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Stem cell biology meets systems biology

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Stem cells and their descendents are the building blocks of life. How stem cell populations guarantee their maintenance and/or self-renewal, and how individual stem cells decide to transit from one cell stage to another to generate different cell types are long-standing and fascinating questions in the field. Here, we review the discussions that took place at a recent EMBO conference in Cambridge, UK, in which these questions were placed in the context of the latest advances in stem cell biology in presentations that covered stem cell heterogeneity, cell fate decision-making, induced pluripotency, as well as the mathematical modelling of these phenomena.

Introduction

In June 2009, the 4th EMBO conference entitled 'Advances in Stem Cell Research: Stem cells, Systems and Synthetic Biology', organised by Austin Smith (University of Cambridge, UK) and Mike Tyers (University of Edinburgh, UK), took place in Cambridge, UK, as part of the scientific communication and networking component of the EuroSyStem Project (www.eurosystemproject.eu). It brought together 151 researchers from 31 countries to discuss and exchange their latest results and ideas on fundamental questions concerning stem cell biology, such as stem cell self-renewal, lineage decisions and commitment, as well as reprogramming.

The meeting highlighted the importance of experimental and systems biological approaches for achieving a better understanding of stem cell behaviour and properties. The increasing influence of computational and modelling methods in the field of stem cell biology was illustrated by the fact that half of all the conference talks contained theoretical results. The computational methods presented were not restricted to data exploration and analysis. Instead, many of the speakers proposed theoretical concepts and mathematical models that aimed to explain quantitatively biological mechanisms.

A key topic addressed by many speakers was the investigation of the mechanisms of cellular state transitions. The heterogeneity of stem cells and stem cell populations, a topic that is closely related to the issue of potentially reversible state transitions, was also discussed as an extremely important topic in stem cell biology. The presentation of recent experimental results and of new mathematical modelling approaches related to these fields was complemented by talks that presented upcoming technologies, such as genome-wide screening approaches or the construction and analysis of synthetic regulatory networks. To provide a concise overview of the topics discussed during this meeting, we briefly summarise the key findings according to the following two themes: (1) mechanisms of cellular state transitions; and (2) cellular heterogeneity of stem cells and stem cell populations.

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Mechanisms and models of cellular state transitions

The question of how cell fate decisions are made is not new. However, research in this area has intensified since the demonstration that pluripotency can be induced in somatic cells by the overexpression of a small number of transcription factors (Takahashi and Yamanaka, 2006; Takahashi et al., 2007; Okita et al., 2007). These experiments proved both the general reversibility of cellular development, which had in principle already been shown by cloning (Wilmut et al., 1997) and by nuclear reprogramming experiments (Eggan et al., 2004; Hochedlinger and Jaenisch, 2002), and that transcriptional regulation is able to reset the differentiation status of cells. This insight strengthened the view that a detailed understanding of the transcriptional network of (stem) cells will enable researchers to control differentiation more efficiently and, therefore, to (re)program cells in order to use them in different clinical applications.

In addition to experimental investigations of different types and mechanisms of cellular state transitions, and in addition to the development of increasingly sophisticated differentiation and (re)programming protocols, theoretical approaches are also required to disentangle and understand the regulatory principles of (stem) cell organisation. Thus, this meeting aimed to bring together a coalition of experimental and theoretical researchers in the field of stem cell biology in order to broaden our understanding of cellular state transitions.

Examples of cell state transitions

At the meeting, different examples of cell state transitions were presented and complemented by discussions of various mechanisms that (potentially) affect or even drive these events.

Margaret Fuller (Stanford University, CA, USA) explained how local signals from the microenvironment influence the behaviour of male germline stem cells (GSCs) in *Drosophila*. Adherens junctions physically attach the GSCs to hub (niche) cells, providing a polarity cue, which results in asymmetric cell division (Yamashita et al., 2007). The cell that remains in contact with the niche cell persists as a stem cell, while the other daughter cell differentiates. Signals involved in GSC maintenance include the STAT (STAT92E – FlyBase) transcription factor, which is induced by the niche cells, and transforming growth factor β (TGFβ) class signals from surrounding cells, both of which repress the expression of important differentiation factors in the GSCs. Interestingly, cells that are starting to differentiate [so-called transient amplifying (TA) cells], can revert to the stem cell fate if they recontact the niche cells, suggesting that extrinsic signals can reverse cell states.

The interpretation of microenvironmental signals was also the focus of the talk by Peter Swain (University of Edinburgh, UK), but his main interest is the theoretical explanation of the typical sigmoidal (i.e. S-shaped) response of many genetic and signalling networks as the concentration of input signals to the network increases. He argues that sigmoidal responses occur because cells infer changes in the state of the extracellular environment from intracellular changes or from local changes at the membrane. If a cell is inferring whether the environment is in one of two possible states, then a sigmoidal response could be understood as the cell's biochemical implementation of a so-called 'Bayesian' inference. That means, the response of a cell is proportional to the probability of the environmental states, given the actual biochemical sensing result.

Takashi Hiiragi (Max-Planck Institute, Muenster, Germany) reported on mechanisms that contribute to the induction of asymmetry during mouse blastocyst development in inner cell mass (ICM) specification. His laboratory developed a fluorescence-based promoter-trap screen in mouse embryos to identify novel players involved in embryonic patterning. Using four-dimensional live imaging of mouse embryos, his group can precisely track lineage segregation during early development. The results he presented on genes that are active specifically in either one of the two earliest lineages (ICM or trophectoderm) hint at the involvement of stochastic processes during embryonic patterning, consistent with this group's previous reports (Dietrich and Hiiragi, 2007; Dietrich and Hiiragi, 2008).

Haematopoietic stem cells (HSCs) are one of the most extensively studied types of stem cell. Although it is well established that definitive (d)HSCs, which maintain the blood system throughout life, develop during embryogenesis at ~E10-11, very little is known about the stages of their differentiation from mesoderm. In this context, Shinichi Nischikawa (Riken Centre of Developmental Biology, Kobe, Japan) presented data describing four distinct stages during mouse haematopoietic development, starting from Flk1 $(Kdr)^+$ Etv2 $(ER71)^+$ blood cells in the yolk sac (E7.0-7.5) to VE-Cad $(Cdh5)^+$ $Runx1^+$ haemogenic endothelial cells, which integrate into the vascular luminal wall of, for example, the dorsal aorta and umbilical artery, before budding off in order to generate CD45 $(Ptprc)^+$ blood cells.

Margaret Buckingham (Institute Pasteur, Paris, France) summarised previous work from her laboratory on skeletal muscle differentiation and its dependence on the Pax3 and Pax7 transcription factors (Relaix et al., 2005). Myogenic progenitor cells derive from multipotent $Pax3^+$ cells in the embryonic somite, in which reciprocal repression between *Pax3* and *Pax7* and a gene that encodes another key transcriptional regulator was demonstrated. Buckingham reported that perturbation of this equilibrium affects the cell fate choice of developing myogenic progenitors. These recent findings provide a model for the maintenance of multipotency and for how cells exit from this state.

Inducing cell state transitions: programming, reprogramming and induced pluripotency

The fact that transcription factors affect cell differentiation has long been known; that they can also facilitate the reprogramming of the cellular state is a more recent discovery (Takahashi et al., 2007; Takahashi and Yamanaka, 2006). Several presentations dealt with the problem of efficient reprogramming protocols in different cell systems.

Thomas Graf (Centre for Genomic Regulation, Barcelona, Spain) presented data concerning the direct reprogramming of committed haematopoietic cells with transcription factors. He showed that a single factor, C/EBPα, can efficiently switch committed B and T cell precursors to acquire a macrophage phenotype (Laiosa et al., 2006; Xie et al., 2004). However, the effects of C/EBPα on the two lineages differed in that it reprogrammed fully mature B lymphocytes, whereas T lineage cells became partially resistant at the double-positive stage. These observations suggest that differentiation plasticity varies widely within the haematopoietic system and that the B lineage is particularly prone to transdifferentiate into macrophages.

Yann Barrandon (Ecole Polytechnique Fédérale de Lausanne, Switzerland) reported how unipotent stem cells derived from different stratified epithelia can be reprogrammed and can gain in potency when exposed to skin morphogenic signals (Claudinot et al., 2005). Moreover, he presented data exploring the functional relationship between thymic epithelial cells (TECs) and multipotent keratinocyte stem cells of the skin using clonal analysis and transplantation assays. Embryonic and postnatal TECs contain clonogenic epithelial cells, which retain morphological, phenotypical and functional properties of multipotent hair follicle stem cells, including the capacity to be serially transplanted and to generate all epithelial derivatives of rodent skin. Collectively, his data demonstrate that epithelial stem cells can modulate their gene expression program and cell fate in response to diverse microenvironments.

Interestingly, epithelial cells not only respond to differentiation cues of their microenvironment but can also influence the microenvironment itself. In this context, Freddy Radtke (Ecole Polytechnique Fédérale de Lausanne, Switzerland) presented data on how loss of Notch signalling in skin epithelium leads to the secretion of multiple cytokines that influence the underlying stroma and even the haematopoietic system. The cytokine-induced changes within this microenvironment cause two medical conditions, known as atopic dermatitis and myeloproliferative disease.

José Silva (University of Cambridge, UK) discussed the reprogramming of neural stem cells (NSCs) into induced pluripotent stem (iPS) cells. Silva reported how NSCs rapidly acquired an undifferentiated morphology after being transduced with the reprogramming factors Oct4 (Pou5f1), c-Myc and Klf4. However, their progression to a true iPS cell state, as characterised by the stable expression of endogenous Oct4 and Nanog and by X-chromosome reactivation in female cells, was only observed when cells were grown in the presence of the 2i medium (which inhibits mitogen-activated protein kinase and glycogen synthase kinase signalling) and leukaemia inhibitory factor (LIF) (Silva et al., 2008). Silva showed that Nanog is necessary for reprogramming NSCs into iPS cells, but is not required for their maintenance. However, when Nanog expression was elevated in epiblast stem cells (EpiSCs), they were able to revert to the 'ground state' of pluripotency (Silva et al., 2008).

Shinya Yamanaka (University of Kyoto, Japan) provided a seminal overview of the generation of mouse iPS cells (Takahashi and Yamanaka, 2006). In particular, he discussed the low efficiency with which iPS cells are generated and the importance of the cells of origin. iPS cells have been generated from multiple cell types, including mouse embryonic fibroblasts (MEFs), tail tip fibroblasts (TTFs) (Takahashi and Yamanaka, 2006), adult hepatocytes and adult stomach cells (Aoi et al., 2008), which raises the question of whether all of these iPS cells are identical. This was investigated by the generation of primary and secondary neurospheres and by assessing their ability to differentiate into different neural lineages using iPS cells generated from diverse cell types. These experiments revealed clear differences in the ability of some iPS cells to differentiate into neuronal lineages, which reflected their different origins.

Identification of regulatory network components

As discussed above, transcription factors and other molecular regulators play an important role in controlling stem cell fate decisions and cellular state transitions. Whereas some of the molecular regulators are already well known, others remain to be identified. There were several presentations that addressed the identification of important regulatory components of transcriptional networks that control self-renewal and/or differentiation.

Bertie Göttgens (University of Cambridge, UK) discussed the control of HSC differentiation by the basic helix-loop-helix

transcription factor Scl (Tal1). Using ChIP-Seq technology to identify the regulatory targets of Scl, his laboratory has generated a genome-wide catalogue of Scl binding events in a stem/progenitor cell line, identified multiple new direct Scl target genes, and reconstructed a transcriptional network that consists of 17 factors and their respective regulatory elements. Göttgens proposed that the coupling of ChIP-Seq in model cell lines with in vivo transgenic validation and bioinformatic analysis is a widely applicable strategy for reconstructing stem cell regulatory networks in which biological material is otherwise limiting (Wilson et al., 2009).

Gerald de Haan (University Medical Centre Groningen, The Netherlands) combined transcriptional profiling and genetic linkage analysis to dissect networks of interacting genes that specify cellular function in four developmentally distinct haematopoietic cell stages (Breitling et al., 2008; Bystrykh et al., 2005). His group evaluated genome-wide RNA expression in highly purified Lin^- Sca1 $(Ly6a)^+$ c-Kit^+ cells with multilineage potential, committed Lin^- $Sca1^-$ c-Kit^+ progenitor cells, erythroid Ter119 $(Ly76)^+$ and myeloid Gr1 $(Ly6g)^+$ precursor cells isolated from C57BL/6 × DBA/2 (BXD) recombinant mouse strains. Variation in transcript abundance was assessed by Illumina Sentrix Mouse-6 chip technology, and genetic linkage analysis identified quantitative trait loci (QTL) that affected the variation in the expression levels of corresponding genes (so-called eQTL). This dataset led to several predictions concerning the dynamic rewiring of the regulatory network, which need to be followed up by hypothesis-driven experimentation.

Guy Sauvageau (Université de Montréal, Canada) presented a retroviral-based strategy to identify components of the regulatory network of stem cells (Bilodeau et al., 2007). His group generated DELES, a library of ES cell clones that contain nested chromosomal deletions. When assayed for differentiation, a number of clones showed defects in embryoid body formation in vitro and in contributing to chimeric tissues in vivo. Their complementation studies, which involve reinserting coding and non-coding DNA via cDNA and modified BAC transfections, respectively, have revealed the potential rescue of a selected family of differentiation phenotypes, proving that this approach to identifying new regulators of ES cell differentiation works and should improve our knowledge of ES cell pluripotency regulation and differentiation.

Another approach to identifying regulatory components of stem cell self-renewal/differentiation was mentioned several times during the meeting: perturbation using RNAi screens. One example is the work of Frank Buchholz (Max-Planck Institute for Molecular Cell Biology and Genetics, Dresden, Germany), who presented the results of a genome-wide RNAi screen for genes that affect ES cell identity via alteration of Oct4 expression (Ding et al., 2009). Factors with the strongest effect on Oct4 expression included components of the Paf1 complex (Paf1C), a protein complex associated with RNA polymerase II. Buchholz demonstrated that Paf1C binds to promoters of key pluripotency genes, where it maintains a transcriptionally active chromatin structure. Paf1C is developmentally regulated and blocks ES cell differentiation upon overexpression. When knocked down in ES cells, Paf1C has similar effects to Oct4 or Nanog depletion, indicating that it has an important role in maintaining ES cell identity.

A further application of RNAi-induced system perturbations to study the dynamic regulation of cell fate changes in mouse ES cells was presented by Ihor Lemischka (The New York Stem Cell Foundation, Black Family Stem Cell Institute, New York, NY, USA). Global changes in histone acetylation and RNA polymerase II transcription, as well as in mRNA and protein abundance, were measured by his group over 5 days after the specific depletion of Nanog, a key pluripotency regulator. These data profiled how the perturbation of a single gene progressively led to distinct changes in the pluripotency network at multiple molecular levels over time and provide a dynamic view of information flow in the epigenome, transcriptome and proteome networks. Florian Markowetz (Cancer Research UK, Cambridge Research Institute, UK) demonstrated how this wealth of data can be used in follow-up studies to address specific biological questions. He concentrated on dependencies between histone acetylation and gene expression and showed how the level of coordination between them increases over time and how changes in histone acetylation are surprisingly predictive of changes in gene expression.

Other screening methods were also presented. For example, Manfred Auer (University of Edinburgh, UK) introduced the 'single-bead' technology (Hintersteiner and Auer, 2008; Meisner et al., 2009), which is part of an integrated chemical biophysics (ICB) platform that can screen up to 400,000 compounds in a single day. Another screening approach was presented by Edda Klipp (Humboldt University Berlin, Germany), who presented a newly initiated project on screening for small molecules that affect pluripotency regulation in ES and iPS cells.

Mathematical modelling of cellular state transitions

To analyse theoretically cellular state transitions and cell fate decisions, one needs to specify a mathematical framework that can formally describe the structural relationship between the regulatory components involved (Barabasi and Oltvai, 2004; Newman et al., 2006) and that can quantify the system dynamics, given a certain network structure (Alon, 2007; Gardner et al., 2000; Huang et al., 2005). The issue of network dynamics was a central theme of this conference, and a number of speakers

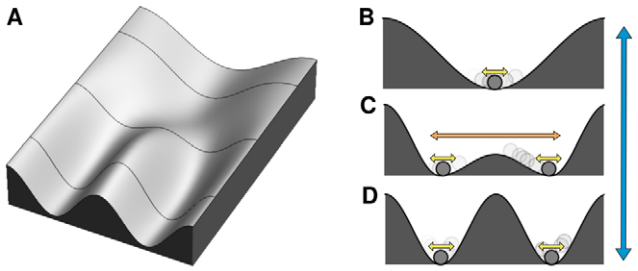

Fig. 1. Attractor concept for the description of cellular states and different levels of variability. (**A**) A three-dimensional illustration of an attractor landscape. The valleys represent stable stationary states (i.e. attractors) generated by a hypothetical regulatory network. Depending on the particular 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. (**B-D**) Selected attractor configurations (i.e. cross-sections of the given landscape) with corresponding variance components. (B) A single attractor, characterised by a small degree of potential fluctuations in cellular characteristics within the attractor ('microheterogeneity'). (C) Two accessible attractors. This configuration allows for heterogeneity within attractors and for potential exchange between the attractors ('macroheterogeneity'). (D) Two separated attractors. Cells are trapped in one of the two possible attractors and cannot exchange between them. However, a third level of heterogeneity (illustrated by the blue arrow) corresponds to potential changes in the attractor landscape itself. This can be achieved by changes in the configuration (e.g. the parameter values) of the network, even without changing the structure/topology of the network.

supported the idea that the theory of dynamical systems and of non-linear dynamics (e.g. Strogatz, 1994) is a suitable framework for describing and analysing cellular state transitions. The key idea of this methodology is to represent cellular states as attractors of dynamical systems. The existence of these attractors, as well as the possibility that cells can change from one attractor (that is, from one cellular state, such as the pluripotent stem cell state) to another (e.g. a particular differentiated cell type) and potentially back (e.g. in the context of iPS generation), depends on the network structure (such as the transcription factors and their regulatory links), the network configuration (such as the strength of transcriptional regulation), the particular state of the cells (e.g. their actual gene expression profile) and on system perturbations (such as non-specific background transcription). It has become popular to illustrate this attractor concept in terms of the 'epigenetic landscape' picture, first published by Waddington (Waddington, 1957). Although this is a useful illustration of the general meaning of the attractor concept, it also has some drawbacks. Most notably, it implies that all attractors are accessible by the cells at any time and that they just need to be 'pushed' into the right attractor 'valley'. However, as emphasised by several speakers (including I. Lemischka, I. Roeder, A. Brock and J. Kurths), attractor landscapes have to be considered as dynamic, rather than static, structures. It should also be emphasised that changes to the attractor landscapes can be caused by changes in the network structure itself (such as the loss of a regulatory pathway) and by parameter changes within the same network structure (such as a change in transcriptional activity) (Fig. 1). The role of stochasticity and heterogeneity as potential mechanisms that affect cellular state transitions was also raised in a number of talks.

Several modelling approaches that followed on from these ideas were presented at the meeting. Ingo Roeder (University of Leipzig, Germany) used a system of differential equations to analyse the experimentally observed heterogeneity of Nanog expression in mouse ES cells (Fig. 2). The theoretical results demonstrate that the variability in Nanog expression can be explained either as a fluctuating change of individual cells between two coexisting attractors induced by small random system perturbations (i.e. 'transcriptional noise'), or by the existence of an oscillating attractor. The model analysis made clear that distinguishing between these two scenarios is not possible based on cell population statistics at a single point in time, but requires the monitoring of the temporal changes of Nanog expression, preferentially in individual cells.

A similar conceptual description of cellular state transitions was presented by Amy Brock (Children's Hospital Boston, MA, USA). In particular, she referred to a publication by Huang et al. in which the differentiation of progenitor cells by chemically distinct stimuli was shown to follow trajectories that initially diverged in genome-wide state space but eventually converged to a similar end state (Huang et al., 2005). Furthermore, based on observations of a phenotypic change common to many cancers – the switch to multi-drug resistance – Brock presented evidence for the hypothesis that cancer states might also correspond to, and be described as, state space attractors (Brock et al., 2009).

Coming from a different angle, but also highlighting the dynamic nature of networks and the power of bifurcation analysis (i.e. the analysis of attractor properties) for understanding regulatory processes in (stem cell) biology, Juergen Kurths (Potsdam Institute for Climate Change, Germany) described a theoretical method to reconstruct correlation structures in networks based on the analysis of synchronisation in complex networks (Arenas et al., 2006). He

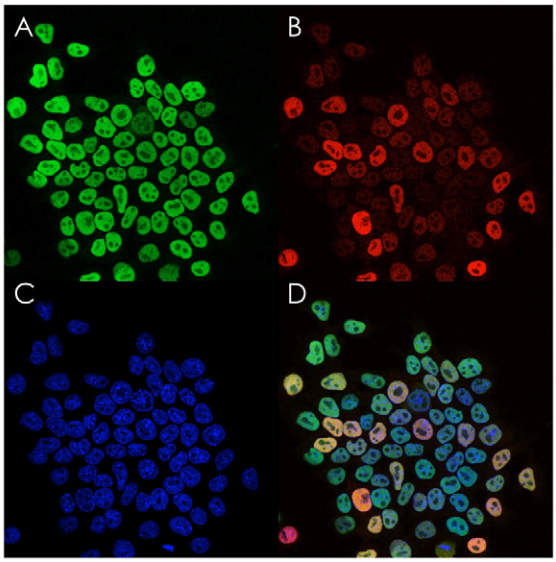

Fig. 2. Heterogeneous expression of Nanog in ES cells. Immunofluorescence staining for (**A**) Oct4 and (**B**) Nanog, and (**C**) staining with DAPI; (**D**) an overlay of A-C. In mouse ES cells, Oct4 staining appears to be relatively homogeneous, whereas Nanog expression levels differ substantially within individual ES cells. Image courtesy of Austin Smith.

also highlighted the potential of synthetic networks as a means of studying the general effect of coupling different types of network motifs (see below).

Along these lines, Joerg Stelling (ETH Zuerich, Switzerland) discussed the rationale, design and engineering of synthetic signalling and decision networks, as applied to biological systems. The design of circuits with complicated behaviour, such as oscillators in mammalian cells, requires detailed theoretical analysis, but in principle such engineering tasks can be achieved. Stochastic noise, however, as mentioned by Stelling, might deteriorate circuit performance.

Complementary to the intracellular perspective, studying cellular state transitions at the intercellular level can provide fundamental insights into the mechanisms that control the self-renewal and differentiation of stem cells. Ben Simons (University of Cambridge, UK) presented a mathematical analysis based on genetic labelling studies, in which individual stem cell clones were tracked. Using concepts from statistical physics, this study demonstrated how the scaling behaviour of clone size distributions and how spontaneous patterning phenomena reveal signatures of stochastic stem and progenitor cell fate. Based on these results, one can draw conclusions about the molecular mechanisms that control the maintenance, repair and regeneration of adult tissues and about the common organisational principles of tissue architecture, pointing to the importance of cell-cell as well as cell-microenvironment interactions.

The view that stem cell organisation is a dynamic process rather than a sequence of predefined developmental steps leads to the idea that stem cell populations are self-organising systems (see also Loeffler and Roeder, 2002; Potten and Loeffler, 1990). In this context, Markus Loeffler (University of Leipzig, Germany) emphasised the importance of a functional definition of stem cells, in which stem cells are characterised by a set of capabilities that can or cannot be used depending on the actual needs imposed by the

system (Loeffler and Roeder, 2002). Such a perspective has several important implications. For example, it requires the application of functional assays to demonstrate that a certain cell can act as a stem cell. Also, it implies that phenotypic characterisations (e.g. by cell surface markers) only provide representative snapshots of a cell's state. As such, they disregard the dynamic component of a cell's status and are, therefore, not a definitive verification of stem cell potential. Within the self-organisation paradigm, stem cell functionality is determined by both the general potential of a particular cell (i.e. its capability) and by cell-cell and cell-microenvironment interactions. That this perspective is able to consistently explain a wide range of experimentally observable phenomena has been illustrated by Markus Loeffler using model simulations of different stem cell systems, such as the haematopoietic system and the intestinal crypt.

Heterogeneity of stem cells and stem cell populations

Stem cells are characterised as cells that can continuously maintain or even self-renew their own population and that can generate a progeny of cells with more restricted properties or potential. Historically, stem cells have been seen as a biologically homogeneous population 'designed' to fulfil these criteria. However, multiple speakers at this conference presented data that clearly show that stem cells are much more heterogeneous than previously thought (see Fig. 2). Whether this heterogeneity represents an intrinsic property that has regulatory functions or whether it results from the exposure of stem cells to a variety of different, small (e.g. microenvironmental) perturbations (potentially summarised and described by stochastic effects) without functional implications was one of the major themes of this conference.

One of the major problems in the characterisation of stem cell heterogeneity is that stem cells and their descendents are mostly analysed at the population, rather than at the single-cell, level. Furthermore, most experiments focus on a few time points and do not trace individual cell identities over time. This, however, excludes another level of heterogeneity, namely the change in the properties of individual cells over time. Timm Schroeder (Helmholtz Centre Munich, Germany) presented new imaging and cell-tracking methods that monitor the fate of individual cells over long periods of time, allowing, for example, the quantification of selected protein expression levels in living stem cells. This novel type of data is used to generate and verify improved models that describe stem cell systems. Schroeder showed how the technology was recently used to demonstrate that embryonic endothelial cells could indeed produce blood cells, proving the previously much debated existence of haemogenic endothelial cells (Eilken et al., 2009).

Another important step in the process of understanding the role of cellular heterogeneity is the characterisation of its different underlying sources. In his talk, Alejandro Coleman-Lerner (University of Buenos Aires, Argentina) presented a method to disentangle different variance components that affect cell fate decisions in yeast. Based on the analysis of a series of experiments, he also discussed the effect of 'transcriptional noise' (i.e. small, unpredictable variations in the transcriptional activity) and its relation to the pheromone response of the cells (Yu et al., 2008).

Phedias Diamandis from Peter Dirk's laboratory (Hospital for Sick Children, Toronto, Canada) presented data on the heterogeneity of human NSCs. Phenotypically uniform populations of human NSCs express low levels of various neurotransmitter (NT) genes. Their heterogeneous expression patterns, Diamandis reported, could be re-established from individual cells. His results indicate that human NSCs demonstrate reversible patterns of NT expression that are intrinsically encoded in this lineage and that only restricted subpopulations can respond to specific NT cues. This suggests that stochastic sampling of different neurochemical states in NSCs might temporally and spatially control fate decisions in response to extrinsic cues, consistent with the general concept of 'lineage priming' in human NSCs.

The concept of 'priming' as a regulatory mechanism to allow a flexible response to different extrinsic (differentiation) signals was also discussed by other speakers. Ingo Roeder discussed, from a mathematical point of view, the previously suggested hypothesis that variable Nanog levels might be used to transiently 'prime' ES cells to respond to differentiation signals (Silva and Smith, 2008). And Amy Brock referred to a joint experimental and theoretical analysis of mouse haematopoietic progenitor cells, showing that within a single cell state, heterogeneity is characterised by slow fluctuations and has functional consequences in the priming of cell lineage commitment (Chang et al., 2008).

Connie Eaves (University of British Columbia, Vancouver, Canada) presented experimental findings that explain three sources of heterogeneity in mouse HSCs. The output of cells in large numbers of individually tracked clones, each derived from a highly purified HSC, revealed at least two distinguishable cell types that both display durable (indefinite) self-renewal ability but different lineage competencies in serial transplantation assays (Dykstra et al., 2007). The relative numbers of these two stem cell types normally change markedly throughout life, although they are stably propagated at a clonal level in vivo. In addition, Eaves reported that two other types of blood stem cells with durable self-renewal ability exist that are distinguished by their different self-renewal activities in transplant assays and their different prevalence in fetal and adult life. The change in prevalence from one type to another appears to be cell-autonomous and to correlate with a change in their cell cycle status (Bowie et al., 2007).

Several talks referred to the fact that cellular heterogeneity is not a static feature, but a systemic property that is subject to considerable changes. One example of a mechanism that is able to induce heterogeneity among stem cells is the activity of telomerase. Lea Harrington (University of Edinburgh, UK) showed that mice and humans heterozygous for telomerase undergo telomere erosion and stem cell depletion, leading to disease and early mortality. In a mouse strain with initially long telomeres, telomere erosion was observed for up to ten generations in *Tert* heterozygous mice. In later generations, however, telomeres unexpectedly re-equilibrated to near wild-type lengths, with no tissue or stem cell dysfunction even upon further heterozygote interbreeding. The re-equilibration occurred via the lengthening of the shortest telomeres by telomerase. Thus, partial telomerase depletion does not invariably lead to disease, and the extension of 'telomerase-accessible' telomeres may ameliorate tissue and stem cell dysfunction.

Conclusions

The conference highlighted the fact that the driving force in current stem cell biology is ultimately to achieve a comprehensive understanding of the regulatory mechanisms of cell state transitions as the basis for a safe, controllable and efficient use of cellular programming, reprogramming and iPS-induction protocols.

An essential component in this process is the identification of molecular regulators and their mutual interactions, that is, of the underlying regulatory networks. In this context, it was emphasised at the meeting that networks should be considered as dynamic structures. As this implies that the properties of the network

components, or even the network structure, are constantly evolving, the flexibility, variability and reversibility of (stem) cell fates will increasingly become the focus of research activities in this field.

Another important theme of the conference was that cellular heterogeneity and the mechanisms of cell state transitions should be considered as internally related, as cells can switch between different states to induce heterogeneity. Therefore, the observed heterogeneity of stem cell populations is highly unlikely to be just the result of random perturbations. Instead, the idea that heterogeneity guarantees the robustness and flexibility of a system is becoming increasingly accepted. In this respect, it has been suggested that cellular state transitions and, therefore, processes such as self-renewal, differentiation or de-differentiation, could also be controlled by the regulation of the degree of heterogeneity within the system. In such a mechanism, sometimes referred to as 'noise' regulation, stochastic fluctuations in the transcriptional activity of cells would be a non-negligible factor.

As a final point, the meeting illustrated very clearly that predictive mathematical models are widely accepted tools that can considerably enhance our understanding of the regulatory principles of stem cell organisation. The conference reflected the general tendency that theoretical methods are becoming an integral part of stem cell biology.

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