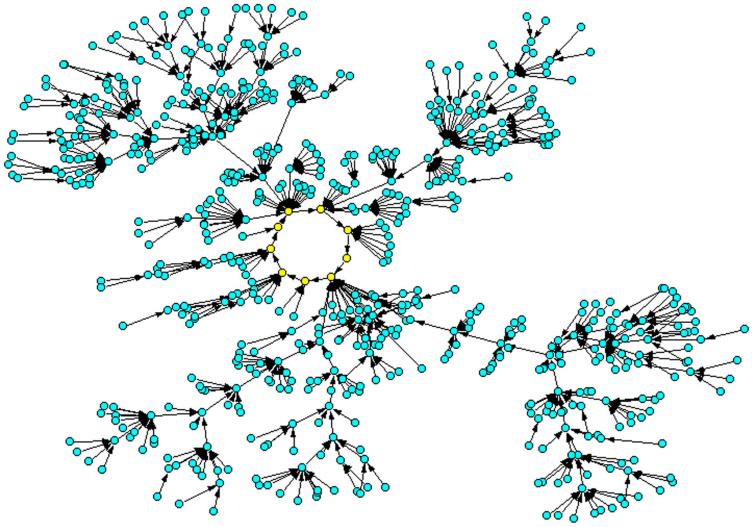


Figure 7. Attractors can be fixed (point) or cyclic. Kauffman (1969) *J. Theor. Biol.* **22:**437 – 467.

Cellular Reprogramming as Navigation Through a Complex Attractor Landscape

In a complex cellular attractor landscape there might be many coexisting stationary attractors (here represented as local minima), each of which might be associated with a unique molecular signature. In this view, cellular reprogramming corresponds to guiding the cell through the landscape from one local minimum to another (shown by the dotted arrows) [7]. As there might be many distinct paths between minima (both direct and through intermediary minima), reprogramming from one cell type to another might be achieved through numerous different routes (Fig. 8).

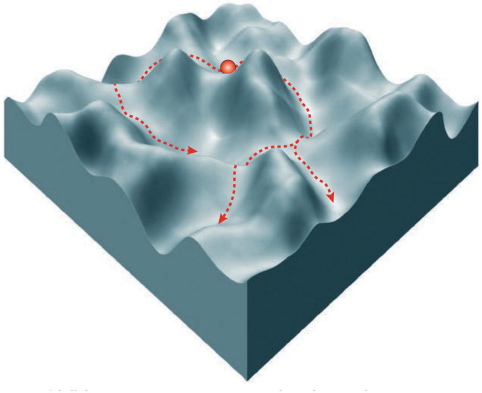


Figure 8. Waddington's epigenetic landscape. Macarthur *et al.* (2009) *Nat. Rev. Mol. Cell Biol.*

The valleys represent stable cell attractor (stationary) states generated by a hypothetical regulatory network. Depending on the particular configuration of the network (e. g. different parameter values, such as transcription or decay rates), a different number and/or different qualities of attractors are possible [3].

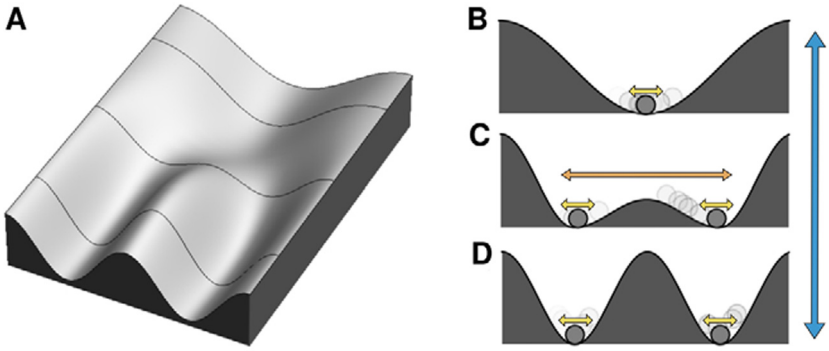


Figure 9. Cell fates or phenotypes as stable attractors (valleys) in regulatory landscape. Roeder and Radtke (2009) *Development.*

Modelling Stem Cell Fate Decisions

Simplified models of key switching mechanisms in pluripotent cells such as the SONs (Sox-2, Oct4, Nanog) network that maintains pluripotency [7], or the GATA-1/PU.1 switch that controls HSC differentiation fate, can be modelled using a number of different mathematical methods. Nonlinear dynamical theory, agent-based modelling, Boolean networks, and machine learning methods are common mathematical modelling techniques that have been applied to modelling gene regulatory networks and cell fate decisions. These simplified models that nonetheless capture the important behaviour of the cell can be useful in understanding fate decision mechanisms and controlling fate decisions artificially e. g. by small molecules. An example of a simple nonlinear rate equation model of the switch controlling HSC differentiation to myeloid or erythroid progenitors has been reported by Andrecut *et al.* recently [8].

Architecture of the self-activation and mutual repression two-gene circuit

HSC differentiate down the erythroid or myeloid pathways depending on the interplay between two key transcription factors, GATA-1 and PU.1. These factors antagonize the expression of the other but stimulate their own expression, a common regulatory switching motif, the bistable latch. This model is represented diagrammatically in figure 10.

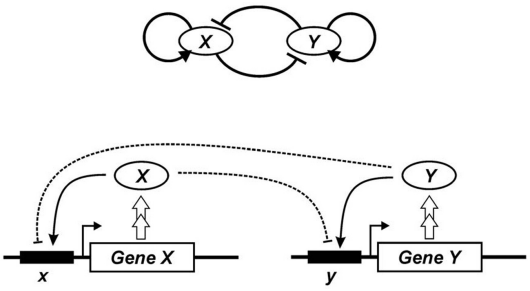
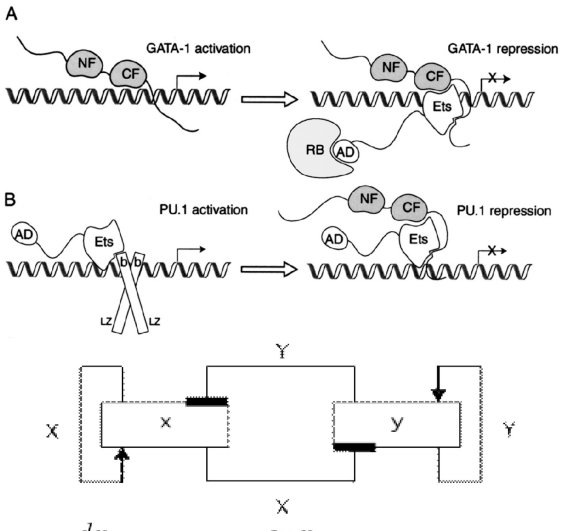


Figure 10. General representation of the bistable switch. Top: coarse-grained circuit scheme for the circuit of two genes X and Y as a dynamical system; bottom: molecular mechanism model amenable for a more detailed chemical reaction kinetics formalism, indicating the variables for the model due to the distinction between genes/promoters (x and y) and the transcription factor proteins (X, Y) [8]. Andrecut *et al.* (2011) *PLoS ONE* **6**(5):e19358.

The mutual antagonism of the two transcription factors, and their autocatalytic stimulation can be represented by a series of rate equations that can be solved numerically. The schematic of this switching system and form of the rate equations is shown in figure 11.



$$\frac{dx}{dt} = \frac{\alpha_0 x}{xy + \alpha_1 x + \alpha_2 y + \alpha_3} - \alpha_4 x$$

$$\frac{dy}{dt} = \frac{\beta_0 y}{xy + \beta_1 y + \beta_2 x + \beta_3} - \beta_4 y.$$

Figure 11. General rate equations describing the mutual antagonism and self-stimulation by the two transcription factors. Andrecut *et al.* (2011) *PLoS ONE* **6:**e19358. *doi:10.1371/journal.pone.0019358;* Winkler *et al.* (2009) *Artif. Life,* **15**(4):411.

When these equations are solved with added noise, this stochastic simulation of the system generates three noisy attractor configurations illustrated in figure 12. These three attractor states can be equated to the uncommitted multipotent attractor, the myeloid attractor with GATA-1 low and PU.1 high, and the erythroid attractor with GATA-1 high and PU.1 low. Interestingly addition of noise to the system generates a manifold of multipotency linking the two committed attractors to the multipotent stem cell state [8]. This may provide a theoretical explanation for the experimentally observed large statistical fluctuations observed in some transcription factors that surprisingly do not trigger a commitment to an associated differentiation pathway.

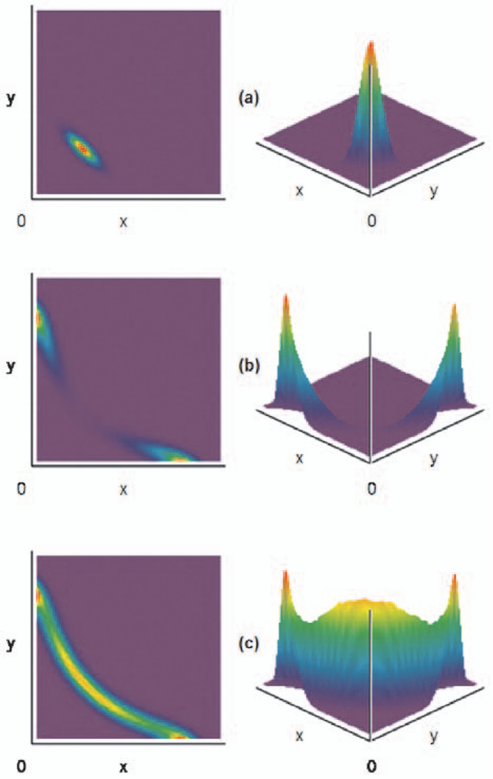


Figure 12. The results of the stochastic simulation of the system for three parameter configurations. **(a)** b.c, **(b)** c.b and **(c)** c = b. (see text for details). Colors (or elevation, respectively) represent the steady state probability distribution (cold-to-warm colors for low-to-high probability for finding the circuit at a given position in the xy-phase plane). Andrecut *et al.* (2011) *PLoS ONE* **6**(5):e19358.

Modelling cell fate decisions is one important issue. However, detecting experimentally at an early stage in commitment which decision has been made is also a key and very difficult problem. For example, stem cells must undergo symmetrical division (to become two stem cells) and asymmetric division (to become one stem cell and one progenitor cell) to maintain the stem cell compartment and provide the progenitor cells that generate the required fully differentiated somatic cells (Fig. 13). It is difficult to detect the symmetry of stem cell division. Coupling of gene expression microarray experiments with modern sparse mathematical feature selection methods can help identify markers of the symmetry of cell division.

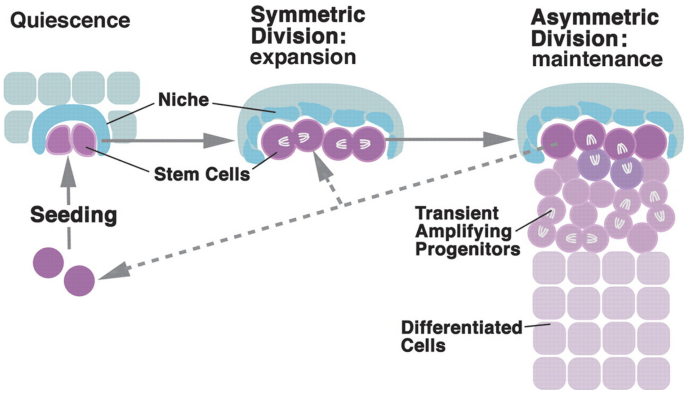


Figure 13. Representation of symmetric and asymmetric stem cell division. *Genes & Dev.* 2007. **21:**3044 – 3060.

Sherley *et al.* recently reported the successful application of this technique to the identification of candidate markers for cell division symmetry [9, 10]. Sparse Bayesian feature selection methods identified a small number of genes from the large number differentially expressed on microarrays derived from the experiments in which the symmetry of cell division was switched artificially by temperature, Zn levels, or p53 expression. Two of the genes identified have striking phenotypes indicative of asymmetric self-renewal in an engineered model cell line. One protein is only down regulated in one sister cell of asymmetric self-renewal divisions as figure 14 illustrates.

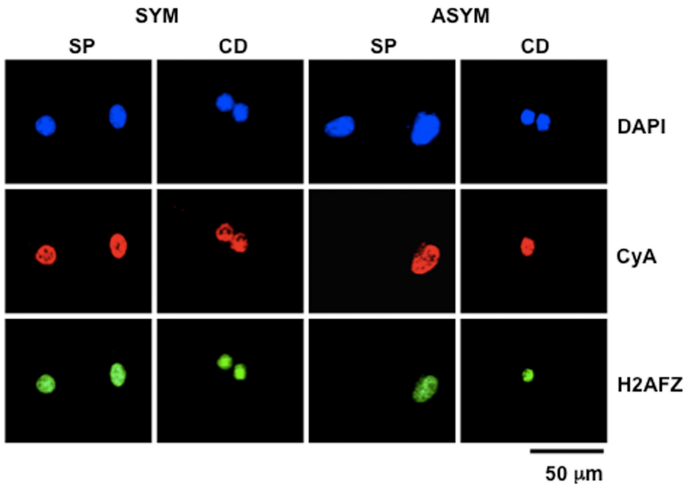


Figure 14. Fluorescently labelled antibodies to one of the cell symmetry marker illustrating localization to one nucleus of a dividing cell (asymmetric division) or both nuclei (symmetric division). Sherley, Smith, Burden, Winkler, *Science,* in preparation.

Small Molecule Modulators of Stem Cell Fate

Gene regulatory networks that control cell fates decisions are also influenced by external factors such as signaling molecules, cell-cell and cell-matrix interactions, surface compliance/modulus or roughness etc. While native growth factors provide key signals to stem cells via surface expressed receptors that can be used to control stem cell fate, it is not practical to immobilize all of these in engineered products like smart surfaces, implants, or in bioreactors. Additionally, viral transfection methods of reprogramming cells have disadvantages in efficiency and potential risks as a result of genomic integration; limitations that small molecules could circumvent. Small organic molecules that can reliably switch or reprogram cells would have a number of important advantages:

- Chemical and thermal stability
- Control over structure
- Low cost
- Control over tethering position
- Ability to control presentation on surfaces
- Spin-off IP value as drugs or reagents
- Molecular specificity, therefore control over off-target effects

Small Molecule Drivers of Fate Transitions

Stem cell fate (survival, proliferation, differentiation, apoptosis) is controlled by a balance between intrinsic internal state of the regulatory network and the presence of external or extrinsic chemical signals provided by e. g.:

- Cytokines
- Growth factors
- Other soluble factors etc.

Cell-matrix and cell-cell interactions via mechanical forces and adhesion factors are also important modulators of fate e. g.:

- Integrins (cell-matrix adhesion)
- Cadherins (cell-cell adhesion)
- Elastic modulus
- Surface patterning
- 2D or 3D environment
- Chemotactic gradients etc.

Surprisingly, given the importance of being able to control stem cell fate decisions, there has been *relatively little* medicinal chemistry research to discover small molecules that can influence cell fate. Notable research efforts include:

- Screening of large chemical libraries for compounds that affect stem cell fate (Figure 15) [11, 12];
- Use of 2i/3i (small molecule antagonists) to maintain 'ground state' pluripotency in mouse [13];
- Rational design of small molecule that mimic protein-protein interactions e. g. small molecule mimetics of cytokines and growth factors that drive adult stem cells down specific differentiation pathways (14 – 16)

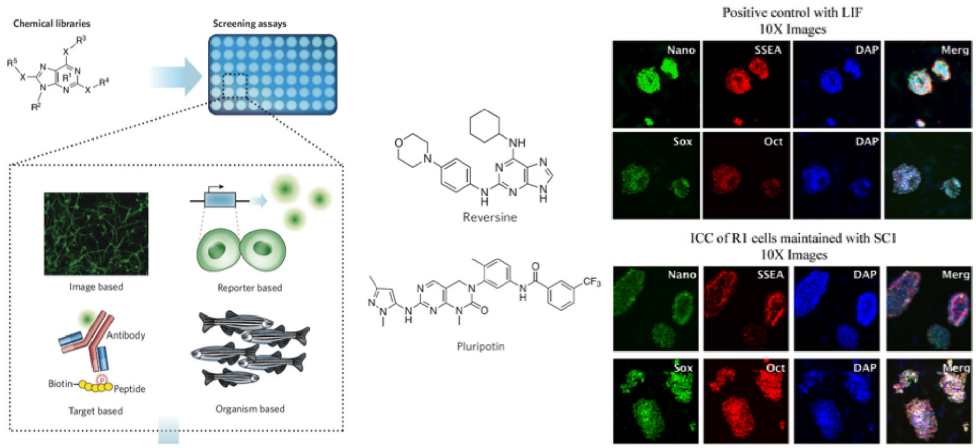


Figure 15. LIF vs. pluripotin for mESC culture. Ding library screening process and two small molecule stem cell effectors discovered. Yue Xu, Yan Shi & Sheng Ding, A chemical approach to stem-cell biology and regenerative medicine

Pluripotent to pluripotent – pluripotency maintenance or self-renewal

Recently, small molecule ligands of the aryl hydrocarbon receptor have been shown to promote self-renewal in HSCs. Regenin1 or SR1 (Fig. 16), and other compounds were identified by screening for compounds that stimulate expansion of CD34+ cells. Subsequently, it was shown that the small molecule is an antagonist of the aryl hydrocarbon receptor (a nuclear receptor); they did not specifically search for an antagonist. lty and SR1 was shown to stimulate up to 50-fold expansion of CD34+ cells that maintain full multi-lineage potential and engraft efficiently in mouse transplant models [17].

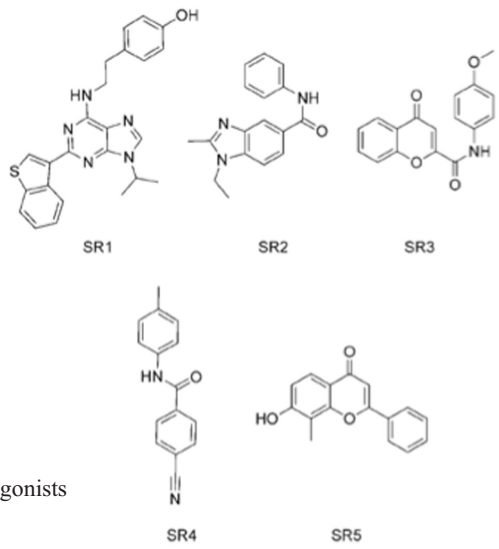


Figure 16. Small molecule antagonists of the AHR.

The Smith group in Cambridge reported a combination of two or three small molecule inhibitors (2i/3i) that maintain mouse ES cells in a pristine 'ground' pluripotent state [13]. These three inhibitors are illustrated in figure 17.

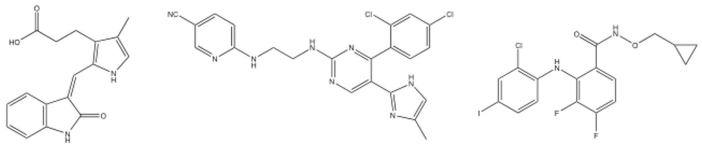


Figure 17. Structure and targets of three small molecule inhibitors that maintain the mES ground state. Ying *et al.* (2008) *Nature* **453:**519; Austin Smith *(WO/2007/113505)* Culture medium containing kinase inhibitors and uses thereof.

Pluripotent to somatic – directed differentiation

Mimetics of cytokines, growth factors, or cell adhesion interactions can be used to direct the differentiation of multipotent cells towards desired terminally differentiated cells. Designing small molecules to mimic or block protein-protein interactions is very difficult, but an increasing number of successes are being reported. These small molecules can affect stem cell differentiation directly or when incorporated into artificial bioreactors, but also have

intrinsic value as new drugs. Our interest has been in designing mimetic of haematopoietic growth factors and adhesion molecules as robust components of smart surfaces, bioreactors, or as haematological drugs.

The important components of bone marrow niche include [18 – 20]:

- The extracellular matrix: network of osteoblasts, collagen, integrins, fibronectin, aggrecan and link
- growth factors: thrombopoietin, stem cell factor, interleukins (IL-3, IL-6), FLT3L, Notch ligands

We initially focused on designing mimetics of the growth factor thrombopoietin (TPO) that would function as agonists or antagonists of its receptor c-Mpl. We chose TPO because it is a key niche growth factor, there are > 15 known chemical classes of TPO mimicking compounds, although there is no X-ray structure of c-Mpl to aid rational design of ligands.

Thrombopoietin plays a critical role in differentiation of HSCs down the megakaryocytic pathway (Fig. 18) [15].

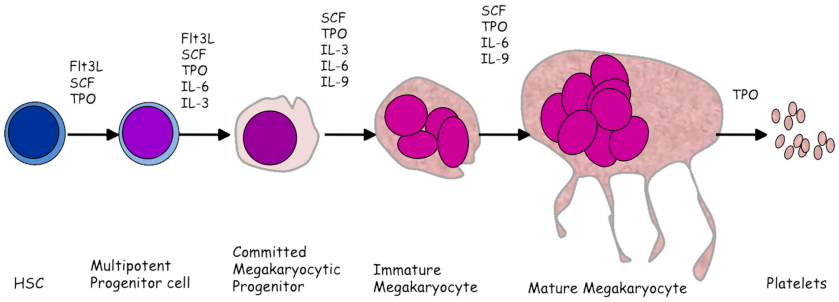


Figure 18. Megakaryocyte pathway promoted by TPO or agonists.

Unusually c-Mpl provides at least two different binding sites for small molecule mimetics. There is a mutation in human and chimpanzee c-Mpl transmembrane domain that places a histidine in the transmembrane helix. This interacts with a large set of small molecules drugs exemplified by Eltrombopag that penetrate the cell membrane, interact with His499 and cause the receptor to dimerise, switch, and facilitate downstream signalling (Fig. 19) [15, 16].

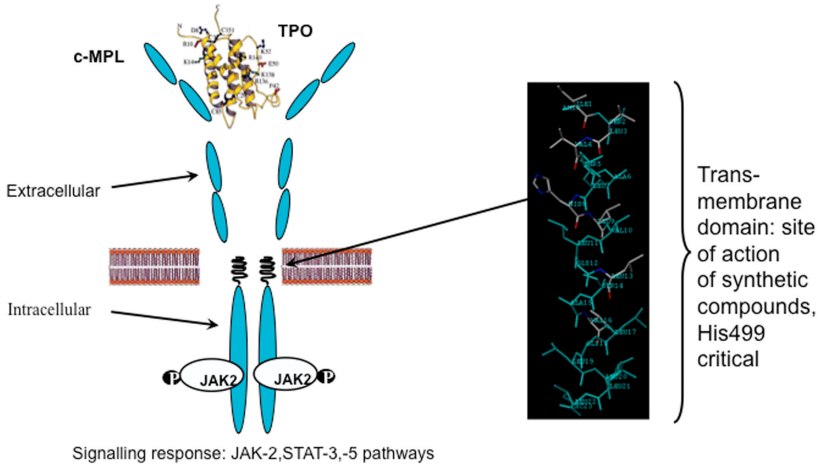


Figure 19. C-Mpl transmembrane domain showing the location of unique His499.

A selection of the chemical classes that interact with this transmembrane domain in c-Mpl is summarized in figure 20. Molecular modelling methods can locate the low energy conformations (shapes) and common structural alignments that allow the molecular features modulating agonist activity to be elucidated (Fig. 21) [16].

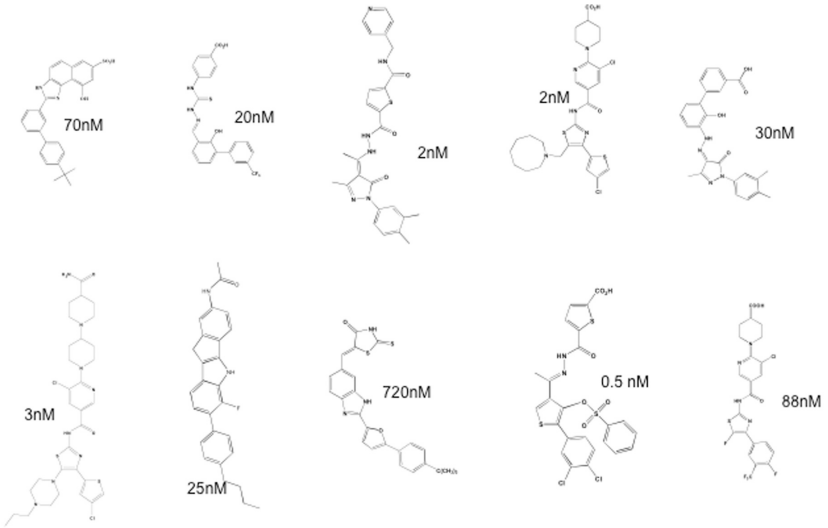


Figure 20. Typical chemical classes of potent small molecule agonists of c-Mpl acting at the transmembrane domain.

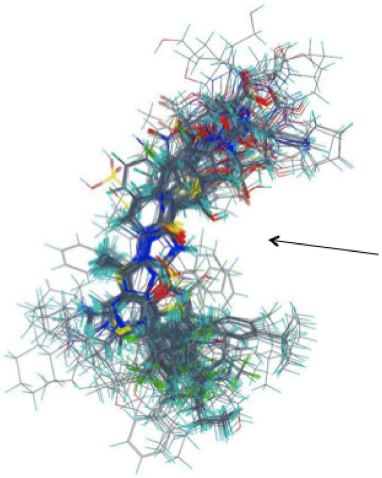


Figure 21. Common structural motifs and alignments used for modelling TPO agonists [16].

The other potential binding site for small molecule mimetics of TPO is at the extracellular domain of the receptor where the natural ligand TPO binds (Fig. 17). Phage display experiments reported by Cwirla *et al.* identified small peptides that act as agonists and antagonists of c-Mpl [21]. We identified a small conserved epitope (RQW) in these peptides and investigated the role of truncations, mutations, cyclization, dimerization and different dimer linker lengths on agonist activity. This culminated in the discovery of several potent c-Mpl agonists with nanomolar activity in the factor dependent primary cell screen (Fig. 22), in primary CD34+ HSCs, and *in vivo* (Fig. 23) [22]. We also generated the first nanomolar antagonist of c-Mpl.

Peptide	Sequence	%TPO	EC_{50} nM
Dimer	IEGPTLRQWLAARA-K-ARAALWQRLTPGEI | β-Ala	121±15	3.7±2.8
Dimer with pendant K	FmocIEGPTLRQWLAARA-K-ARAALWQRLTPGEIFmoc | β-Ala	105 143	1.0 0.8
Dimer with pendant K and LC-biotin	FmocIEGPTLRQWLAARA-K-ARAALWQRLTPGEIFmoc | β-Ala | K | LC-biotin	102	11
Dimer with pendant K and PEG(2000)-biotin	FmocIEGPTLRQWLAARA-K-ARAALWQRLTPGEIFmoc | β-Ala | K | PEG(2000)-biotin	108 116 113	1.8 3.4 7.5
Truncated Dimer with pendant K	Fmoc-LRQWLAARA-K-ARAALWQRL-Fmoc | β-Ala | K	126	840
Linear-Linear Dimer	AcIEGPTLRQWLAARA**GKG**ARAALWQRLTPGEIAm	85	122
Retroinverso Linear-Linear Dimer	AcIEGPTLRQWLAARA**GKG**IEGPTLRQWLAARAAm	111	3.8
Linear-Truncated Linear	AcIEGPTLRQWLAARA**GKG**ARAALWQRLAm	85	790

Figure 22. Agonist activity of TPO-mimetic peptides.

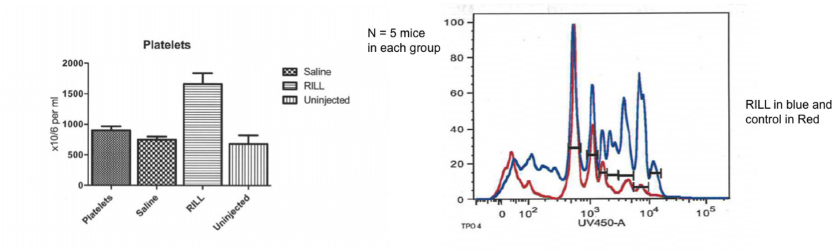
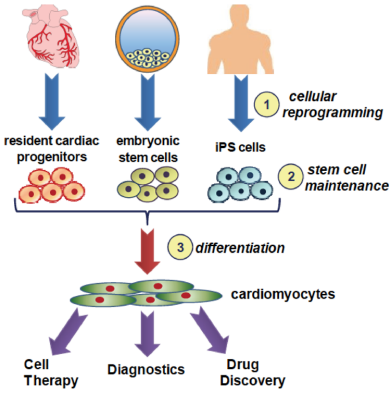


Figure 23. *In vivo* agonist activity of small molecule TPO mimetic. There is a dramatic increase in platelets (left) and megakaryocyte ploidy (right).

Hao *et al.* recently reported small molecules that promote differentiation of ES or iPS cells into cardiomyocytes suitable for cell therapy, cardiac diagnosis, or as screens for new cardiac drug discovery (Fig. 24) [23].



Molecule	Name	Target	reference
	Pluripotin/SC1	Dual inhibitor of RasGAP/ERK1	Chen, *et al.*, PNAS 2006 Ref 18
	CHIR99021	GSK3 inhibitor	Ying *et al.*, Nature 2008 Ref 19
	PD0325901	MEK inhibitor	Ying *et al.*, Nature 2008 Ref 19
	IQ-1	Phosphatase PP2A Inhibitor (Wnt modulator)	Miyabayashi *et al.*, PNAS 2007 Ref 20

Table 1. Small molecules for maintenance of pluripotent stem cells

Figure 24. Small molecules for SC-based cardiology. Hao *et al.* (2011). Chemical Biology of Pluripotent Stem Cells: Focus on Cardio-myogenesis, in *Embryonic Stem Cells* – Recent

Somatic to pluripotent: Induced pluripotent stem (iPS) cells

Yamanaka was the first to reprogram somatic cells to pluripotency [23, 25]. The main approaches used or proposed to date are (Figure 23):

- Use of retroviruses to transduce mouse fibroblasts with Oct-3/4, SOX2, c-Myc, and Klf4a, the four key pluripotency genes essential for the production of pluripotent stem cells. As c-Myc is oncogenic, 20% of the chimeric mice developed cancer.
- Retroviral mediated reactivation of the same four endogenous pluripotent factors, but selected Nanog as a pluripotency marker. Yamanaka created iPS cells without c-Myc, less efficient, but reduced the risk of cancer.

- Using small compounds that can mimic the effects of transcription factors. While this is not possible yet, the ultimate goal is to discover a cocktail of reprogramming factors and compounds that efficiently and reliably reprogram somatic cells to iPS cells
- Reprogramming through the use of drug-like chemicals activating specific molecular targets, not mimicking transcription factors
- Use of naked DNA, RNA, siRNA and related approaches.

Small molecule compounds may be able to compensate for a reprogramming factor that does not effectively target the genome or fails at reprogramming for another reason, raising reprogramming efficiency. They also avoid the problem of genomic integration, which in some cases contributes to tumorogenesis.

Huangfu *et al.* [26] found that the histone deacetylase inhibitor valproic acid increased reprogramming efficiency 100-fold (compared to Yamanaka's traditional transcription factor method) and proposed that this compound was mimicking the signalling caused by the transcription factor c-Myc without being oncogenic. A similar type of compensation mechanism was proposed to mimic the effects of Sox2. Likewise, Ding *et al.* [27] inhibited histone methyl transferase with the small molecule BIX-01294 in combination with the activation of calcium channels in the plasma membrane to increase reprogramming efficiency. It is foreseeable that such experiments will continue to find small compounds that improve efficiency rates.

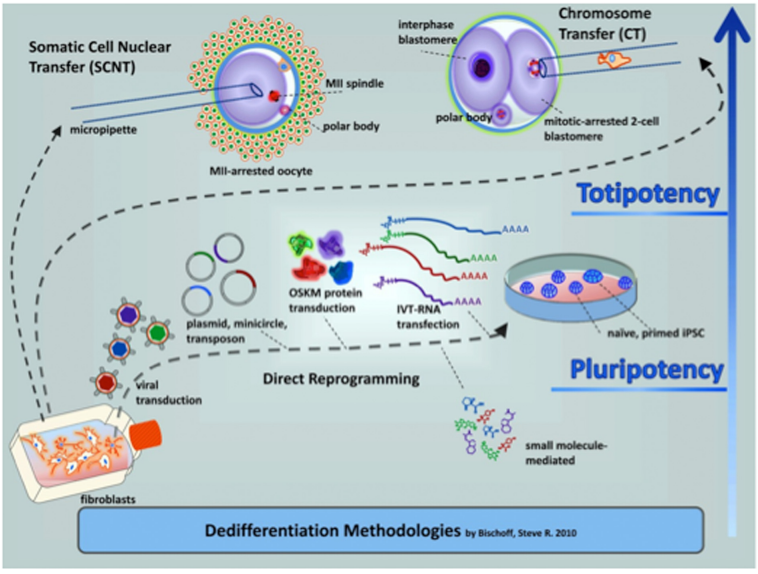


Figure 25. Gene, viral and small molecule methods for dedifferentiation and somatic cell reprogramming to pluripotency. From: *Advances in Pluripotent Stem Cell-Based Regenerative Medicine*, Atwood (Ed.), ISBN: 978-953-307-198-5, (23)

Somatic to somatic

The recent recognition of the plasticity of cell identity and the nascent capabilities to reprogram some cells into other types leads inevitably to speculation on whether any cell can in principle be reprogrammed into anything else. This is termed direct reprogramming and is attracting considerable interest. Li *et al.* [28] have recently demonstrated that transient overexpression of reprogramming factors in fibroblasts leads to the rapid generation of epigenetically activated cells (unstable intermediate populations). These can be coaxed to back into various differentiated state(s), ultimately giving rise to fully differentiated cells entirely distinct from the starting population, as figure 26 illustrates.

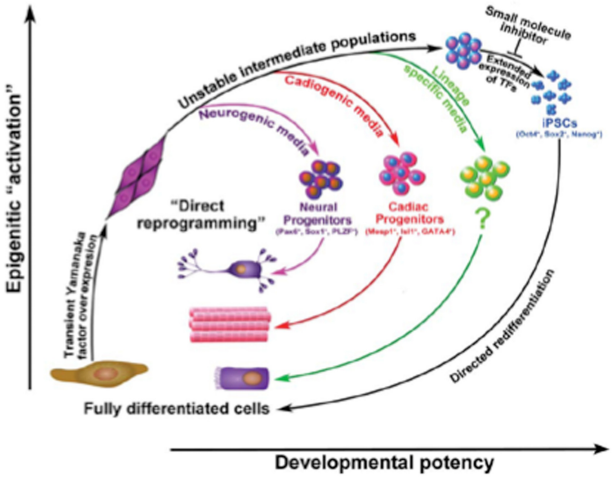


Figure 26. Direct reprogramming of somatic cells to other cell phenotypes using small molecules. Li *et al.* (2012) *Stem Cells* **30:**61 – 68.

A related report by Kim *et al.* [29] outlined a strategy for discovery of small molecules with potential for limb regeneration. They also involved a high throughput screen of chemical libraries to identify molecules capable of altering proliferation and gene expression profiles in urodele amphibian skeletal muscle cells. The small molecules BIO (glycogen synthase-3 kinase inhibitor), lysophosphatidic acid (pleiotropic activator of G-protein-coupled receptors), SB203580 (p38 MAP kinase inhibitor), or SQ22536 (adenylyl cyclase inhibitor) induced proliferation. These proliferating cells were multipotent and possessed a markedly different gene expression pattern than lineage-restricted myoblasts. Genes related to signal transduction and differentiation were particularly affected (Fig. 27). Some molecules were found to promote skeletal muscle dedifferentiation and differentiation into alternate cell types.

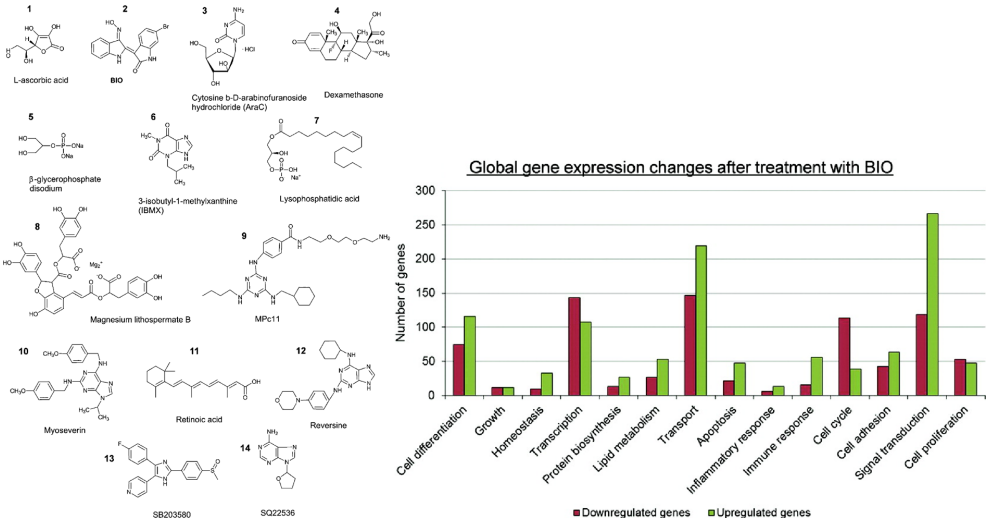


Figure 27. Small molecules discovered by chemical library screens that promote dedifferentiation of muscle cells into multipotent cells with markedly different gene expression profiles. Kim *et al.* (2012) *ACS Chem. Biol.* ASAP.

Aberrant pluripotent to somatic or death

The recent recognition that aberrant stem cell, cancer stem cells, play an important role on many forms of cancer has provided a promising new approach to therapy. Several groups are investigating targeting cancer stem cells directly using small molecules [30]. While this research is at a very early stage, if cancer stem cells can be selectively identified, killed, or forced to differentiate treatments may be more effective and recurrence of drug resistant tumour less likely. Ischenko *et al.* [30] reported that long-term treatment of gliomasphere cells with the cyclopamine (Fig. 26) alone killed all cancer stem cells in culture, and induced the regression of glioma tumors established from the gliomasphere cells in nude mice *in vivo*, without detectable secondary effects.

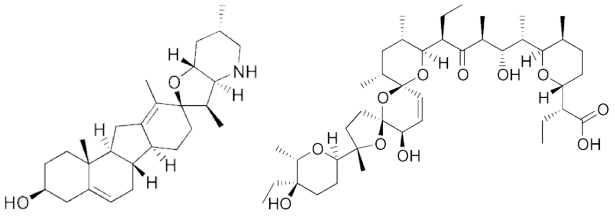


Figure 28. Cyclopamine (left) and Salinomycin (right). Ischenko *et al.* (2008) *Current Medicinal Chemistry*/Wiki Commons.

Gupta *et al.* [31] also adopted a small molecule library screening approach to discover compounds targeting breast cancer stem cells. They reported that salinomycin reduces the proportion of breast cancer stem cells in culture by > 100-fold compared to another cytotoxic cancer drug paclitaxel, Salinomycin also inhibits mammary tumour growth *in vivo* and reduce the expression of breast cancer stem cell-related genes.

Perspective

Clearly, it is early days in the discovery, design, and use of small organic molecules and peptides to control cell fate. Research over the past five years has been very encouraging. It is likely that reprogramming of somatic cells to pluripotency will be achievable largely or entirely by small molecules in short to medium time frames. There is increasing evidence that physical and chemical cues can control cell fate, and that transdifferentiation of one somatic cell type to another without isolating any multipotent intermediate may also be possible in the medium term. There are good prospects that this will be achieved using a combination of topographical, mechanical and/or chemical cues. This has interesting implications for tissue replacement and engineering, providing hope that many injuries, illnesses and congenital defects can be treated effectively where current therapies do not exist or provide a poor outcome. One of the most exciting prospects is selective targeting of cancer stem cells by small molecule drugs. If this is realized, treatments for cancers could improve dramatically because the underlying cause of many tumours is eliminated. Time will tell how many of these exciting possibilities come to fruition. Finally, while this chapter was being finalized, it was announced that the Nobel Prize in Medicine had been awarded to Gurdon and Yamanaka in recognition of their discovery of cellular reprogramming.

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