

Stem Cells in 2009

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There is intense contemporary interest in the identity, stability, and potential of stem cells, in the body or in the lab. The unusually comprehensive view provided by the 7th annual meeting of the ISSCR provides a framework to summarize recent progress.

More than 3100 researchers traveled to Barcelona in July for the 2009 annual meeting of the International Society for Stem Cell Research. To reflect the enthusiasm of this young and rapidly growing scientific society, we use a style that is informal but hopefully informative to summarize the main themes that were discussed. The meeting started with a focus on the brain and ended with pluripotency. Along the way, participants from 56 different countries (see [Figure 1](#)) discussed advances in many areas of stem cell biology.

Brain Disease and Repair

The excitement in the stem cell field is derived from many sources, one of the most significant being relevance to human health and disease. Advances in stem cell biology are often presented as bringing us closer to therapies for neurodegenerative disease. To provide a context for this goal, the opening session focused on the development and regeneration of the brain. Nancy Wexler (Columbia University, New York City) opened with a description of how the presence of Huntington's disease (HD) in her family led her to the Lake Maracaibo region of Venezuela. Using videos of patients and their families, Nancy showed how the disease alters movement and how the effects are distinct in different people. The large pedigree gathered around Lake Maracaibo led to HD being the first genetic disease mapped using the techniques of DNA chemistry. HD is one of several brain disorders caused by an expansion of a polyglutamine region that is often associated with abnormal protein aggregates or inclusions in the nuclei of cells. Even though HD is highly penetrant, individuals with the same number of repeats have very different patterns of disease. Contemporary analysis of the Venezuela kindred is focused on the identification of other genomic regions that modify the severity of the disease. Elena Cattaneo (University of Milan) continued this theme. She described a series of experiments that started with cellular models and culminated in clinical studies showing mutant *htt* alters the expression of a growth factor (BDNF) and the receptor TrkB that promote neuronal survival ([Zuccato et al., 2008](#)). These changes in gene expression are linked to activation of the REST/NRSF repressor that regulates fundamental features of neural differentiation. REST interacts physically and genetically with other members of a protein complex that represses transcription. This complex includes the methyl-CpG-binding protein 2 (MeCP2). Loss or gain of *MeCP2* expression causes a range of neurological and

psychiatric disability, Rett syndrome. Fred 'Rusty' Gage (Salk Institute, La Jolla) discussed a novel function of MeCP2 in the developing brain. Long interspersed nuclear elements-1 (Line-1 or L1) are derived from retrotransposons and represent >15% of the human genome. When they are transcribed, these RNAs can retrotranspose into new genomic locations where they cause somatic mosaicism that is associated with clinical disease. L1 promoters have CpG islands, and their transcription increases as neural precursors differentiate. It is now clear that there are increased numbers of L1 insertions in the genome of human brain cells, implying that loss of *MeCP2* is mutagenic, providing a potentially novel cause of disease ([Coufal et al., 2009](#)). The interaction of multiple genetic loci is a confounding issue for most diseases. In HD and Rett, stem cell biology is providing new tools to understand how mutations lead to the pathological mechanisms.

The mechanisms controlling stem cell differentiation in the central and peripheral nervous system were presented by Yukiko Gotoh (University of Tokyo) and Marianne Bronner-Fraser (Caltech, Pasadena). Their presentations showed that our growing ability to define specific steps in the neural lineage provides fundamental rules controlling cell fate and survival in the brain. The generation of dopamine neurons from stem cells has stimulated a great deal of work on the *ex vivo* development of this cell type, but recent work suggests that grafted dopamine neurons acquire disease in Parkinson's patients. Etienne Hirsch (Salpêtrière Hospital, Paris) presented clear evidence that dopamine neurons are killed by CD4⁺ T cells ([Brochard et al., 2009](#)). A similar finding, a non-cell-autonomous cause of neuronal death, has been obtained in models of amyotrophic lateral sclerosis (ALS). In this case, motor neurons die when the initiating mutation is expressed in either immune or vascular cells, but not in cells of the brain ([Yamanaka et al., 2008](#)). Cell therapy is often presented as the justification for the major effort to generate human neurons from pluripotent cells. As these presentations showed, neurodegeneration is caused by a pathology that involves multiple cell types. Regenerative medicine will have to develop an understanding of the cell interactions and widely dispersed pathology that put patients at risk.

In the Endoderm

Allan Spradling (Carnegie Institute, Baltimore) showed that the *Drosophila* hindgut has quiescent stem cells that can divide in

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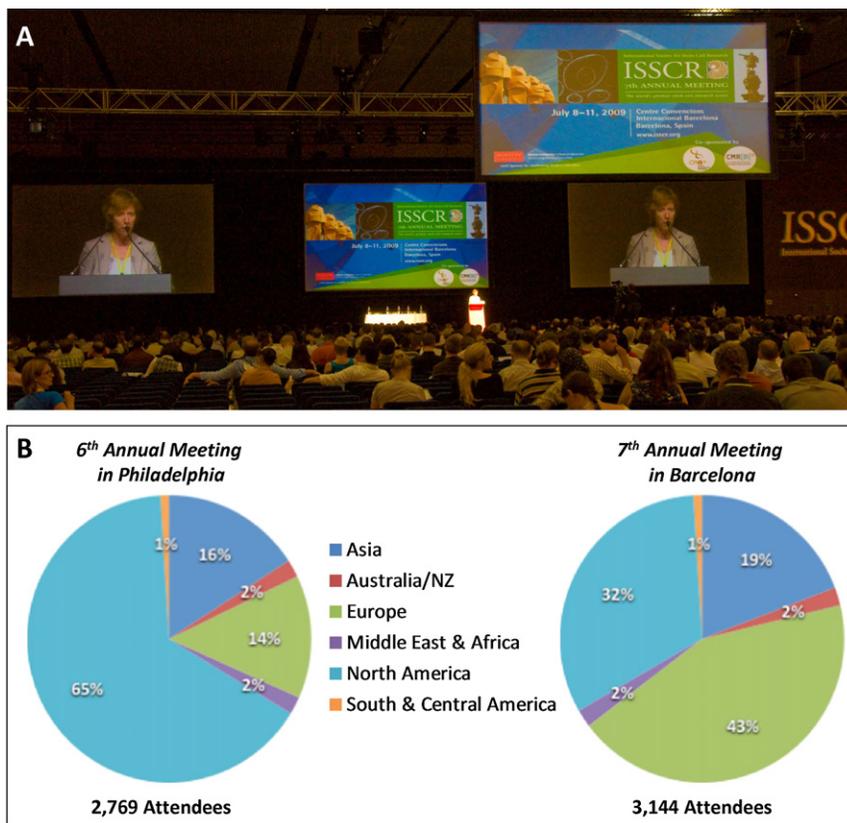


Figure 1. The 7th Annual Meeting in Barcelona Drew a Large and Diverse Audience of Attendees

(A) Fiona Watt opening the meeting. The difference in scale between the video images and the distant brightly lit figure of the President of our society gives a visual sense of the size of the meeting room and the scope of the program.

(B) The geographic origin of the participants at the 6th Annual Meeting in 2008 versus the 7th Annual Meeting in 2009.

Along the whole length of the endoderm, outgrowths of the gut tube form specialized organs. From the perspective of human disease, the liver and pancreas are among the most significant of these endodermal structures. Claude Bernard showed that the liver pumps sugar into the blood. Through the production of insulin, the pancreas takes sugar out of the blood. Pancreatic dysfunction leads to high levels of glucose in the blood, and the frequency of diabetes is stimulating many different approaches to generate or control the insulin-producing pancreatic β cells. There are two major types of cells in the pancreas: the exocrine cells secrete enzymes into the

response to injury and promote repair (Fox and Spradling, 2009). In mammals, the existence of intestinal stem cells has been known since the work of LeBlond and colleagues in the 1960s. However, until this year, there has been no rigorous demonstration of the clonal growth of intestinal stem cells in vitro. Han Clevers (Hubrecht Institute, Utrecht) described work showing that single cells from the adult intestinal crypt would proliferate form structures that contain both a crypt and a villus, the two morphological domains of many regions of the gut. These model intestinal units would self-renew in vitro, clearly meeting the two in vitro criteria for stem cells. This process occurs in the absence of other niche cells, perhaps because Paneth cells at the crypt base produce all the factors necessary (Sato et al., 2009).

The clonal expansion in vitro occurs when a specific cell type expressing the *LGR5* gene was isolated. *LGR5* (leucine-rich-repeat containing G protein-coupled receptor 5) was isolated as a Wnt signaling target gene in the intestine. *LGR5*-positive cells are normally seen in the base of the crypt and, as expected for a stem cell, their marked progeny are found throughout intestinal crypts and villi (Barker et al., 2007). Interestingly, Cappechi and colleagues have reported a similar lineage-tracing strategy to mark the progeny of cells expressing the polycomb gene *Bmi1*. *Bmi1*⁺ and *LGR5*⁺ cells are normally distinct, but both cell types give rise to entire crypts (Sangiorgi and Cappechi, 2008). The lineage-tracing data suggest that these two putative intestinal stem cell types can give rise to one another. It is clear that the precision of in vivo tracking and clonal in vitro assays are prompting new assessment of the potential of intestinal cells.

gut, and the endocrine cells secrete insulin and other hormones into the blood. At one time, these cell types were thought to be developmentally distinct, but Sara Ferber (Sheba Medical Center, Tel-Hashomer) suggested that exocrine or liver cells can be transduced into endocrine cells. The insulin-producing β cells themselves can divide in vivo, providing another target for regulation (Dor et al., 2004). Harry Heimberg (Vrije University Brussel, Brussels) used a pancreatic injury model (duct ligation) to identify another precursor (NGN3⁺ ductal cells) that has the ability to produce new β cells (Xu et al., 2008). Heimberg is now looking to see if *LGR5* marks this putative stem cell population in the pancreas, following the suggestion that *LGR5* marks stem cell populations in many tissues.

The development of a cell therapy based on endocrine pancreatic cells would require large amounts of tissue. The reports that both mouse and human ESCs can generate pancreatic endocrine cells suggest that pluripotent cells may be a suitable source. To achieve the controlled production of differentiated cell types from pluripotent human cells, the several steps in this ex vivo development must be defined and optimized. Doug Melton (Harvard University, Boston) described the identification of a small molecule that boosts the production of an early endodermal cell (Borowiak et al., 2009). Melton argued that it was possible to develop robust technologies for each step of the lineage from a pluripotent stem cell to a functional β cell.

The lung is an outgrowth of the endoderm at a more anterior location. Mark Krasnow (Stanford University) also employed drug-inducible lineage tracing to identify separate epithelium, smooth muscle, and endothelial clones during lung development

in vivo. The data suggest that the sequential activation of three subroutines of lung epithelial lineage control the branching morphology that is characteristic of the lung (Metzger et al., 2008). Stay tuned for further advances when this approach is combined with a recent detailed identification of dividing cells in the developing mouse lung (Rock et al., 2009). These experiments make the lung one of the best-understood organs in the developing mouse. Using a dramatic combination of stem cells and tissue engineering, Paola Macchiarini (Hospital Clinico de Barcelona) replaced the damaged primary bronchial airway tube in a 30-year-old human. Macchiarini took a decellularized tracheal tube and then seeded it with autologous respiratory epithelial stem cells on the inside and autologous chondrocyte precursor cells on the outside (Macchiarini et al., 2008). This bio-engineered organ produced recovery of breathing in the patient.

In the Mesoderm

Hematopoietic stem cells are often considered the “gold standard” in terms of the quality and scope of the experiments that validate the stem cell population at different stages of development and adult life. Despite the apparent maturity of this field, there are many open questions. One of these is the specification and precise identity of hematopoietic stem cells at different stages of development. Ana Cumano (Pasteur Institute, Paris) investigated the origin of these cells by using c-kit as a marker for detailed tracing experiments in the developing murine embryo. She defined a unique combination of surface markers and transcription factors in the region of the embryo where HSCs are first found, the Aortic-Gonadal-Mesonephric (AGM) region. These data define differences between embryonic hematopoietic stem cells and their counterparts at later stages in development in the fetal liver or the adult bone marrow (Bertrand et al., 2005).

Andreas Trumpp (DKFZ, Heidelberg) identified two HSC populations: one that normally divides once every 30 days (“active”; 85% of HSCs), and another that divides perhaps five times over the lifetime of the animal (“dormant”; 15%) (Wilson et al., 2008). Only active HSCs make progenitors under baseline conditions, and dormant HSCs are activated following stress (5-FU treatment). Treatment with interferon alpha (IFN α 1) can make dormant HSCs go from G0 to G1, effectively activating them. He suggested that “priming” with IFN α 1 before starting chemotherapy might be a way of killing tumor cells and the dormant cancer stem cells and reported that this has worked in six patients with leukemia (Essers et al., 2009).

In normal hematopoiesis, HSCs generate multiple distinct cell types. An important feature of this model is that cells are committed to different branches of the blood lineage. Meinrad Busslinger (Institute of Molecular Pathology, Vienna) discussed the transcription factor Pax5, which emerges as a critical “gate-keeping” molecule, suppressing the lineage-inappropriate expression of genes during lymphocyte differentiation. From their first discovery, the pattern of expression of Pax transcription suggested a role in specifying cell type. Other work, particularly on the role of Pax6 in specification of eye development, confirms that Pax genes can define cell type. Pax5 has a continuing role maintaining distinctions between different branches of the lineage in the blood. Detailed analysis of the transcriptional control of the Pax5 gene during development shows that Polycomb action represses the gene until the

early B cell stage of development (Decker et al., 2009). B cells can easily switch fates without Pax5. Further insight into the stability of hematopoietic lineages will come from analysis of the gate-keeping functions of Polycomb and Pax genes.

In the blood, there is good evidence for an early founder cell that also generates vascular cells. This is also true for skeletal muscle. Work from Margaret Buckingham (Pasteur Institute) has shown that Pax3 and Pax7 specify two types of muscle precursor. They have now demonstrated that a balance between PAX3/7 and FOXC2 in somites was critical for the choice between skeletal muscle and vascular fates in a mesodermal stem cell. From bone marrow, an adherent cell can be isolated that grows in cell culture and supports the survival of HSCs. This cell has been extensively studied and is known to generate bone, smooth muscle, and fat cells. A similar cell can be found in other tissues, including skeletal muscle, and it seemed possible that many tissues contain these mesenchymal stem cells (MSCs) in addition to the tissue-specific stem cell. Paolo Bianco (University of Rome) made a strong case that MSCs are restricted to the bone marrow and that other tissues do not generate MSCs. Further work in this area may define tissue-specific stem cells at a new level of precision.

In most mammalian tissues, we know little about the mechanisms that control the number of stem cells. Debbie Yelon (Skirball Institute, New York) is analyzing the network of signals that control the size of the cardiac progenitor pool in zebrafish. By developing a chemical caging-based method to fate-map cardiac progenitor cells, she showed that the number of cardiac progenitor cells largely determines heart size (Keegan et al., 2004). Other data shows that hedgehog is required to maintain the cardiac progenitor pool and that retinoid signaling plays a repressive role in regulating heart size (Keegan et al., 2005). These inductive and repressive signals regulate the size of the heart in development and place any future work on cardiac stem cells in the context of the entire development of the organ, a mega-niche.

The practical consequences of understanding stem and progenitor cells in the blood were the subject of presentations by Leonard Zon (Children’s Hospital Boston) and Claudio Bordignon (Vita-Salute San Raffaele University, Milan). The blood from the umbilical cord of newborn infants contains HSCs, but there are too few to reconstitute the marrow of an adult. Using the zebrafish system, Leonard Zon found that prostaglandins enhance the ability of HSCs to engraft. Remarkably, these results were confirmed in a mouse model, and a clinical trial is underway only 2 years after the first discovery (Goessling et al., 2009; North et al. 2009). Claudio Bordignon presented clinical results of studies attempting to solve the problem of graft-versus-host disease, which is an important problem after bone marrow transplantation. Bordignon described a gene therapy that selectively removes cytotoxic T cells and reduces the incidence of graft-versus-host disease in several different clinical settings (Ciceri et al. 2009). The speed of progress is encouraging and shows the benefit of manipulating a well-defined cellular target.

The Niche

The germ cells have a case for being the once and future stem cell. Genetic studies have defined the interacting cell types

and molecular mechanisms that support the production of sperm from germ cells in *Drosophila*. In the *Drosophila* testis, there are fewer spermatogenic stem cells as flies get older. Leanne Jones (Salk Institute) reported that overexpression of *unpaired* (the factor controlling spermatogenic stem cells that is released from the hub cell niche in the testes) allows rejuvenation of these old spermatogenic stem cells.

The specific sites supporting HSCs in development speaks to the importance of local cell interactions, the niche. Hanna Mikkola (UCLA) and Trista North (Harvard University) described effects of blood flow on the maturation of blood stem cells during mouse and zebrafish development, respectively. The *NCX1* gene is a voltage-dependent calcium channel that may be the target for drugs that are widely used to treat heart disease. Loss of *NCX1* gene causes embryonic lethality at embryonic day 9.0 in the mouse. In the mutant mouse, the heart never functions, so HSCs remain at the site where they are initially made—the origin niche. Mikkola reported that independent sites generate blood, including the yolk sac, AGM region, and the placenta (with the placenta having 15× more blood stem cells than the other two sites; Rhodes et al., 2008). This group is using their ability to isolate HSCs to establish an artificial niche and to ask if this niche will promote the differentiation of HSCs from human ESCs. Although blood stem cells are present in the AGM without blood flow, they fail to suppress the endothelial markers that reflect their lineage origin. In zebrafish, blood flow also regulates HSC development. HSCs are generated at an endothelial surface where gas exchange occurs, and North showed that administration of exogenous nitric oxide could rescue the maturation of blood stem cells in zebrafish without blood flow (North et al., 2009).

In adult mammals, HSCs are usually found in the bone marrow where they interact with bone cells and vascular elements. Shane Mayack (Joslin Diabetes Center) found that the osteoblast niche of blood stem cells in the bone marrow of old mice changes the numbers and differentiation properties of these blood stem cells (Mayack and Wagers, 2008). However, heterochronic parabiosis of old and young mice led to rejuvenation of blood cells in the older mouse. This result is similar to the rejuvenation of adult muscle stem cells, as discussed at the meeting by Irina Conboy (UC Berkeley; Conboy et al., 2005).

The Wnt signaling pathway plays a central role in regulating the differentiation of the skin, the largest and one of the most dynamic organs in the body. Tcf3, a BHLH transcription factor, acts downstream of the Wnt/ β -catenin to regulate both uncommitted and differentiating cells in the developing skin. Elaine Fuchs (Rockefeller University, New York) showed that Tcf3 and Tcf4 have Wnt-independent and different roles in the timing of stem cell cycling in the adult skin (Nguyen et al., 2009). Although traditionally the stem cells in the bulge region of the hair follicle have been thought to initiate a new hair cycle, Valentina Greco (Rockefeller University) demonstrated that the hair-growth cycle is initiated by FGF7 that stimulates proliferation of cells in the hair germ (between the bulge and the dermal papilla), which contribute to the growing hair follicle before the contribution of new cells from the bulge begins (Greco et al., 2009). In the blood, the skin, and other tissues, the aging of the niche is coming into sharper focus.

Technical Advances In Vitro and In Vivo

Technical development is a central part of our field. In some cases, the motivation is clinical advance, and in others, curiosity is the motivating force. One of the limiting factors for blood stem cell transplantation is the inability to substantially expand blood stem cells in culture. Peter Zandstra (University of Toronto) argued that quantitative modeling of feedback signals in culture from the progenitor and differentiated blood cell progeny of the stem cells may provide a better way to expand blood stem cells in vitro (Kirouac and Zandstra, 2008). He suggested that the niche could be reconstructed in a bioreactor by manipulating both positive and negative feedback signals to the self-renewing umbilical cord blood stem cells. Eric Deneault (University of Montreal) used a gene-engineering approach to look for factors that would increase blood stem cell self-renewal. He found more than a dozen new factors that increased blood stem cell self-renewal, and the majority of these were validated in vivo in reconstitution assays after transplant of the manipulated blood stem cells. Perhaps most surprising, two of the factors (*Fos* and *STP1*) did not work directly but increased the recruitment of host blood stem cells (Deneault et al., 2009). Thus, even in a screen for intrinsic control of stem cells, the importance of cell interaction was found.

At any time, the cells of the blood come from a restricted set of the stem cells. Fernando Camargo (Harvard University) has improved on the previous methods for marking distinct stem cell lineages by using a drug inducible transposase to mark blood stem cells without irradiation or transplantation. Camargo used clonal tracking to show that only a small fraction of blood stem cells actively make progeny at any one time. An elegant technological advance gives insight into the control of precursor cell proliferation in living humans. Jonas Frisen (Karolinska Institute, Stockholm) realized that the limited period in the 1950s and 1960s of above-ground nuclear testing would label humans with carbon-14 and allow a human version of the label-retaining experiment that has been used in many experimental models to identify stem cells. They showed that the loss of the C14 label in DNA in the dentate gyrus of the adult human hippocampus from people who have died at various times since the nuclear testing shows that these neurons are being replaced. Their most recent results suggest a small but interesting turnover of cardiomyocytes in the human heart (Bergmann et al., 2009). Frisen speculated that this approach could be used to define the effects of different environments and different genomes on precursor cell turnover in human tissues. New technologies continue to promise rapid advances in the brave new world of stem cell biology.

Pluripotency and Self-Renewal

The current interest in human pluripotent stem cells rests on the belief that they can be maintained in a stable state in cell culture over many cell divisions. In a lecture, dedicated to the late Anne McLaren, Janet Rossant (Hospital for Sick Children, Toronto) presented data on the earliest cell-fate decisions in the mammalian embryo—differentiation into trophoblast and primitive endoderm cells. Her results show that, in contrast to mouse ESCs that appear to be largely independent of FGF signaling, trophoblast stem cells and endoderm stem cells are particularly dependent on this signaling pathway.

A remarkable feature of these results is that major embryonic and extra-embryonic cell types of the developing embryo can be expanded as cell lines. The embryo is derived from pluripotent cells and there are now four pluripotent cell types that have been derived from the mouse: embryonic stem cells (ESCs), embryonic germ (EG) cells, epiblast stem (EpiS) cells and induced pluripotent stem cells (iPSCs). Like human ESCs, FGF promotes the self-renewal of mouse EpiS cells. Mouse ESCs and EpiS cells are also distinct in their responses to LIF. Another important distinction is the inability of the EpiS cell to incorporate into the inner cell mass after injection into the blastocyst. EpiS cells were initially derived from mouse embryos after they implant 5.5 days after fertilization. Azim Surani (Cambridge University) reported that EpiS cells can be derived from the post-implantation mouse embryo even 7.5 days after fertilization. He also showed that these cells can spontaneously reprogram into ESCs that can generate chimeric mice after blastocyst injection. This transition was achieved without exogenous genetic manipulation when the cells were simply placed in conditions that support ESCs (Bao et al., 2009). Another advance was suggested by Catherine Browne (Griffith University, Brisbane), who reported that pluripotent stem cells could be isolated from the adult mouse olfactory mucosa without genetic manipulations. This derivation of pluripotent stem cells from adult tissues has implications for the mechanisms for pluripotency.

EG cells are derived from primordial germ cells (PGCs) that give rise to the gametes. Recent work identifies *Lin28* as a regulator of PGC differentiation and as a reprogramming factor (West et al., 2009). *Lin28* through inhibition of the microRNA let-7 regulates PGC specification at an early stage readily accessed in differentiating ESCs. Richard Gregory (Children's Hospital Boston) showed that *Lin28* regulates its target miRNA let-7 by promoting its degradation through the addition of a terminal Uridine (uridylation; Hagan et al. 2009). In a reversal of the historic truth, the access we have to the relevant cells in culture may make germ cell differentiation a general model to understand how microRNAs regulate cell-fate decisions.

In the testis of mammals, large numbers of sperm are generated through an intermediate spermatogonial stem cell. Fruit flies and mammals share fundamental aspects of germ line control. During spermatogenesis in *Drosophila*, the *PIWI* genes were first identified as regulating germline stem cell self-renewal. Haifan Lin (Yale University, New Haven) discussed new data on how the PIWI proteins regulate polysome formation and, through this post-transcriptional mechanism, control the self-renewing cell divisions in the seminiferous tubule (Wang et al., 2009). In sperm, DNA is condensed to an extent that transcription is massively inhibited providing an unusual opportunity to define post-transcriptional controls of self-renewal.

The use of stem cell-based systems to define new advances in molecular biology was continued by Richard Young (MIT, Cambridge). He proposed an alternative view of transcriptional initiation in ESCs that involves an initial short antisense transcript, a role for MYC in regulating a step that permits transcriptional elongation of the coding RNA, and assigns new roles for chromatin components in transcriptional control (Seila et al., 2008). This rapid increase in our detailed knowledge of chromatin structure currently suggests iPSCs and ESCs are virtually identical in terms of their epigenetic status.

A dramatic illustration of epigenetic plasticity is the generation of iPSCs from terminally differentiated cell types through reprogramming. Konrad Hochedlinger (Harvard University) and Shinya Yamanaka (Kyoto University) both focused on the major roadblock in reprogramming—the generally low efficiency of the process (0.1%–1%). Hochedlinger proposed four possible reasons: (1) stem/progenitor cells in starting tissues are easier to be reprogrammed, yet they exist in low abundance; (2) low efficiency of viral infection; (3) genetic alteration due to viral insertion; and (4) reprogramming is a stochastic process. Using the hematopoietic lineage as a testing ground, the Hochedlinger lab has defined the efficiency of reprogramming at different stages of the lineage. Hematopoietic stem cells are reprogrammed at 15.8% efficiency. Pro B cells can be reprogrammed at 11.6% efficiency yet mature B cells can only be reprogrammed at ~0.04% efficiency, 300-fold lower than their progenitors. Likewise, myeloid precursors can be reprogrammed at 27% efficiency, yet granulocytes can only be reprogrammed at 0.1% efficiency (Eminli et al., 2009). These observations suggest that most differentiated cells have limited replicative potential, thus, low reprogramming efficiency.

Shinya Yamanaka, Konrad Hochedlinger, and Hongkui Deng (Beijing University) discussed data showing that p53 and associated components of stress response signaling p21, UTF1, and Ink4a/Arf limit the efficiency of generating iPSCs (Hong et al., 2009; Zhao et al., 2008). Yamanaka then further compared iPSCs derived from embryonic fibroblasts, adult tail tip fibroblasts, adult hepatocytes, and gastric epithelial cells for their differentiation potential by teratoma formation, chimerism, and germline competency. In addition, the ability of iPSCs to differentiate into neural cells was assessed by generating so-called secondary neurospheres that were subsequently transplanted into the striata of mice (SNS assay). Whereas iPSCs from different origins gave rise to similar results on regular teratoma studies, they show very different behavior in chimera and SNS assays. Although mouse embryonic fibroblasts and gastric epithelial cells give rise to iPSCs that are similar to embryonic stem cells in their teratoma-forming propensity in the SNS assay, iPSCs derived from hepatocytes show intermediate propensity and iPSCs from the adult tail fibroblasts show the highest propensity to form teratomas in the SNS assay (Miura et al., 2009). These results suggest that iPSCs derived from different donor cells vary substantially in their differentiation and tumor-forming properties.

Cancer

Of course, mouse ESCs teach us that a teratoma is not necessarily a “full-blown” tumor. There are many forms of developmental variation, and they will all be relevant to a field that aspires to control development. The shortening of telomeres is a clear genetic change, and Maria Blasco (CNIO, Madrid) extended her previous work showing that telomere shortening is epigenetically regulated in epidermal stem cells. She demonstrated that telomere loss activates p53 and impaired mobility (Flores and Blasco, 2009). She then demonstrated that during fibroblast reprogramming into iPSCs, telomeres do not reach expected length immediately but achieve this after multiple passages (Marion et al., 2009). Blasco concluded that iPSCs could only be generated when adult cells have long telomeres, whereas

those with deficient telomeres undergo p53-dependent cell death.

Barriers in epigenetic reprogramming were also discussed by Juan Carlos Izpisua Belmonte (Center of Regenerative Medicine, Barcelona). He showed that p53-deficient cells are amenable to reprogramming and that cells deficient for *FANC*, a DNA repair gene, can only be reprogrammed at low frequencies seem to support this hypothesis. Strikingly, genetically “repaired” *FANC* (after reintroduction of the gene) regained not only full reprogramming potential but also the ability to differentiate into hematopoietic cells, suggesting that gene therapy and iPS technology may provide a novel therapeutic strategy (Raya et al., 2009).

Promyelocytic leukemia (PML) is caused by fusion of the *PML* nuclear protein with *RAR α* that regulates the function of the tumor suppressor PTEN. Luciano Di Croce (Center for Genomic Regulation, Barcelona) has determined how this modified transcription factor is capable of interacting with key components of the epigenetic machinery including PRC2, but may also have identified a specific temporal mode of action where both the histone demethylase UTX and Zrf1 connect DNA methylation and histone modification with transcriptional silencing (Morey et al., 2008). Eduard Batlle (IRB, Barcelona) explored the intestinal stem cell (ISC) origin of colorectal cancer (CRCs) and the role of Eph-ephrin and Wnt signaling pathways in its progression. Batlle reviewed that the majority of human colorectal cancers (CRCs) are initiated by mutations in the tumor suppressor gene *Apc*, which switches on the Wnt pathway in a constitutive fashion. He then reported that beta-catenin and most Wnt target genes induced by *APC* mutations in CRCs, such as the *EphB1*, -2, and -3 receptors of ephrinB ligands, are expressed at the highest levels in ISCs in normal intestine and then progressively decrease in transient amplifying progenitors and more differentiated intestinal epithelial cells. Using EphB2 as a marker allows the isolation of ISCs and more differentiated intestinal cells. Remarkably, during initial CRC progression, beta-catenin and EphB receptors expend their expression along the crypt epithelium (Cortina et al., 2007). However, EphB2 and EphB3 suppress CRC progression beyond the early stages of tumor development. Most colorectal cancers silence the expression of EphB receptors around the adenoma-carcinoma transition, despite constitutive Wnt signaling. These observations illustrate how the link between ISCs and CRCs may require an understanding of the different roles EphB receptors play during early and malignant stages of colorectal cancer progression.

This theme of connecting stem cells to cancer was continued by Thea Tlsty (UCSF) and Jane Visvader (Walter & Eliza Hall Institute, Melbourne), both focusing on the mammary tissue. Building on their previous isolation of mouse mammary gland stem cells that are similar to basal subtype, Jane Visvader showed that GATA3 promotes the transition a more committed progenitor cell and that Notch promotes further differentiation to the luminal lineage. To extend these conclusions to humans, the Visvader group has also isolated human basal stem/progenitor cells, luminal progenitors, and mature luminal cells; determined their molecular signatures; and examined how these molecular signatures change during the BRCA-induced early cancer groups (Lim et al., 2009).

Thea Tlsty explored how mammary epithelial stem cells acquire epigenetic plasticity that predisposes them to become tumors. She reported that mammary epithelial cells from women

free of cancer, when examined in vitro, contain a subpopulation of variant mammary epithelial cells that can continuously grow, bypassing stress-response mediated arrest. This growth is caused by methylation of the promoter of *p16INK4a* leading, in turn, to epigenetic and genetic mosaicism, including telomere instability (Dumont et al. 2008). Importantly, this clonal expansion phase of tumorigenesis is supported by TGF β signals from stromal cells that promote the epithelial-to-mesenchymal transition and malignancy. Key features of tissue and cancer stem cells seem to be regulated by similar molecular and cellular rules.

Are There General Rules?

But is cell identity and number controlled by stable evolutionary rules? Jeremy Brockes (University College, London) described the dependence of adult limb regeneration in salamander on neural innervation. Nerves induce the expression of a factor (AG2) in Schwann cells at the margin of a cut limb. AG2 is then expressed in glandular cells that are critical for regeneration of the limb skeleton. Interestingly, the neural innervation itself induces the dependence of limb regeneration on neural innervation. Preventing nerve growth into the limb during early development allows limb regeneration in the adult without AG2 and without neural innervation (Kumar et al., 2007). The regenerating newt limb gave us another surprise. Elly Tanaka (Center for Regenerative Therapies Dresden) described the regeneration of the limb in the developing newt. Using transgenic newts where fluorescent transgenes marked different cell lineages, she showed that the cells in the blastema that regenerate the limb are not multipotent stem cells but, rather, committed progenitors for muscle, skin, and Schwann cells (Kragl et al., 2009). In the newt, limbs are imprinted by nerves, and committed cells can be activated. Another surprise came from Yann Barrandon (EPF, Lausanne), who suggested transdifferentiation from one epithelial population to another was possible.

What will it take to define general rules of development? Olivier Pourquie (Stowers Institute for Medical Research, Kansas City) showed that mouse mesodermal tail bud stem cells are segmented into somites at a specific determination point as they migrate rostrally through the action of a clock that oscillates every 2 hours and controls segmentation through the sequential activation of Notch/FGF versus Wnt. Pourquie suggested that defining the state of thousands of molecules through time was the way to understand somite formation (Aulehla and Pourquie, 2008). Pourquie encouraged us to define the general rules of development by constructing databases containing the molecular changes that define cell lineages in different species. The energy and insight of the science presented in Barcelona suggests that he will have enthusiastic support from around the world. For an update on all these exciting ideas, come to San Francisco in 2010.

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