Development 137, 2455-2460 (2010) doi:10.1242/dev.049130
© 2010. Published by The Company of Biologists Ltd

Shushing down the epigenetic landscape towards stem cell differentiation

Justin K. Ichida, Evangelos Kiskinis and Kevin Eggan*

Summary
In February 2010, researchers interested in stem cell biology gathered in Keystone, Colorado, USA to discuss their findings on the origins and behaviors of pluripotent and multipotent stem cells, and their therapeutic potential. Here, we review the presentations at that meeting and the questions that emerged concerning how a stem cell 'decides' to self-renew or differentiate, what their distinct properties are and how this information can be used to develop novel therapies.

Key words: Differentiation, Induced pluripotent stem cells, Reprogramming

Introduction
The timing, topic and location were ideal for the Keystone 2010 conference on 'Stem Cell Differentiation and Dedifferentiation', which was co-organized by Fiona Watt (CRUK Cambridge Research Institute, Cambridge, UK) and Shinya Yamanaka (Kyoto University, Kyoto, Japan). It was at the same meeting 4 years previously that Yamanaka first announced the induction of pluripotency in differentiated cells using defined factors (Takahashi and Yamanaka, 2006). Since then, Yamanaka's method for changing cellular phenotypes using transcription factors has been applied to the reprogramming of diseased human fibroblasts, the differentiation of stem cells into specific cell types of interest and the more direct reprogramming of one somatic cell type into another (Hochedlinger and Plath, 2009). As this field matures, it was an ideal time to review the progress made and to discuss the challenges that remain.

The cell biologist Hal Waddington described cellular differentiation as a ball rolling through an epigenetic landscape (Waddington, 1957). This descending ball, repeatedly choosing between distinct couloirs and valleys, eventually comes to rest at a point near the mountain's base. As the ball lacks any further 'developmental potential energy', it remains there as a terminally differentiated cell. Between the advances described at this conference and afternoons spent skiing in summit county, one could not help but wonder how easy it has become to traverse Waddington's landscape. Whether it is a reprogramming gondola carrying a differentiated cell back to pluripotency – shushing down the mountain by directed differentiation in order to produce a specific cell type or hiking over terrain from the 'valley' of one lineage directly to that of another – the speakers at this year's Keystone Symposium illuminated the ease by which cellular identity can now be manipulated.

Rather than presenting the meeting in chronological order, we have organized our review around several central questions that were collectively addressed at this meeting. These include: 'what is the best method for producing induced pluripotent stem cells (iPSCs) and are they equivalent to embryonic stem (ES) cells?' (Fig. 1); 'what is the provenance of an ES cell and why do human and mouse stem cells have distinct properties?'; 'what are the molecular circuits that control stem cell self-renewal and differentiation?'; 'how do these circuits integrate signals from injured or aging tissues?'; and, finally, 'can we control stem cell differentiation to allow the preparation of therapeutically relevant cell types with real utility?'.

A gondola back to the summit or to a distinct peak?
A central topic of this year's meeting was whether iPSCs are equivalent to ES cells and whether the methods used for reprogramming dramatically alter the resulting stem cells. Both Yamanaka and James Thomson (University of Wisconsin, WI, USA) touched on this issue in their keynote addresses. Thomson shared a perspective on their approach for generating iPSCs and described a collaboration with Shuchin Zhang (University of Wisconsin, WI, USA) in which they determined whether these pluripotent cells could be differentiated into motor neurons (Hu et al., 2010). This provocative talk suggested that a fundamental deficit exists in the ability of human iPSCs to differentiate: several iPSC lines failed to generate motor neurons at the efficiency exhibited by ES cell lines, even when they lacked transgenic insertions (Hu et al., 2010).

The theme of abnormal iPSC differentiation continued in Yamanaka's talk. He described attempts to differentiate mouse iPSCs into secondary neurospheres (Miura et al., 2009). When this experiment was performed with iPSCs that carried a *Nanog*::GFP reporter, in some lines, the reporter of pluripotency failed to be extinguished (Miura et al., 2009). Intriguingly, reporter expression was not uniform across all cells but, rather, was restricted to what Yamanaka labeled as 'unsafe' cells within the line. When cell lines were subcloned and expanded, they gave rise to heterogeneous populations of either 'safe' or 'unsafe' iPSCs. These 'unsafe' cells were more likely to generate a teratoma after transplantation into the mouse brain (Miura et al., 2009). The difference between the safe and unsafe populations remains to be determined.

Christoph Bock (Harvard University, MA, USA) from Alex Meissner's group continued with this theme and described a collaboration between four groups – Alex Meissner's, Kevin Eggan's (Harvard University, MA, USA), Hynek Wichterle's and Chris Henderson's (both at Columbia University, NY, USA) – to determine the variation in the properties of human iPSCs and ES cells. The goal was to determine the robustness of iPSC lines for disease modeling and to produce a 'scorecard' for quality controlling these cell lines. Bock described genome-wide gene expression and DNA methylation patterns for a large cohort of cells, as well as their propensity for differentiating towards specific lineages. Although substantial variability in the ability of iPSCs to differentiate was

The Howard Hughes Medical Institute, Stowers Medical Institute, Harvard Stem Cell Institute and the Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA 02138, USA.

*Author for correspondence (keggan@scrb.harvard.edu)

DEVELOPMENT

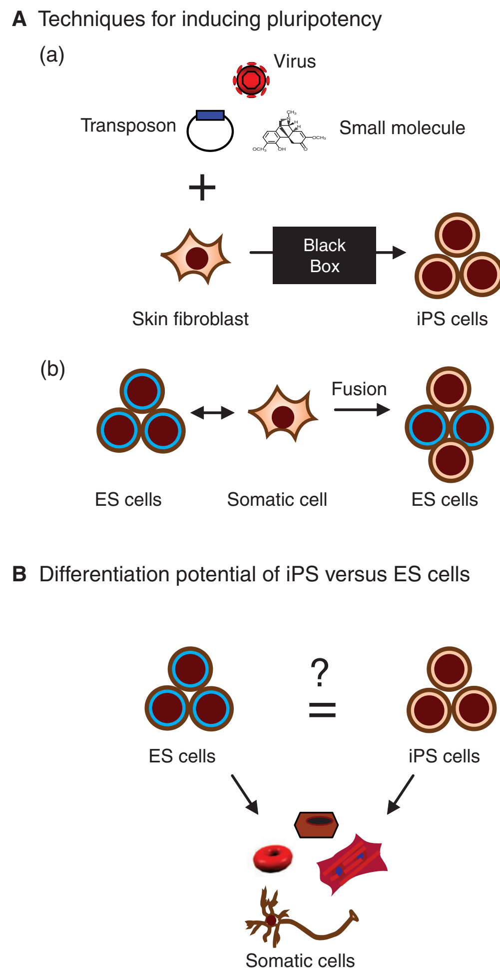

Fig. 1. Concepts in stem cell reprogramming and differentiation. (**A**) (a) Genetic, small-molecule, and (b) fusion-based methods can be used to induce pluripotency in somatic cells and to study reprogramming. (**B**) Genome-wide comparisons of induced pluripotent stem (iPS) cells and embryonic stem (ES) cells highlight the issue of whether iPS cells are equivalent to ES cells with respect to differentiation potential.

observed between different human iPSC lines, this variation seemed to be explained by variation that also exists among human ES cell lines. Ron Mckay (National Institute of Neurological Disorders and Stroke, MD, USA) also seemed to find that iPSCs behave much as ES cells do. The variation described in pluripotent cell lines suggests that a large number of both human iPSCs and ES cells must be compared in order to determine whether any difference in differentiation potential is specific to iPSCs.

This group of speakers also discussed efforts to improve the efficiency of reprogramming techniques. Most iPSCs are produced through retroviral transduction (Takahashi and Yamanaka, 2006) (Fig. 1A). The problem with this approach is that the reprogramming genes, particularly *MYC* (cMyc), are potent oncogenes (Okita et al., 2007). If the viral vectors become re-expressed in mice produced from these cells, it can lead to tumor initiation (Okita et al., 2007). Additionally, this method is inefficient, limiting its utility. Rudolf Jaenisch (Whitehead Institute, MA, USA) and Azadeh Golipour (Samuel Lunenfeld Research Institute, Toronto, Canada) presented two new genetic methods for reprogramming, while Eggan presented a method that replaces the reprogramming genes with small molecules (Fig. 1A). Jaenisch described mice in which the reprogramming genes are present at a single locus that can be induced with doxycyclin (Carey et al., 2010). This approach dramatically improves the efficiency of reprogramming, and, as Jaenisch demonstrated, given enough time, it enables any cell to be reprogrammed (Hanna et al., 2009).

By contrast, Golipour uses transposons to reprogram cells (Kaji et al., 2009; Woltjen et al., 2009). Transposons efficiently induce reprogramming and can be later removed (Fig. 1A). Golipour reported her colleagues' genetic RNAi screen to identify genes that influence reprogramming, which uncovered a key role for BMP signaling and the induction of mesenchymal-to-epithelial transition (MET) during early reprogramming. Golipour showed that this process is linked to the BMP-dependent induction of miR-205 and of the miR-200 family of microRNAs, which are key regulators of MET. Similarly, Eggan's group have discovered through their reprogramming approach, which is designed to replace each reprogramming gene with distinct small molecules, that inhibitors of the activin arm of TGFβ signaling enhance reprogramming, allowing it to occur in the absence of both Myc (cMyc) and Sox2 (Ichida et al., 2009). As James Thomson had presented CHIP-seq and gene expression data reinforcing the observation that BMP and activin signaling are reciprocal and have antagonistic effects on one another in ES cells (Xu et al., 2008), these two presentations seem to confirm each other's observation.

Talks by Helen Blau (Stanford University, CA, USA) and Amanda Fisher (Imperial College, London, UK) reminded the audience that the generation of iPSCs is not the only method for reprogramming. Blau described a genetic screen in which mouse ES cells were fused with somatic cells to form stable heterokaryons (Bhutani et al., 2010) (Fig. 1A, part b). She reported that reprogramming by cell fusion proceeds more rapidly than it does with defined factors, suggesting that this approach would be useful for identifying genes required for the reprogramming process. An RNAi knockdown screen by this laboratory identified AID, a deaminase that can act on 5-methyl cytosine to cause DNA demethylation, as a requirement for reprogramming in this cell-fusion system. Loss of AID function inhibited demethylation and reprogramming, whereas increased levels of AID improved reprogramming efficiency (Bhutani et al., 2010).

Amanda Fisher also used cell fusion to study reprogramming (Pereira and Fisher, 2009). Using various mutant mouse ES cells, she demonstrated that Polycomb proteins from the PRC2 complex were essential for reprogramming. In an effort to define the role of PRC2, Fisher and colleagues identified Jarid2 as a novel component of the complex. She went on to provide evidence that Jarid2 serves a crucial function in ES cell pluripotency and reprogramming, primarily through the transcriptional priming of 'bivalent' chromatin domains (Landeira et al., 2010; Pasini et al., 2010; Pereira et al., 2010). The genes that bear these chromatin domains, which contain both activating and repressive post-translational modifications to histones, are thought to be poised for expression during differentiation and development. These talks eloquently demonstrated that heterokaryon formation coupled with genetic intervention allows insight to be gained into reprogramming.

What's in a name?

Another topic of conversation revolved around what human and mouse ES cells are and where they come from. Although human and mouse ES cells are both pluripotent, they have distinct properties. Mouse cells grow in dome-like colonies, rely on leukemia inhibitory

DEVELOPMENT

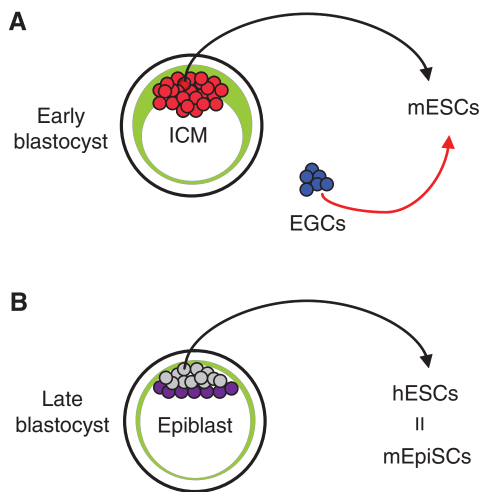

Fig. 2. The origins of mouse and human embryonic stem cells. (**A**) Mouse ES cells (mESCs) may arise directly from the inner cell mass (ICM) of the early mouse blastocyst or from ICM-derived embryonic germ cells (EGCs). (**B**) Human ES cells (hESCs) are likely to arise from the epiblast of the late blastocyst and are more similar to mouse epiblast stem cells (mEpiSCs).

factor (LIF) signaling for self-renewal, can differentiate into primitive endoderm and contribute to the developing embryo when placed into the blastocyst (Silva et al., 2008; Silva and Smith, 2008). By contrast, human ES cells, and their in vivo orthologs, mouse epiblast stem cells, grow in flat epithelial sheets, require FGF for their renewal and, although they are pluripotent in vitro, they do not participate in development when placed into the embryo (Brons et al., 2007; Tesar et al., 2007). Based on these properties, it is tempting to speculate that mouse ES cells are reminiscent of the inner cell mass (ICM), whereas epi-stem cells and human ES cells are epiblast cells, which self-renew in culture (Fig. 2).

Austin Smith (The Wellcome Trust Centre for Stem Cell Research, Cambridge, UK) reported his group's experiment in which they treated mouse blastocysts and mouse ES cell lines with a cocktail of three small-molecule inhibitors of differentiation. Interestingly, these compounds seemed to have similar effects both in vivo and in vitro: they promote renewal and division, while inhibiting differentiation into primitive endoderm (Silva and Smith, 2008). These results are consistent with mouse ES cells having an ICM origin. However, in a series of talks by Thomas Zwaka (Baylor College of Medicine, TX, USA) and by his trainee Li Fang Chu, an alternative model of mouse ES cell origins was presented. In this model, mouse ES cells are derived from germ cells that arise in the outgrowing ICM. The evidence for this proposal came from lineage tracing experiments in which ES cells were derived from embryos expressing Cre recombinase under control of the *Blimp1* promoter. When this gene becomes expressed in germ cells of the embryo, it activates a *lacZ* reporter. Intriguingly, all ES cells derived from such embryos were *lacZ* positive, suggesting that ES cells are germ cell derived.

This is an interesting model, as it is possible to derive embryonic germ cells, which share many properties with ES cells, from the migrating germ cells later in development. Accordingly, Takashi Shinohara (Kyoto University, Kyoto, Japan) described the isolation of spermatogenic stem cells, which, when transplanted back into the testis, can form sperm. At an extremely low efficiency, these cells interconverted into pluripotent cells (Takehashi et al., 2010). Together, these results suggest the interesting possibility that all germ line competent pluripotent cells are derived from germ cells.

This attractive hypothesis could also explain another developmental conundrum: why female ES cells have two active X chromosomes. Germ cells in the developing female undergo X chromosome reactivation. Could it be that similar mechanisms are at play in mouse ES cells? Cold water was thrown on this model by the work of Azim Surani (The Wellcome Trust/CRUK Gurdon Institute, Cambridge, UK). He attempted to derive mouse ES cells from *Blimp1*-deficient embryos, which lack all germ cells (Ohinata et al., 2005). He reasoned that if ES cells come from germ cells then he should not be able to derive them from these embryos. However, in their preliminary experiments, this did not seem to be the case.

By contrast, human ES cells appear to be epiblast derivatives rather than germ line-derived stem cells. Consistent with this notion, Hans Scholer (Max-Planck Institute for Molecular Biomedicine, Münster, Germany) presented his group's attempts to isolate human male germ line stem cells, which appears to be much harder to do than in mice. Although he was able to isolate cells that had the properties of those previously described in the literature as being human germ line stem cells (Conrad et al., 2008), he could not demonstrate their interconversion to pluripotency. Very careful analysis of these cells seemed to indicate that, rather than being germ line stem cells, they were instead only testicular fibroblasts. Scholer went on to explain where the previous group had been led astray by the behavior of these cells in various assays. One could not leave his talk without feeling that the previously reported pluripotent conversion of these cells (Conrad et al., 2008) had been justifiably thrown into doubt.

Although the conclusion that human ES cells most closely resemble epiblast cells seems on stable ground (Brons et al., 2007; Tesar et al., 2007), the work on X chromosome inactivation in human ES cells described by Jaenisch did throw this conclusion somewhat into question. In his talk, Jaenisch described work carried out in his lab that indicates that human ES cells grown under normal conditions have an inactive and an active X chromosome. In this sense, they would be similar to differentiating cells of the epiblast. However, when he grew an early sub-clone of these cells in low oxygen, his group observed signs of biallelic expression of X-linked genes. This would seem to suggest that, under some conditions, human ES cells resemble mouse ES cells in their state of dose compensation. Consistent with this conclusion, when these human ES cells were differentiated, one chromosome became inactivated. These experiments suggest that there may be a ground state for human pluripotency, which is not commonly accessed and more closely resembles that found in the mouse (Ying et al., 2008).

Fred Gage (The Salk Institute, CA, USA) presented work on his group's efforts to distinguish adult neural stem cells (NSCs) according to their site of origin and also outlined a proposal to generate iPSC lines from a variety of primates. Their goal is to use human and non-human primate NSCs to derive various neuronal subtypes in an attempt to understand why human and non-human primate brains differ from one another. Alternative splicing variants of genes encoding proteins such as neuropsin, which is involved in learning and memory (Li et al., 2004), have been described to exist between species. Could iPS cells provide an invaluable insight into the evolution of the human brain?

This cadre of presentations was rounded off by Anthony Atala (Wake Forest University, NC, USA), who updated the audience on his work with human fetal amniotic stem cells (De Coppi et al., 2007). These cells seem to be similar to human ES cells in that they

DEVELOPMENT

may have some limited capacity to give rise to endoderm, mesoderm and ectoderm in vitro. Although they express low levels of OCT4 and some other pluripotency factors, it was unclear whether these proteins are found in the nucleus where they would exert their function. These cells do not form teratomas when injected into nude mice, which is attractive for transplant approaches. When hearing this talk, we wondered whether multipotent neural crest progenitors might have sloughed off the embryo into the amniotic fluid. Owing to the diverse differentiation capacities of the neural crest (Joseph et al., 2004), these cells could, in principle, form some of the cell types described in this talk.

Ski school: mechanics of self-renewal and differentiation

In addition to talks on reprogramming and pluripotent stem cell provenance, there were a number of talks on the mechanistic aspects of why stem cells behave as they do. Ihor Lemischka (Mount Sinai Hospital, NY, USA) updated the audience on his studies of the ES cell transcriptome and proteome. His goal is to understand the systems-level control of self-renewal and differentiation. The informatics tools for these experiments continue to become more and more sophisticated. Especially of note were bioinformatic interfaces that could portray 4D changes in global RNA and protein levels (Lu et al., 2009). Colin Melton (University of California, San Francisco, CA, USA) also discussed the post-transcriptional control of stem cell behavior (Melton et al., 2010). He described published work on two miRNA families that are expressed in ES cells and that have antagonistic effects on one another in stem cell differentiation and renewal (Melton et al., 2010).

Larry Stanton (Genome Institute, Singapore) shared structure/function experiments on the transcription factors Sox2 and Sox17. Sox2 is a component of the transcription factor network that drives the self-renewal of ES cells and is included in the cocktail of reprogramming factors described by Yamanaka. By contrast, Niakan and colleagues have shown that Sox17 is important for driving the differentiation of mouse ES cells towards primitive endoderm (Niakan et al., 2010). Strikingly, Stanton observed that Sox17 and Sox2 had almost identical consensus binding motifs, and went on to show that with just a single amino acid substitution in their DNA-binding domains, the functions of Sox2 (iPS induction) and Sox17 (endoderm induction) could be swapped.

Fiona Watt shifted the focus of the meeting to lineage-committed progenitors. Her work included a collaborative effort with bioengineers to grow epidermal stem cells on surfaces that had distinct extracellular matrices printed in patterns to dictate the shape in which each cell grew (Gautrot et al., 2010). Their goal was to determine how physical constraints modified a cell's behavior. Surprisingly, the substrate to which the cells adhered only modestly affected their behavior. By contrast, the shape that cells were constrained to occupy strongly influenced their differentiation state. These effects seemed to be mediated through changes in the cytoskeleton (Gautrot et al., 2010). It will be interesting to further understand this phenomenon, as it is likely to be a general feature of stem cells. Catherin Niemann (University of Cologne, Germany) described her work on the sebaceous gland stem cells and their maintenance. Using K15-Cre as a lineage-tracing tool, she showed that single bulge cells can reconstitute the sebaceous glands. Interestingly, this process is independent of hair follicle regeneration by bulge cells during the hair cycle (Niemann, 2009).

Toshio Suda (Keio University, Tokyo, Japan) continued the discussion of the stem cell micro-environment by describing his work on hypoxia in the blood-forming hematopoietic niche. His work described the behavior of hematopoietic stem cells in the hypoxia-inducible factor 1α (HIF1α) knockout mouse (Kurihara et al., 2010). Interestingly, these mice had decreased stem cell quiescence and lost stem cells more rapidly as they aged. Although these studies suggest the sensing of hypoxia may be a key regulator of blood stem cell behavior, it needs to be resolved whether this mutation, which must place additional and unknown pressure on other aspects of hematopoiesis, could be exerting some indirect effect (Hosokawa et al., 2010).

Amy Wagers (Harvard University, MA, USA) also described her intriguing work on the aging of the stem cell niche. Her experiments with animal parabiosis suggest that there are circulating factors that modify the behavior of stem cells. Remarkably, when she conjoins old and young animals in a heterochronic parabiosis experiment, circulating factors from the young animal enter the old animal and rejuvenate it. Although her work on rejuvenation of the skeletal muscle had already been published (Conboy et al., 2005), here she focused on the aging of the blood stem cell niche. She indicated that osteoblasts supporting the stem cells change during aging. Serum from young animals seems to reverse many of the deleterious changes to old osteoblasts, opening the door to the possible purification of the circulating factor or factors. She also described a small molecule screen to identify compounds that could revert the phenotype of the osteoblasts to a more youthful state. Inhibitors of TGFβ signaling seemed to score well in this screen.

Deepak Srivastava (Gladstone Institute of Cardiovascular Disease, CA, USA) described in his talk the factors that control the behavior of cardiac progenitors and focused primarily on the discovery of a novel connection between the Notch and WNT signaling pathways (Kwon et al., 2009). This connection seems to be mediated by modulation of glycogen synthase kinase 3β (GSK3β) stability. Interestingly, this new signaling pathway may be at the heart of the self-renewing ability of many somatic stem cell types.

Skiing with purpose: shushing towards therapeutic goals

The final theme explored at this meeting was the therapeutic use of stem cells. Chad Cowan (Harvard University, MA, USA) described his efforts to use transcription factors to direct the reprogramming of fibroblasts and ES cells into both hepatic and adipose tissues. His goal is to use these cells for metabolic studies and for understanding drug-mediated toxicity. Cowan also presented attempts to use iPSCs to investigate the mechanistic contribution of various polymorphisms towards diseases, such as myocardial infarction. He closed by challenging the audience to consider using iPSCs to model the widespread effects of single nucleotide polymorphisms (SNPs) in many disease processes.

E. Edward Baetge (Novocell, CA, USA) also spoke about metabolic disorders and shared Novocell's progress towards the directed differentiation of human ES cells into pancreatic β-cells (Kroon et al., 2008). Their goal is to produce β-cells for transplantation into individuals with diabetes. Although they can now relieve diabetes in a mouse model, several challenges remain. β-Cells produced using their method seem to express multiple hormones and may be immature. Although placing them for some time in vivo allows them to mature, they will probably need to discover how to mature them in vitro (Kroon et al., 2008).

Hideyuki Okano (Keio University School of Medicine, Japan), Hans Keirstead (University of California, Irvine, CA, USA) and Ron McKay all gave talks on how stem cells might be used to treat neurological conditions. Okano described the neurogenic potential

DEVELOPMENT

of stem cells, derived from both human fetal sources (Yamane et al., 2010) and from ES cells (Kumagai et al., 2009), after their transplantation into the mouse spinal cord. He provided compelling evidence that the generation of glial cells was more important for the recovery of the animal than the production of new neurons. He also described genetic manipulation of marmoset embryos. His lab has now generated marmosets that carry an α-synuclein mutation that causes Parkinson's disease. They are currently rearing the animals to determine whether they will succumb to neural degeneration (Sasaki et al., 2009).

Ron Mckay kept the discussion focused on the midbrain and described their efforts to produce bona fide dopaminergic neurons. The method he described relied on WNT and SHH signaling to first generate floor plate and then midbrain neurons (Joksimovic et al., 2009; Ravin et al., 2008). Meanwhile, Hans Keirstead shared his group's progress in producing oligodendrocytes (Hatch et al., 2009; Sharp et al., 2010). His talk focused primarily on the requirements producing ES cells and ES-derived neurons to use as therapeutic products (Hatch et al., 2009; Sharp et al., 2010) for treating spinal cord injury and spinal muscular atrophy (SMA).

Amy Sinor (Harvard University, MA, USA) also described the use of motor neurons for therapeutic purposes, but her approach was very different from that of Keirstead's. She described efforts in Lee Rubin's lab (Harvard University, MA, USA) to use these motor neurons for drug discovery. In the childhood disease SMA, motor neurons degenerate (Lorson et al., 2010). Many researchers believe that SMA could be reversed if a compound could be identified that increased the expression levels of a survival factor for motor neurons, which is hypomorphically expressed in these cells. Using an image-based screen, Sinor found a number of interesting compounds that seem to have this activity. Sinor's talk was followed by a talk from Zhong Zhong (GlaxoSmithKline, China), who is taking a similar approach to Sinor, reflecting an interest among large pharmaceutical companies in using ES cells as drug screening tools.

Rita Perlingeiro (Lillehei Heart Institute, MN, USA) described her group's work on the function of PAX transcription factors in skeletal muscle stem cells. Most notably, she described the effects of ectopically expressing *Pax3* in mouse ES cells, which induced the cells to take on a myogenic phenotype. When injected into a dominant model of muscular dystrophy, these cells contributed to an increase in the strength of diseased muscle (Darabi et al., 2009). This work, although promising, would presumably require a non-genetic method for inducing *Pax3* in stem cells before transplantation, perhaps using a small-molecule approach.

Before derivatives from iPS cells or ES cells move towards the clinic, large-animal models will be needed to test both their safety and efficacy as well as to develop surgical techniques. A talk by Jing Liao (Shanghai Institute of Biochemistry and Cell Biology, Shanghai China) described the derivation of pig iPSCs (Wu et al., 2009). As the pig is an excellent model for surgical transplantation, these cells could prove to be incredibly valuable. Pig iPSCs, which appear to be similar to human iPSCs in their gene expression program and differentiation capacity, should be invaluable for producing populations of cells for testing new transplantation therapies.

Nobuko Uchida (StemCells, CA, USA) described his work on a mouse model of infantile neuronal ceroid lipofuscinosis, a neurodegenerative disease that results from the excessive accumulation of lipopigments in the body's tissues (Tamaki et al., 2009). In this work, his group explored the protective effects of transplanting human fetal nervous system stem cells into the mutant mice. These cells, which are grown as neurospheres and are free from concerns over teratoma formation, seem to protect a variety of neuronal cell types by actively migrating throughout the nervous system after transplant, supporting the host cells that would otherwise be damaged by this previously untreatable enzyme deficiency (Tamaki et al., 2009).

Gabriela Gebrin Cezar (University of Wisconsin, WI, USA) is trying to understand the entirety of small-molecule constituents residing within cells. This collection of molecules that she calls the 'metabolome' could be a very sensitive indicator of physiological changes within a cell. She hopes to use this approach to discover biomarkers for disease that could aid in both the development of diagnostics and in clinical trial design (Cezar et al., 2007). This approach has already been helpful in understanding how glial cells become toxic to motor neurons in amyotrophic lateral sclerosis (ALS) (Marchetto et al., 2008).

Although talks on translational stem cell biology reflected the early stage of efforts in the development of new therapeutics, they demonstrated a broadening viewpoint in the ways that stem cells might be deployed for treating disease. No longer are thoughts limited purely to the notion of cell replacement therapy. In addition to this potentially valuable approach, disease modeling, drug discovery, improved toxicological studies and modulation of inflammatory processes all seem to be very promising approaches.

Conclusion

In summary, it is remarkable how quickly the stem cell field has managed to move forward since Yamanaka's breakthrough discovery several years ago. Stem cell science seems to be on the verge of moving out of its fascinating, but awkward, adolescence into a stage of maturation where cellular behavior can be precisely controlled for a better understanding of developmental biology and for regenerative medicine.

Acknowledgements

We thank all authors who agreed to share unpublished data in this meeting review and regret that owing to space constraints we were not able to discuss some presentations in greater detail. J.I. is a Stan and Fiona Druckenmiller, New York Stem Cell Foundation Postdoctoral Fellow. E.K. is a European Molecular Biology Organization Postdoctoral Fellow. K.E. is an Assistant Investigator of the Stowers Medical Institute, an HHMI Early Career Scientist and Fellow of the MacArthur Foundation.

References

Bhutani, N., Brady, J. J., Damian, M., Sacco, A., Corbel, S. Y. and Blau, H. M. (2010). Reprogramming towards pluripotency requires AID-dependent DNA demethylation. *Nature* **463**, 1042-1047.

Brons, I. G., Smithers, L. E., Trotter, M. W., Rugg-Gunn, P., Sun, B., Chuva de Sousa Lopes, S. M., Howlett, S. K., Clarkson, A., Ahrlund-Richter, L., Pedersen, R. A. et al. (2007). Derivation of pluripotent epiblast stem cells from mammalian embryos. *Nature* **448**, 191-195.

Carey, B. W., Markoulaki, S., Beard, C., Hanna, J. and Jaenisch, R. (2010). Single-gene transgenic mouse strains for reprogramming adult somatic cells. *Nat. Methods* **7**, 56-59.

Cezar, G. G., Quam, J. A., Smith, A. M., Rosa, G. J., Piekarczyk, M. S., Brown, J. F., Gage, F. H. and Muotri, A. R. (2007). Identification of small molecules from human embryonic stem cells using metabolomics. *Stem Cells Dev.* **16**, 869-882.

Conboy, I. M., Conboy, M. J., Wagers, A. J., Girma, E. R., Weissman, I. L. and Rando, T. A. (2005). Rejuvenation of aged progenitor cells by exposure to a young systemic environment. *Nature* **433**, 760-764.

Conrad, S., Renninger, M., Hennenlotter, J., Wiesner, T., Just, L., Bonin, M., Aicher, W., Buhring, H. J., Mattheus, U., Mack, A. et al. (2008). Generation of pluripotent stem cells from adult human testis. *Nature* **456**, 344-349.

Darabi, R., Baik, J., Clee, M., Kyba, M., Tupler, R. and Perlingeiro, R. C. (2009). Engraftment of embryonic stem cell-derived myogenic progenitors in a dominant model of muscular dystrophy. *Exp. Neurol.* **220**, 212-216.

De Coppi, P., Bartsch, G., Jr, Siddiqui, M. M., Xu, T., Santos, C. C., Perin, L., Mostoslavsky, G., Serre, A. C., Snyder, E. Y., Yoo, J. J. et al. (2007). Isolation of amniotic stem cell lines with potential for therapy. *Nat. Biotechnol.* **25**, 100-106.

Gautrot, J. E., Trappmann, B., Oceguera-Yanez, F., Connelly, J., He, X., Watt, F. M. and Huck, W. T. (2010). Exploiting the superior protein resistance of polymer brushes to control single cell adhesion and polarisation at the micron scale. *Biomaterials* **31**, 5030-5041.

Hanna, J., Saha, K., Pando, B., van Zon, J., Lengner, C. J., Creyghton, M. P., van Oudenaarden, A. and Jaenisch, R. (2009). Direct cell reprogramming is a stochastic process amenable to acceleration. *Nature* **462**, 595-601.

Hatch, M. N., Nistor, G. and Keirstead, H. S. (2009). Derivation of high-purity oligodendroglial progenitors. *Methods Mol. Biol.* **549**, 59-75.

Hochedlinger, K. and Plath, K. (2009). Epigenetic reprogramming and induced pluripotency. *Development* **136**, 509-523.

Hosokawa, K., Arai, F., Yoshihara, H., Iwasaki, H., Hembree, M., Yin, T., Nakamura, Y., Gomei, Y., Takubo, K., Shiama, H. et al. (2010). Cadherin-based adhesion is a potential target for niche manipulation to protect hematopoietic stem cells in adult bone marrow. *Cell Stem Cell* **6**, 194-198.

Hu, B. Y., Weick, J. P., Yu, J., Ma, L. X., Zhang, X. Q., Thomson, J. A. and Zhang, S. C. (2010). Neural differentiation of human induced pluripotent stem cells follows developmental principles but with variable potency. *Proc. Natl. Acad. Sci. USA* **107**, 4335-4340.

Ichida, J. K., Blanchard, J., Lam, K., Son, E. Y., Chung, J. E., Egli, D., Loh, K. M., Carter, A. C., Di Giorgio, F. P., Koszka, K. et al. (2009). A small-molecule inhibitor of tgf-Beta signaling replaces sox2 in reprogramming by inducing nanog. *Cell Stem Cell* **5**, 491-503.

Joksimovic, M., Yun, B. A., Kittappa, R., Anderegg, A. M., Chang, W. W., Taketo, M. M., McKay, R. D. and Awatramani, R. B. (2009). Wnt antagonism of Shh facilitates midbrain floor plate neurogenesis. *Nat. Neurosci.* **12**, 125-131.

Joseph, N. M., Mukouyama, Y. S., Mosher, J. T., Jaegle, M., Crone, S. A., Dormand, E. L., Lee, K. F., Meijer, D., Anderson, D. J. and Morrison, S. J. (2004). Neural crest stem cells undergo multilineage differentiation in developing peripheral nerves to generate endoneurial fibroblasts in addition to Schwann cells. *Development* **131**, 5599-5612.

Kaji, K., Norrby, K., Paca, A., Mileikovsky, M., Mohseni, P. and Woltjen, K. (2009). Virus-free induction of pluripotency and subsequent excision of reprogramming factors. *Nature* **458**, 771-775.

Kroon, E., Martinson, L. A., Kadoya, K., Bang, A. G., Kelly, O. G., Eliazer, S., Young, H., Richardson, M., Smart, N. G., Cunningham, J. et al. (2008). Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. *Nat. Biotechnol.* **26**, 443-452.

Kumagai, G., Okada, Y., Yamane, J., Nagoshi, N., Kitamura, K., Mukaino, M., Tsuji, O., Fujiyoshi, K., Katoh, H., Okada, S. et al. (2009). Roles of ES cell-derived gliogenic neural stem/progenitor cells in functional recovery after spinal cord injury. *PLoS ONE* **4**, e7706.

Kurihara, T., Kubota, Y., Ozawa, Y., Takubo, K., Noda, K., Simon, M. C., Johnson, R. S., Suematsu, M., Tsubota, K., Ishida, S. et al. (2010). von Hippel-Lindau protein regulates transition from the fetal to the adult circulatory system in retina. *Development* **137**, 1563-1571.

Kwon, C., Qian, L., Cheng, P., Nigam, V., Arnold, J. and Srivastava, D. (2009). A regulatory pathway involving Notch1/beta-catenin/Isl1 determines cardiac progenitor cell fate. *Nat. Cell Biol.* **11**, 951-957.

Landeira, D., Sauer, S., Poot, R., Dvorkina, M., Mazzarella, L., Jørgensen, H. F., Pereira, C. F., Leleu, M., Piccolo, F. M., Spivakov, M. et al. (2010). Jarid2 is a PRC2 component in embryonic stem cells required for multi-lineage differentiation and recruitment of PRC1 and RNA Polymerase II to developmental regulator genes. *Nat. Cell Biol.* (in press).

Li, Y., Qian, Y. P., Yu, X. J., Wang, Y. Q., Dong, D. G., Sun, W., Ma, R. M. and Su, B. (2004). Recent origin of a hominoid-specific splice form of neuropsin, a gene involved in learning and memory. *Mol. Biol. Evol.* **21**, 2111-2115.

Lorson, C. L., Rindt, H. and Shababi, M. (2010). Spinal muscular atrophy: mechanisms and therapeutic strategies. *Hum. Mol. Genet*. Epub ahead of print.

Lu, R., Markowetz, F., Unwin, R. D., Leek, J. T., Airoldi, E. M., MacArthur, B. D., Lachmann, A., Rozov, R., Ma'ayan, A., Boyer, L. A. et al. (2009). Systems-level dynamic analyses of fate change in murine embryonic stem cells. *Nature* **462**, 358-362.

Marchetto, M. C., Muotri, A. R., Mu, Y., Smith, A. M., Cezar, G. G. and Gage, F. H. (2008). Non-cell-autonomous effect of human SOD1 G37R astrocytes on motor neurons derived from human embryonic stem cells. *Cell Stem Cell* **3**, 649-657.

Melton, C., Judson, R. L. and Blelloch, R. (2010). Opposing microRNA families regulate self-renewal in mouse embryonic stem cells. *Nature* **463**, 621-626.

Miura, K., Okada, Y., Aoi, T., Okada, A., Takahashi, K., Okita, K., Nakagawa, M., Koyanagi, M., Tanabe, K., Ohnuki, M. et al. (2009). Variation in the safety of induced pluripotent stem cell lines. *Nat. Biotechnol.* **27**, 743-745.

Niakan, K. K., Ji, H., Maehr, R., Vokes, S. A., Rodolfa, K. T., Sherwood, R. I., Yamaki, M., Dimos, J. T., Chen, A. E., Melton, D. A. et al. (2010). Sox17 promotes differentiation in mouse embryonic stem cells by directly regulating extraembryonic gene expression and indirectly antagonizing self-renewal. *Genes Dev.* **24**, 312-326.

Niemann, C. (2009). Differentiation of the sebaceous gland. *Dermatoendocrinology* **1**, 64-67.

Ohinata, Y., Payer, B., O'Carroll, D., Ancelin, K., Ono, Y., Sano, M., Barton, S. C., Obukhanych, T., Nussenzweig, M., Tarakhovsky, A. et al. (2005). Blimp1 is a critical determinant of the germ cell lineage in mice. *Nature* **436**, 207-213.

Okita, K., Ichisaka, T. and Yamanaka, S. (2007). Generation of germline-competent induced pluripotent stem cells. *Nature* **448**, 313-317.

Pasini, D., Cloos, P. A., Walfridsson, J., Olsson, L., Bukowski, J. P., Johansen, J. V., Bak, M., Tommerup, N., Rappsilber, J. and Helin, K. (2010). JARID2 regulates binding of the Polycomb repressive complex 2 to target genes in ES cells. *Nature* **464**, 306-310.

Pereira, C. F. and Fisher, A. G. (2009). Heterokaryon-based reprogramming for pluripotency. *Curr. Protoc. Stem Cell Biol.* **Chapter 4**, Unit 4B 1.

Pereira, C. F., Piccolo, F. M., Tsubouchi, T., Sauer, S., Ryan, N. K., Bruno, L., Landeira, D., Santos, J., Banito, A., Gil, J. et al. (2010). ESCs require PRC2 to direct the successful reprogramming of differentiated cells toward pluripotency. *Cell Stem Cell* (in press).

Ravin, R., Hoeppner, D. J., Munno, D. M., Carmel, L., Sullivan, J., Levitt, D. L., Miller, J. L., Athaide, C., Panchision, D. M. and McKay, R. D. (2008). Potency and fate specification in CNS stem cell populations in vitro. *Cell Stem Cell* **3**, 670-680.

Sasaki, E., Suemizu, H., Shimada, A., Hanazawa, K., Oiwa, R., Kamioka, M., Tomioka, I., Sotomaru, Y., Hirakawa, R., Eto, T. et al. (2009). Generation of transgenic non-human primates with germline transmission. *Nature* **459**, 523-527.

Sharp, J., Frame, J., Siegenthaler, M., Nistor, G. and Keirstead, H. S. (2010). Human embryonic stem cell-derived oligodendrocyte progenitor cell transplants improve recovery after cervical spinal cord injury. *Stem Cells* **28**, 152-163.

Silva, J. and Smith, A. (2008). Capturing pluripotency. *Cell* **132**, 532-536.

Silva, J., Barrandon, O., Nichols, J., Kawaguchi, J., Theunissen, T. W. and Smith, A. (2008). Promotion of reprogramming to ground state pluripotency by signal inhibition. *PLoS Biol.* **6**, e253.

Takahashi, K. and Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **126**, 663-676.

Takehashi, M., Kanatsu-Shinohara, M. and Shinohara, T. (2010). Generation of genetically modified animals using spermatogonial stem cells. *Dev. Growth Differ.* **52**, 303-310.

Tamaki, S. J., Jacobs, Y., Dohse, M., Capela, A., Cooper, J. D., Reitsma, M., He, D., Tushinski, R., Belichenko, P. V., Salehi, A. et al. (2009). Neuroprotection of host cells by human central nervous system stem cells in a mouse model of infantile neuronal ceroid lipofuscinosis. *Cell Stem Cell* **5**, 310-319.

Tesar, P. J., Chenoweth, J. G., Brook, F. A., Davies, T. J., Evans, E. P., Mack, D. L., Gardner, R. L. and McKay, R. D. (2007). New cell lines from mouse epiblast share defining features with human embryonic stem cells. *Nature* **448**, 196-199.

Waddington, C. H. (1957). *The Strategy of the Genes.* London: Geo Allen and Unwin.

Wilkinson, J. M., Machleidt, T., Echeverria, V. M., Vandenheuvel-Kramer, K., Honer, J., Zhong, Z. and Bi, K. (2008). HTS assays using a disease-relevant cell model for interrogating the MAP kinase pathway initiated by multiple receptors. *Assay Drug Dev. Technol.* **6**, 351-359.

Woltjen, K., Michael, I. P., Mohseni, P., Desai, R., Mileikovsky, M., Hamalainen, R., Cowling, R., Wang, W., Liu, P., Gertsenstein, M. et al. (2009). piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. *Nature* **458**, 766-770.

Wu, Z., Chen, J., Ren, J., Bao, L., Liao, J., Cui, C., Rao, L., Li, H., Gu, Y., Dai, H. et al. (2009). Generation of pig induced pluripotent stem cells with a drug-inducible system. *J. Mol. Cell Biol.* **1**, 46-54.

Xu, R. H., Sampsell-Barron, T. L., Gu, F., Root, S., Peck, R. M., Pan, G., Yu, J., Antosiewicz-Bourget, J., Tian, S., Stewart, R. et al. (2008). NANOG is a direct target of TGFbeta/activin-mediated SMAD signaling in human ESCs. *Cell Stem Cell* **3**, 196-206.

Yamane, J., Nakamura, M., Iwanami, A., Sakaguchi, M., Katoh, H., Yamada, M., Momoshima, S., Miyao, S., Ishii, K., Tamaoki, N. et al. (2010). Transplantation of galectin-1-expressing human neural stem cells into the injured spinal cord of adult common marmosets. *J. Neurosci. Res.* **88**, 1394-1405.

Ying, Q. L., Wray, J., Nichols, J., Batlle-Morera, L., Doble, B., Woodgett, J., Cohen, P. and Smith, A. (2008). The ground state of embryonic stem cell self-renewal. *Nature* **453**, 519-523.