VIEWPOINT

Stem cells assessed

Cédric Blanpain, George Q. Daley, Konrad Hochedlinger, Emmanuelle Passegué, Janet Rossant and Shinya Yamanaka

Abstract | The increasing momentum of stem cell research continues, with the better characterization of induced pluripotent stem (iPS) cells, the conversion of differentiated cells into different cell types and the use of pluripotent stem cells to generate whole tissues, among other advances. Here, six experts in the field of stem cell research compare different stem cell models and highlight the importance of pursuing complementary experimental approaches for a better understanding of pluripotency and differentiation and an informed approach to medical applications.

Six years on from the initial derivation of induced pluripotent stem cells (iPS cells)[1], new studies have highlighted important differences between iPS cells and embryonic stem (ES) cells, including their epigenetic landscapes. How comparable is the developmental status of iPS cells and ES cells, and how do you view the potential of iPS cells as an alternative to ES cells for use in research, disease modelling and therapies?

Cédric Blanpain. Aside from some minor differences, iPS cells and ES cells from mice exhibit very similar epigenetic landscapes. It was possible to define the similarities and differences between the epigenetic landscapes of mouse ES cells and iPS cells because the naive state of pluripotency and the early steps of ES cell commitment have been characterized with great precision for mouse ES cells.

By contrast, much less is known about the naive state of human ES cells. In addition, for obvious ethical reasons, the 'gold standard' assay to assess pluripotency *in vivo* in mice — the demonstration of contribution to all cell lineages, including germline transmission — cannot be used for human ES cells.

There is no doubt that iPS cell technology will further improve and will be widely used for research, disease modelling and therapies. The challenge now is to better define the epigenetic landscapes of human pluripotent cells and subsequently improve the reprogramming methods, so that iPS cells almost exactly resemble the naive state of human ES cells. Once this goal is achieved, it is very likely that iPS cells will replace human ES cells for most applications.

George Q. Daley. When randomly chosen ES cells and iPS cells are compared against one another by microarray and low-resolution methylation analyses, the differences among ES cells are as significant as the differences between ES cells and iPS cells. Especially when iPS cells are reprogrammed from optimal sources (for example, from embryonic fibroblasts) with highly efficient transgene-free methods, the resulting iPS cells are virtually indistinguishable from ES cells by functional and molecular criteria. Thus, I would argue that ideal generic iPS cells are comparable in all ways to ES cells. However, in a practical sense, individual clones of iPS and ES cells may manifest important differences, especially when derived in one laboratory where unique, local technical practices produce consistent differences between the cells. We have generated a large series of pluripotent stem cells from the same mouse strain, including iPS cells generated from dermal fibroblasts of aged mice, and ES cells from embryos made by nuclear transfer as well as naturally fertilized blastocysts[2]. We even compared lines made from 'secondary' systems that carry the same transgene integration. We saw consistent differences in the differentiation capacity of these cells, and by using genome-wide, high-resolution methylation analyses we could consistently distinguish among the distinct cell types, even when blinded to the identity of the cells[2]. We were likewise able to distinguish human iPS cells from different tissues[3]. So, although I conclude that ES cells and iPS cells are theoretically comparable, in practical use, when iPS cells are derived from distinct tissues of aged individuals, conditions that are less than optimal for reprogramming, the resulting cells can harbour distinct and diagnostic epigenetic signatures that reflect the technical limitations of reprogramming. This is an important feature to recognize when trying to model disease, especially when reprogramming cells from patients of advanced age. As we refine reprogramming techniques, I anticipate that the epigenetic memory that distinguishes iPS cells from different tissue sources will be more effectively erased. I think we are still too early in the preclinical phase of both ES cell and iPS cell technology to envision precisely which sources will prove optimal. I predict that for some conditions, 'off-the-shelf' cell products based on ES cells will prove useful, whereas for other disorders more personalized iPS cell therapies will be called for.

Konrad Hochedlinger. Although a number of molecular differences have been reported between ES cells and iPS cells, recent data suggest that experimental variables, such as genetic background, passage number, viral integrations, derivation conditions and line-to-line variability among them, can markedly affect the epigenetic and functional properties of stem cells and might account for many of the previously seen differences. I therefore think that it will be important to continue the work on both human ES cells and iPS cells until we have a clear understanding of their similarities and differences, with the aim to assess whether any possible differences have adverse effects on their therapeutic potentials.

The developmental potency of human ES cells and iPS cells has not yet been evaluated beyond teratoma formation, which is not a stringent assay. However, certain mouse iPS cell lines can generate entire animals and thus pass the strictest developmental assay. This indicates that at least some iPS cell lines are functionally equivalent to ES cells. It might therefore be possible to identify those iPS cell lines with desired traits by using optimized culture conditions and potential markers.

Emmanuelle Passegué. By deriving ES cells, we are freezing a very early and transient stage of embryonic development *in vitro*, which allows us to dissect the general principles and to underpin the mechanisms that control pluripotency and lineage specification. By reprogramming differentiated cells into iPS cells, we are learning about the plasticity of fate commitment and the complex molecular networks and underlying epigenetic mechanisms that

The contributors*

Cédric Blanpain is a researcher of the Belgian Fund for Scientific Research (FRS/FNRS) and an investigator of the Walloon Excellence in Life Science and Biotechnology (WELBIO) at the Université Libre de Bruxelles (ULB), Belgium. He carried out his postdoctoral studies in the laboratory of Elaine Fuchs, Rockefeller University, New York, USA. His laboratory is studying the role of stem cells during development, homeostasis and cancer.

George Q. Daley is the Samuel E. Lux IV Professor of haematology and oncology and Director of the Stem Cell Transplantation Program at Children's Hospital Boston, Massachusetts, USA. He is also Professor of Biological Chemistry and Molecular Pharmacology and Pediatrics at Harvard Medical School, Boston, Massachusetts, USA, and an investigator of the Howard Hughes Medical Institute, Chevy Chase, Maryland, USA.

Konrad Hochedlinger is an associate professor at Harvard's Department for Stem Cell and Regenerative Biology, Cambridge, Massachusetts, USA, and a member of the Massachusetts General Hospital Cancer Center and Center for Regenerative Medicine, Boston, Massachusetts, USA. His laboratory is studying the molecular mechanisms of cellular reprogramming. Using induced pluripotent stem cells and embryonic stem cells from mice and humans, his laboratory tries to understand how transcription factors induce epigenetic remodelling of the genome and ultimately cell fate change.

Emmanuelle Passegué is an associate professor of medicine at the Eli and Edythe Broad Center for Regenerative Medicine and Stem Cell Research at the University of California, San Francisco (UCSF), California, USA. She trained with Irving Weissman at Stanford University, California, USA, and joined the UCSF faculty in 2006. Her laboratory investigates the mechanisms controlling haematopoietic stem cell function during normal haematopoiesis and in haematological malignancies.

Janet Rossant is a developmental biologist interested in lineage formation in the mammalian blastocyst and its derived stem cell lines. She leads the Ontario Stem Cell Initiative, a consortium of scientists, engineers, clinicians and academic and commercial partners dedicated to applying stem cell discovery to treating disease.

Shinya Yamanaka received his Ph.D. from Osaka City University, Osaka-shi, Japan, and he worked at the Gladstone Institute of Cardiovascular Disease, San Francisco, California, USA. He took up a professorship at Nara Institute of Science and Technology, Ikoma, Japan, in 1999 and at Kyoto University, Japan, in 2004. He now serves as Director of the Center for iPS Cell Research and Application (CiRA), Kyoto, Japan. His laboratory works on the establishment of generation and evaluation methods of clinical-grade induced pluripotent stem cells.

*Listed in alphabetical order.

ensure lineage specification. Both are fantastic model systems that are not yet fully interchangeable and might never be made so. Therefore, there is still a need to use both for research, disease modelling and therapeutic purposes, and to define which one provides the best results for specific applications.

Janet Rossant. The excitement around iPS cell generation has been enormously invigorating for the field of stem cell research. However, we still do not fully understand the process of reprogramming to pluripotency. It is perhaps not surprising that we are discovering that iPS cell generation can lead to genetic and epigenetic changes that may not reflect the endogenous pluripotent state. However, improved understanding of the temporal progression of reprogramming and better biomarkers of the true pluripotent state will bring ES cell and iPS cell research closer together. iPS cells remain a major breakthrough in being able to access early stages of human development and disease in a petri dish. As we understand more about ES cells, we will be able to compare and contrast them with iPS cells until we have a robust system to generate patient-specific pluripotent cells by direct reprogramming from patient samples.

Shinya Yamanaka. This important question remains controversial. Some researchers have argued that iPS cells can be distinguished from ES cells in terms of their gene expression and DNA methylation patterns, whereas others have reported that both types of pluripotent stem cells have overlapping variations. We noticed that the former groups compared small numbers of clones (generally fewer than ten) of iPS cells and ES cells, whereas the latter groups studied more clones. In our own experience, we have found that many iPS cell clones are indistinguishable from ES cells.

Q *Recent studies have reported the successful production of different cell types, both through* transdifferentiation *and through* reconstitution approaches *using stem cells. How do the therapeutic promise of these and other approaches compare, and which applications may ultimately be most realistic?*

C.B. Stem cells are already used successfully in regenerative medicine, albeit only for a very limited number of treatments such as haematopoietic stem cell transplantation after cancer therapy and transplantation of *in vitro*-reconstituted skin to severely burnt patients. Besides these rare proven stem cell therapies, a lot of studies are going on around the world assessing the potential clinical benefits of a variety of different types of stem cells, for which the safety and the efficacy remains to be determined.

Although iPS cell technology generates great enthusiasm, it remains challenging to differentiate iPS cells into fully mature and functional cells that can be used to replace damaged or deficient cells in clinical settings. We clearly need to establish reproducible protocols to obtain functional and transplantable cells for medical therapy. The functional integration of transplanted cells into tissues also remains a challenge.

The reactivation of endogenous progenitor cells, which naturally mediate tissue repair, is a very interesting alternative approach to cell therapy, although a much better understanding of how stem cells are normally activated during endogenous tissue repair is a prerequisite to achieving this goal.

G.Q.D. Although promising and certainly appealing from a practical perspective, transdifferentiated cells remain rather poorly characterized and not well understood. These cells have not yet been dissected with the refined global genetic and epigenetic analyses that have been applied to reprogrammed iPS cells, and I worry that directly converted cells will ultimately be shown to be at best partially converted, with significant distortions of the heterochromatin and euchromatin landscapes. However, if proven to be epigenetically identical to their native tissues, I think the cells will have enormous value for research and perhaps one day as therapies.

K.H. I believe that it is too early to tell if there is just one winner. It might be that transdifferentiation is more practical and technically easier for the generation of certain cell types, such as muscle and pancreatic β-cells, whereas reprogramming into iPS cells and subsequent *in vitro* differentiation works

better for the derivation of other cell lineages (for example, gut and lung cells). We still have a poor understanding of the type of cell that is actually generated by transdifferentiation compared with the respective cell type in the body. However, I am optimistic that the similarities and differences between primary cells and cells derived by transdifferentiation or *in vitro* differentiation will soon be delineated.

A major limitation of transdifferentiation has been the low yield of cells of interest — that is, the number of neurons, cardiomyocytes and so on that can be generated from a skin biopsy. However, the recent insight by several laboratories that fibroblasts can be dedifferentiated into self-renewing neural stem cells is a promising step forward and might eliminate this barrier, at least for the neural lineage.

E.P. Both transdifferentiation and direct differentiation following transplantation are very promising approaches for tissue repair. The clinical success of haematopoietic stem cell transplantation indicates that direct differentiation is a valid therapeutic strategy, at least for tissues with high cellular turnover. It might turn out that local transdifferentiation will ultimately be more successful for tissues with low cellular turnover, such as the brain or the heart. The future will tell us, but at this stage of the regenerative medicine field many experimental possibilities should be investigated.

J.R. New studies on transdifferentiation of adult cells directly to neurons, heart muscle cells, blood cells and more are intriguing. Will we be able to generate more mature differentiated cells for direct stem cell therapy by this route rather than by laborious forward differentiation from pluripotent cells? Will we be able to reprogram cells safely *in situ* in the tissue of interest by transgene or small molecule expression? Will these cells be truly functional? Will the process be efficient enough to overcome the possible lack of proliferation of the transdifferentiated cells? Can we reprogram differentiated cells directly to stable, self-renewing, tissue-specific progenitors? We do not know enough about the rules of engagement in transdifferentiation at this time to be able to make a judgment call on the best route forward.

S.Y. All scientific technologies have pros and cons. For some applications, iPS cells may be useful if we can create human leukocyte antigen (HLA)-haplotyped stock of appropriately selected iPS cell lines as a source for cell transplantation. But direct reprogramming may be better for other applications to obtain a desired cell type by *in vivo* conversion from another somatic lineage. In any case, it is necessary to do our best to promote both of these closely related technologies for the sake of the patients that are eagerly awaiting new therapies.

Q *Early developmental pathways have been shown to differ in primates versus mice, the latter being a key model organism for stem cell research. How do the benefits to be gained from studies in model systems weigh in against these possibly limiting differences?*

C.B. The mouse has been a key model organism for the study of stem cell biology. The ability of mice to be genetically modified with great precision has allowed us to establish important paradigms in developmental and stem cell biology that are relevant to human development.

Although there are clear differences between mice and humans, many key developmental decisions have been highly conserved during mammalian evolution, and mouse models have provided great insights into different developmental and regenerative processes in humans.

Obviously, primates and humans are much more alike, and the experiments conducted on primates would be more relevant to humans. However, the greater sense of self-recognition of primates led some countries either to forbid the use of primates for biomedical research, or to restrict it to rare cases of important human threats for which no other animal model could be used. The limitations of mouse models for certain aspects of stem cell therapy and the restriction of primate research led to the development of other animal models, such as pigs, to assess the efficacy of stem cell therapy. There is not a single ideal model for stem cell therapy, and the best animal model should probably be defined for each application.

G.Q.D. We have long appreciated the limitations and the value of animal models for teaching us about human biology. Although there are indeed pathway differences, the overall architecture of tissue and organ development has many common, evolutionarily conserved principles. We are finding that studying development comparatively across many organisms — worms, flies, fish, mice and, increasingly, human cells — provides the richest insights.

K.H. I think that the discovery of iPS cells in mice and their extension to humans and other species is the best example that key networks of pluripotency are conserved and one can therefore gain important insights into human development, pluripotency and reprogramming from studying these principles in mice. Moreover, because of work with mouse ES cells and epiblast stem cells, we have been able to generate human ES cells with traits of mouse ES cells (so-called naive state pluripotency) by experimental manipulation. However, there are clear biological differences between humans and mice, and therefore one has to be careful in directly extrapolating any new findings from mice to primates. A point in case is the differential susceptibilities of mouse and human cells to oncogenes, which has affected some cancer studies. In spite of these caveats, I think that the benefits of using mice as a model system (for example, the availability of genetically tractable developmental assays, short gestation time and the fact that they are affordable to many researchers) by far outweigh their limitations.

E.P. The importance of the mouse model system for understanding stem cell biology and tissue repair cannot be overstated. Its strength is the ability to manipulate and interrogate both cells and host with a vast array of cellular, molecular and genetic tools that are not available and might never become available in other primate models. It allows us to formulate testable hypotheses before testing in larger animal models or directly in clinical trials. Although, of course, the mouse model system does not substitute for real human experimentation, it is an essential part of this translational research process. Unfortunately, mouse modelling is more and more disappearing from the 'translational pipelines' supported by the agencies funding the field of regenerative medicine. This might have adverse consequences, especially for understanding the often unanticipated results of human clinical trials.

J.R. There are clearly differences in timing and morphology between rodent and primate embryos, and some differences in signalling pathway usage that may explain why mouse and human ES cells do not have identical molecular and cellular properties. However, the developmental pathways driving tissue and organ differentiation seem to be mostly conserved. The power of mouse genetics means that functional roles for different genes and pathways can be tested rigorously in mice and provide strong

candidates for further analysis in humans. Much of the current success in driving differentiation of human ES cells down developmental lineages has been based on years of experiments identifying conserved pathways in mice and other vertebrates.

S.Y. We identified the four reprogramming factors (OCT4 (also known as POUF51 and OCT3), SOX2, KLF4 (Krüppel-like factor 4) and c-MYC) in mice and then found that the same four factors can be used to make human iPS cells[1]. This is our best example to show the importance of model systems. However, the development of new research models should remain a priority to ensure that the most accurate results can be achieved.

Q *What have been the main technical hindrances for stem cell research, and which advance would be most significant to overcome them?*

C.B. In terms of basic stem cell research, the main technical hindrances have been stem cell heterogeneity and the visualization of stem cells within their *in vivo* microenvironment. The development of single-cell assays enabling the marking and transcriptional profiling of a single stem cell and the development of novel *in vivo* imaging techniques to visualize stem cells in action *in vivo* within their native niche should overcome these current limitations. Moreover, the vast amount of data from transcriptional and epigenetic profiling of stem cells needs to be integrated in a more global approach, allowing the building of precise gene networks that regulate stem cell identity and function.

Regarding the therapeutic applications of stem cells, one of the major technical hindrances is the proper differentiation of stem cells into mature and functional cells that can be engrafted and functionally integrated into the damaged tissues. A better understanding of how stem cells differentiate into a particular cell type and become functionally mature *in vitro* and *in vivo* is crucial to overcome these current limitations. In addition, a much better understanding of how the endogenous repair is orchestrated will greatly help to improve stem cell therapies.

G.Q.D. Our understanding of pathways for directed differentiation and, in particular, the developmental maturation of ES cells to adult somatic tissues remains woefully inadequate. We cannot even begin to imagine highly successful engraftment therapies until we can better control tissue fates. Then the enormous challenges of integration of donor tissues into the host loom large. These challenges are indeed why my laboratory is focused on blood development, where the architecture of tissue engraftment is not limiting.

K.H. Exciting advances over the past few years have provided new surface markers and reporter alleles to prospectively isolate epithelial stem cells from many tissues, including intestine, stomach, lungs and skin. Moreover, culture conditions have been defined that preserve some of these stem cells in a self-renewing multipotent state, which has facilitated mechanistic studies and even allowed the expansion of stem cells for transplantation purposes (for example, skin, cornea, testis and colon). Despite their successful use in bone marrow transplantation, haematopoietic stem cells have so far been refractory to long-term culture. Identifying conditions that preserve their self-renewal potential and multipotency would therefore be a significant advance. In addition, optimized protocols for single-cell genomic and epigenomic analyses would be extremely helpful to overcome some of these limitations and also to address the heterogeneity of stem cell populations. Last, I think that improved live-cell imaging technologies to track stem cells *in vivo* will provide novel insights into tissue homeostasis and cancer.

Two of the remaining challenges with regard to potential applications of ES cells and iPS cells are the derivation of differentiated cell types at high efficiency, purity and maturity (in disease modelling and therapy) as well as protocols to successfully engraft these cells *in vivo*. Exciting new advances have been made over the past few years in terms of generating mature cell types from ES cells and iPS cells (for example, gut and lung cells) and engrafting *in vitro*-derived human cells in animal models at high efficiency (for example, dopaminergic neurons).

Glossary

Blastocysts
Structures that are formed during early embryogenesis in mammals. The fertilized embryo undergoes cleavage to produce blastomeres, which, after a fixed number of cell divisions, become compacted together. The outer cells form an epithelium (the trophoblast) that separates from the internal group of cells, which constitute the inner cell mass (ICM). The resulting structure comprising the trophoblast and the ICM is called the blastocyst.

Epiblast
The inner layer of the developing embryo that originates from the inner cell mass (ICM) and gives rise to the fetus.

Euchromatin
A form of chromatin that is lightly packed and often transcriptionally active during interphase.

Heterochromatin
Highly compacted chromatin that is transcriptionally inactive. Includes structural regions of the chromosome that lack genes ('constitutive' heterochromatin, for example, centromeres) as well as genes that are silenced in a given cell type ('facultative' heterochromatin).

Induced pluripotent stem cells
(iPS cells). Somatic cells that have been induced to become pluripotent through ectopic expression of four transcription factors — OCT4, SOX2, MYC and KLF4 (Krüppel-like factor 4).

Naive state
A state of pluripotency in which cells are fully unrestricted and can give rise to all cell types of the embryo and later adult. This state is present only transiently during mammalian development, in the pre-implantation epiblast, and after culture of mouse embryonic stem cells in the presence of inhibitors of glycogen synthase kinase 3 (GSK3) and extracellular signal-regulated kinase (ERK).

Niche
Specialized microenvironment in which stem cells reside. The nice produces signals that regulate stem cell identity and maintenance.

Nuclear transfer
An experimental method to reprogram differentiated cells back to pluripotency. In this method, mammalian somatic cell nuclei are transplanted into a previously enucleated oocyte (unfertilized egg).

Organotypic
A culture system in which the tissue, removed from an organ, continues to differentiate and develop as if it was in the original organ.

Orthotopic
Transplantation of tissue or cells from a donor into its normal position in the body of the recipient.

Reconstitution approaches
Differentiation of pluripotent stem cells into a tissue or an organ.

Teratoma
An encapsulated, non-malignant tumour that comprises tissue or organ components resembling normal derivatives of all three germ layers.

Transdifferentiation
The use of transcription factors (and, in some cases, chemical factors) to convert a differentiated cell type into another differentiated cell type, even between developmentally distant cells (belonging to different germ layers): for example, the conversion of fibroblasts to neurons.

E.P. The field of stem cell research has been blossoming over the past 5 years and has constantly been at the frontier of new scientific discovery. The development of organotypic culture systems, of orthotopic transplantation techniques and, more recently, of *in vivo* lineage tracing approaches have offered a deeper understanding of tissue hierarchy and of the relative role of long-lived stem cells and transient progenitor cells during different stages (during adult versus embryonic development, and in steady state, regeneration and injury-mediated tissue repair). Our understanding of the developmental pathways and of the opposing and self-reinforcing transcription factors controlling stem cell function has also considerably increased, as has our ability to manipulate these networks to improve tissue repair. Thus, clear progress has been made on the long road of using stem cells for regenerative medicine. However, one of the current challenges is the ability to repair damaged tissue *in situ* and to improve its function without eliciting rejection or causing more damage. Overcoming these obstacles will require cross-disciplinary approaches that bridge bioengineering with immunological studies and the development of medical devices for cell implantation and cell tracking, as well as fundamental investigations of stem cell function in tissue maintenance for limiting genomic instability and eventual cancer development.

J.R. Human ES cells are still hard to grow, dissociate and clonally propagate. Deriving more robust cells with properties similar to mouse ES cells would still be a technical advance. Differentiation to fully mature cell types also continues to be a challenge and may need to be re-examined by asking whether immature precursors could be engineered to express the function of interest for a particular clinical application, even if the entire *in vivo* cell phenotype cannot be achieved. Finally, developing appropriate preclinical and clinical models for tissue replacement therapy is a growing need.

S.Y. There are still many hurdles, but I would like to highlight two issues. The first is the *in vitro*-directed differentiation of ES cells and iPS cells into several lineages, such as haematopoietic progenitor cells and insulin-producing β-cells. To achieve these goals, I think it is crucial to develop proper culture conditions that allow long-term maintenance of these somatic cells. The second hurdle I would like to underscore is how to make tissues or organs from stem cells. This is a continuing challenge for the future, although recent studies carried out by two Japanese groups are promising. One group successfully generated three-dimensional structures resembling eyes and adenohypophysis from mouse ES cells[4,5]. Another group generated a rat pancreas in a mouse by interspecific blastocyst injection of pluripotent stem cells[6]. These approaches may be applicable to humans in the future.

Q *The laws regarding stem cell research are often criticized as being too restrictive and confusing. Do you think they are necessary, and what one change in legislation might improve this situation for researchers?*

C.B. Laws should be made to protect individuals and to establish standards and rules on the basis of strong scientific and ethical considerations. The laws concerning stem cell research vary from country to country, with the exception of some universal rules, such as the prohibition of human cloning. The cultural differences in public opinion between different countries add to the richness of our world. It will be important to keep respecting such cultural differences even if they complicate the harmonization of stem cell regulations.

Concerning stem cell therapy, it is imperative that the laws worldwide are enforced to meet the highest standards: this would include rigorous preclinical testing, enrolling patients with their informed consent and conducting controlled and approved clinical trials with the hope of therapeutic benefits from the treatment. Today, patients are sometimes treated with unproven stem cell therapies outside clinical trials, which is unacceptable.

The laws should also be made to protect intellectual property, which stimulates investment and consequently accelerates the release of new treatments for patients. For all these issues, it is important that the lawmakers are very well informed by the most competent scientists before passing laws on stem cell research.

G.Q.D. The biggest limitation remains federal restrictions on funding, which has stifled the field in the past decade and slowed down the wider exploitation of pluripotent stem cells in research. Now, although the use of iPS cells is less restrictive, we have run head-on into the major National Institutes of Health (NIH) budget constraints owing to the economic crisis. At a time when stem cell biology is so enormously exciting and promising, we lack the resources to fully capitalize on the promise. We can only hope this will change as the economy recovers.

K.H. With the lift of the 2001 restrictions on human ES cell research, legislation is not a major problem anymore. However, there are several human ES cell lines that still remain outside the NIH system. Consequently, federal funding is not available to study these lines. Because many of these cell lines have been derived through pre-implantation genetic diagnosis and carry mutations that have been associated with diseases, it would be very informative to study them in addition to the other approved cell lines using NIH funds.

E.P. Laws and ethical debates are required to define a society regardless of whether it is for a scientific or a private-right question. Rulings based on faith and religious beliefs instead of on scientific facts have indeed hindered the pace of stem cell research, especially with regard to using human ES cells. Clarifying the legislation might certainly help researchers to fully explore the potential of these stem cells for human therapy. It is equally important to continue to educate the public regarding the scientific reality associated with the derivations and use of human ES and iPS cells.

J.R. The legislative environment for stem cell research is very different in different jurisdictions, making a blanket answer to this question difficult. In general, I am in favour of clear national guidelines on stem cell research that can be implemented across all disciplines and apply to all researchers, whether in the public or private sector. Guidelines set and constantly reviewed by an independent and respected national body are better than regulations couched in legislation. Laws are hard to draft so that they encompass all current and future possibilities and hard to change when scientific or societal circumstances change.

S.Y. Scientific technologies are double-edged swords: they can provide many benefits for humanity, but at the same time can be harmful. Thus, regulations are necessary. I think that it is essential for scientists to exert their best efforts to explain the pros and cons of their developing technologies to the public using language that is easy to understand, but as accurate as possible. Therefore, this field of research needs talented science communicators who can explain both the merits and disadvantages of their research.

Cédric Blanpain is at the Walloon Excellence in Life Science and Biotechnology (WELBIO), Interdisciplinary Research Institute (IRIBHM), Université Libre de Bruxelles (ULB), 808, route de Lennik, BatC, C6-130, 1070 Brussels, Belgium.
e-mail: Cedric.Blanpain@ulb.ac.be

George Q. Daley is at The Children's Hospital and Dana Farber Cancer Institute Karp Family Research Building 7214, 300 Longwood Avenue Boston, Massachusetts 02115, USA.
e-mail: daley.lab@childrens.harvard.edu

Konrad Hochedlinger is at the Howard Hughes Medical Institute (HHMI) and Harvard Department of Stem Cell and Regenerative Biology, 7 Divinity Avenue, Cambridge, Massachusetts 02138, USA; and the Massachusetts General Hospital (MGH), Cancer Center and Center for Regenerative Medicine, 185 Cambridge Street, Boston, Massachusetts 02114, USA.
e-mail: khochedlinger@helix.mgh.harvard.edu

Emmanuelle Passegué is at the University of California, San Francisco, Department of Medicine, Division of Hematology and Oncology and the Eli and Edythe Broad Center of Regeneration, Medicine and Stem Cell Research, 35 Medical Center Way, RMB-1017, Box 0667, San Francisco, California 94143, USA.
e-mail: passeguee@stemcell.ucsf.edu

Janet Rossant is at the The Hospital for Sick Children, Departments of Molecular Genetics, and Obstetrics and Gynecology, University of Toronto, 555 University Avenue, Toronto, Ontario M5G 1X8, Canada.
e-mail: janet.rossant@sickkids.ca

Shinya Yamanaka is at the Center for iPS Cell Research and Application, Kyoto University, 53 Kawahara-cho, Shogoin Yoshida, Sakyo-ku, 606-8507 Kyoto, Japan; and the Gladstone Institute of Cardiovascular Disease, 1650 Owens Street, San Francisco, California 94158-2261, USA.
email: yamanaka@cira.kyoto-u.ac.jp

doi:10.1038/nrm3371
Published online 8 June 2012

1. Takahashi, K. & Yamanaka, S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **25**, 663–676 (2006).
2. Kim, K. *et al.* Epigenetic memory in induced pluripotent stem cells. *Nature* **16**, 285–290 (2010).
3. Kim, K. *et al.* Donor cell type can influence the epigenome and differentiation potential of human induced pluripotent stem cells. *Nature Biotech.* **29**, 1117–1119 (2011).
4. Eiraku, M. *et al.* Self-organizing optic-cup morphogenesis in three-dimensional culture. *Nature* **472**, 51–56 (2011).
5. Suga, H. *et al.* Self-formation of functional adenohypophysis in three-dimensional culture. *Nature* **480**, 57–62 (2011).
6. Kobayashi, T. *et al.* Generation of rat pancreas in mouse by interspecific blastocyst injection of pluripotent stem cells. *Cell* **142**, 787–799 (2010).

Acknowledgements
E.P. is a California Institute for Regenerative Medicine (CIRM, San Francisco, California, USA) New Faculty member and a scholar of the The American Society of Hematology (ASH).

Competing interests statement
The authors declare competing financial interests; see Web version for details.

FURTHER INFORMATION
Cédric Blanpain's homepage:
http://blanpainlab.ulb.ac.be/index.htm
George Q. Daley's homepage:
https://daley.med.harvard.edu
Konrad Hochedlinger's homepage:
http://www.hms.harvard.edu/dms/BBS/fac/hochedlinger.php
Emmanuelle Passegué's homepage:
http://passeguelab.ucsf.edu
Janet Rossant's homepage:
http://www.sickkids.ca/research/rossant
Shinya Yamanaka's homepage:
http://www.cira.kyoto-u.ac.jp/yamanaka_group/?lang=en
ALL LINKS ARE ACTIVE IN THE ONLINE PDF