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Front Cover (*Paperback*): Human stem cells differentiating to neurons. Staining for β-tubulin and DAPI. Courtesy of Juan Carlos Izpisúa Belmonte at the Center for Regenerative Medicine, Barcelona. ***Back Cover*** (*Paperback*): A brain lobe from a *Drosophila* third-instar larva labeled with Dlg (*red*), BrdU (*green*), and DAPI (*blue*). The large red cells are the neural stem cells of the central brain. (Courtesy of Jakob von Trotha and Andrea H. Brand.)

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Summary: Present and Future Challenges for Stem Cell Research

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Stem cell research is being driven forward at an intense pace by creative interactions among scientists working in different fields. These include developmental and reproductive biology, regeneration, genomics, live cell imaging, RNA biology, and cancer biology, to name a few. Numerous model systems and techniques are being exploited, and lab scientists are teaming up with bioengineers and clinicians. The ferment of ideas that makes the field so exciting was in full evidence throughout the Symposium. However, many challenges still need to be overcome to translate basic discoveries into therapeutic outcomes that will save lives and fulfill the promises that have been made. This chapter summarizes some of the highlights of the Symposium and indicates future directions that are being taken by leaders in the field.

There are few topics in the biological sciences that have generated such an intense foment of ideas, legitimate therapeutic promise, irresponsible hyperbole, informed public debate, and political controversy as stem cell research. Perhaps only the human genome project has come close to capturing the imagination of such a broad swath of the scientific and lay community. This excitement, combined with the extraordinarily rapid progress of stem cell research during the past few years, has fueled many international meetings. However, there was nothing jaded about the 73rd Cold Spring Harbor Symposium entitled Control and Regulation of Stem Cells. This is because Cold Spring Harbor has a special cachet when it comes to meetings. Not only is the science of the highest quality, but the atmosphere is always special; the eclectic spirits watching from the walls, the natural and artistic beauty of the environment, and the well-orchestrated hospitality help to create an environment that brings out the best in participants. And so the meeting was a great success; new discoveries were unveiled, connections were made, and collaborations were initiated. The reports in this Symposium volume convey some of this energy, but the full excitement will have to be imagined. Likewise, this summary can only provide a brief overview of the important themes that were discussed and the questions that were raised for the coming years.

DEFINING STEM AND PROGENITOR CELLS: BREAKING DOWN OLD STEREOTYPES AND BUILDING A NEW CONSENSUS

One of the consequences of the increased breadth of stem cell research is that investigators are studying a much wider range of model organisms and tissues than previously. When studies were focused on just a few examples, such as the hematopoietic system, small intestine, hair follicle, and *Drosophila* male and female germ line, the definition of a stem cell was relatively straightforward. The properties of what might now be called "classical" stem cells are summarized in Figure 1. They are relatively less differentiated and quiescent cells that reside in a local microenvironment or "niche" that controls their behavior. During the lifetime of the organ, the stem cell population both self-renews and produces daughter progenitor cells that differentiate into one or more postmitotic specialized cell types. The progenitors can themselves self-renew and proliferate extensively, earning themselves the title of transit-amplifying (TA) cells. However, the time span for TA self-renewal is significantly shorter than for stem cells. Most importantly, the "classical" definition of a stem cell includes a stringent requirement for a functional activity: A single stem cell has the potential to maintain or regenerate an entire organ or tissue during the lifetime of the organism.

As new organs have been examined from a stem cell perspective, and greater scrutiny has been applied to old favorites, several new concepts have emerged, as discussed during the Symposium. One such concept is that organs, even relatively small ones, may contain more than one kind of stem cell, each controlled by a different regulatory mechanism. A good example is the *Drosophila* ovary. Once considered only as the home for the germline stem cell (GSC), it is now known to contain two other stem cell populations: the escort stem cell (ESC) and the follicular stem cell (FSC) (Xie et al.; Spradling et al.). Mammalian skeletal muscle has long been known to harbor a population of "satellite cells" that lie just underneath the basal lamina. Studies now show that there are at least two kinds of adult satellite cell: myogenic stem cells that only give rise to muscle and multipotent cells that can give rise to fat and fibroblasts as well (Cerletti et al.). Another example of a tissue containing multiple stem cell types is the mammalian epidermis. In this case, distinct pools of stem cells with different properties reside in the interfollicular epidermis, the hair follicles, and the sebaceous glands (Watt and Collins; Fuchs and Nowak). These pools are thought to be derived from different progenitors during the embryonic development of the epider-

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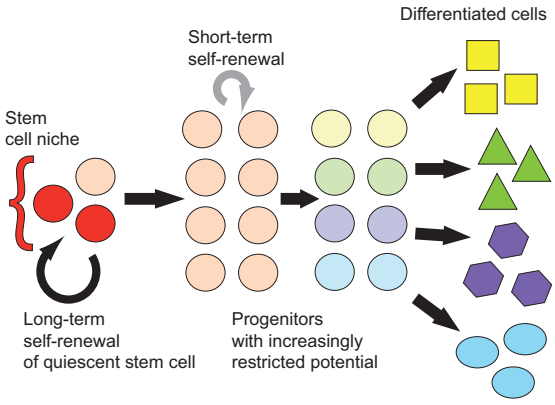

Figure 1. Schematic representation of a tissue maintained through the activity of a "classical" stem cell and its descendants. This archetypal stem cell (*red*) is anchored in a three-dimensional niche (*red bracket*) and is quiescent, with a low proliferative rate. If division is very infrequent, BrdU incorporated into the DNA of the stem cell is retained during long chase periods. Upon division, individual stem cells may generate two identical stem cells, one stem cell and one multipotent progenitor (*pink*) that gives rise to differentiated progeny, or to two progenitors. The particular outcome may depend on intrinsic asymmetry in the segregation of determinants into the two products of a stem cell division, or on extrinsic differences in the environment inside and outside the niche. In either case, the capacity for self-renewal of the stem cell population persists for all, or a substantial fraction of, the lifetime of the organ (*curved black arrow*). Progenitors can divide rapidly as transit-amplifying (TA) cells, but their capacity for self-renewal is limited (*curved gray arrow*). Finally, progenitor cells give rise to terminally differentiated mature cell types (*multicolored shapes*).

mis. Likewise, there is now evidence that different populations of mesodermal cells in the mammalian heart originate in different embryonic heart fields and the epicardial organ (Nakano et al.).

Another emerging concept in stem cell biology is that even apparently homogeneous populations of stem cells are, when examined closely, heterogeneous in terms of their behavior and/or developmental potential. One example highlighted in the Symposium is the population of neural stem cells that resides in the subventricular zone (SVZ) of the lateral ventricle in the mammalian brain. It now appears that stem cells in different locations along the lateral wall express different transcription factor combinations. These impose different positional identities on the stem cells in the different spatial domains and regulate the fate of the daughter cells that arise from them (Alvarez-Buylla et al.). Another example of apparently homogenous multipotent cells having different positional identities was provided by the work of Elly Tanaka on blastema cells in the regenerating salamander limb (Kragl et al.). These relatively undifferentiated mesenchymal cells are located in the distal stump of the amputated limb and give rise to the replacement parts. Contrary to what was once thought, these blastema cells are not pluripotent. Rather, cells derived from one lineage (e.g., muscle) apparently give rise to the same lineage in the regenerated limb. Moreover, blastema cells still retain positional identity relative to the proximodistal (PD) axis of the original limb and always give rise to cells with more distal identity. How positional memory is encoded in stem and progenitor cells, when this identity is acquired, and whether it can be changed experimentally are important questions for the future.

One preconceived idea about "classical" stem cells that has changed during the last few years is that they must be quiescent (Fig. 1). One example of stem cells breaking this mold was provided by the work of Hans Clevers on mouse small intestine (Barker et al.). His findings support the idea that the gene *Lgr5*, encoding a G-protein-coupled receptor, marks a population of relatively undifferentiated epithelial stem cells in the base of the crypt, intermingled with Paneth cells. The main evidence that these cells are stem cells comes from in vivo lineage-tracing studies using an *Lgr5-EGFP-ires-CreER*^{T2} "knockin" allele to drive recombination of a *Rosa26R*^{lacZ} reporter gene. Ribbons of cells expressing β-gal were seen running from the base of crypts to the top of the villi for as long as 14 months following activation of the reported allele by a pulse of tamoxifen. Moreover, the lineage label was seen in all differentiated cell types in the villus. Significantly, the *Lgr5*-expressing crypt cells are not quiescent, as expected if they are "classical" stem cells. Rather, they appear to actively divide about once every 24 hours. Previous studies of the dynamics of cell turnover in the small intestine have suggested that the stem cells are localized just above the base of the crypt, in what is known as the +4 position, and that these cells divide infrequently. In support of this idea, recent studies have used expression of the gene *Bmi1* as a marker of the +4 stem cells (Sangiorgi and Capecchi 2008). If a *Bmi1-CreER* knockin allele is used to drive recombination of a lineage reporter, then lines of cells are also seen running up a crypt for at least a year after induction. Bmi1$^+$ stem cells are not found throughout the small intestine but only in the most anterior region. Taken together, these results are compatible with several models. First, there may be two different stem cell populations in the anterior small intestine, one in the +4 position and another in the crypt base, with no functional difference between them under any conditions. Alternatively, the +4 cells, because of their relative quiescence, may be able to survive certain stressful physiological conditions under which the Lgr5$^+$ cells are damaged or lost. During recovery, the +4 cells would then give rise to new populations of Lgr5$^+$ cells. Under these conditions, the Lgr5$^+$ cells would be more like "long-term self-renewing progenitor cells" than classical stem cells (Fig. 1).

Given the complexities that are emerging from recent studies of adult tissues, it is clear that we need to be very precise when defining cells as stem cells, TA cells, or long-term self-renewing committed or differentiated progenitors. This is especially true for tissues that normally have a slow rate of turnover, such as the islets of the pancreas and the liver and the bronchioles of mouse lung (Rawlins et al.). There is a real need for more models and for new nomenclature to cover the different scenarios that may occur (Fig. 2).

In deriving models for the role of stem cells in adult organs, many different criteria must be taken into consid-

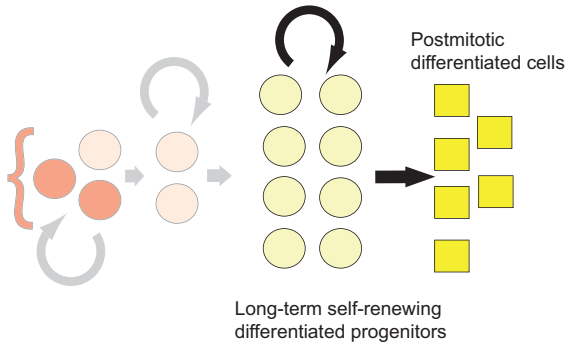

Figure 2. Schematic representation of a tissue maintained primarily through the activity of long-term self-renewing differentiated cells. In this simplified model, a tissue is maintained by the long-term self-renewal (*black curved arrow*) of cells that express differentiated markers and may give rise to one or more postmitotic cell types (*yellow*). This strategy operates during the steady-state maintenance of a tissue that has a slow rate of turnover. However, in response to extensive tissue damage or certain physiological states, the tissue may draw upon a pool of quiescent classical stem cells (*red*) and their progeny (*peach*). These cells also have long-term self-renewal capacity (*gray curved arrows*) but do not normally contribute to tissue maintenance (*short gray arrows*). If the repair conditions are taken into consideration, the yellow cells could be classified as long-term self-renewing committed progenitors. This model can be applied to Clara epithelial cells in the bronchioles of mouse lung (Rawlins et al.). It is also relevant to β cells in the islets of mouse endocrine pancreas that can self-renew during long periods (Brennand et al. 2007; Teta et al. 2007). However, islet tissue can also be regenerated from pancreatic ducts in response to severe damage to the pancreas (Xu et al. 2008).

eration. First, it was emphasized again and again in the Symposium that studies must be based on quantitative, in vivo cell lineage-tracing studies. Ideally, lineage tracing needs to be performed at both the single-cell as well as the population level. Although single-cell lineage tracing is routine in *Drosophila*, new or more sophisticated tools are being developed for organisms such as the mouse and zebra fish. For example, we need the ability to (1) follow the fate of more than one cell type at the same time and (2) image stem cells and their divisions in real time in intact living tissues. A very impressive example of real-time imaging presented in the Symposium was the tracing of labeled spermatogonial cells in mouse testis (Yoshida). We also need more markers for stem cells and their progeny. In addition, a real challenge for the future is to devise models that reveal the full range of physiological conditions under which stem cells are expected to behave and for which they will have been selected during the long evolution of the species. Finally, we need more assays to test the ability of candidate stem cells to regenerate a complete tissue. This ability is the ultimate and perhaps only universal property of a stem cell. Moreover, it is also the most relevant regarding the quest for cell replacement therapy and for building replacement organs.

FUNCTIONAL ASSAYS FOR STEM CELLS

The gold-standard assay for stem cell function is the ability of a single hematopoietic stem cell (HSC) to reconstitute the hematopoietic system of an irradiated mouse (Chao et al.). HSC engraftment can also be used in the zebra fish, and Len Zon described the development of a transparent zebra fish to help investigators study how labeled cells home to and engraft into target tissues (Huang and Zon). A number of other transplantation assays have been reported over the years and the use of some of these assays to study stem cells was described in the Symposium. For example, muscle satellite cells can be transplanted into Mdx mutant mice, which are a model for Duchenne's muscular dystrophy (Cerletti et al.). In another example, spermatogonial stem cells can colonize the testis of an infertile recipient mouse (Brinster and Zimmermann 1994; Kanatsu-Shinohara et al.; Yoshida). A beautiful assay for reconstitution of mouse mammary gland is available. The assay involves introducing genetically marked mouse mammary cells sorted into different populations into the mammary fat pads of prepubertal mice that have been "cleared" of endogenous mammary cells. Lineage-tracing studies show that a single mouse mammary stem cell (MaSC) can reconstitute the entire mammary gland in this assay (Asselin-Labat et al.). Moreover, serial transplantation shows evidence for the long-term reconstitution ability of these cells. One challenge for the future is to "humanize" this assay so that it can be adapted for use with human mammary cells.

Two different assays were described for testing the ability of specific epithelial cells in mouse prostate to function as prostate tissue stem cells (PrSCs). The different conclusions regarding the identity of the PrSC reached using these assays highlight the need for multiple approaches to studying stem cell function. For example, Owen Witte's lab (Lukacs et al.) has focused on using an assay, initially developed by Cuhna, in which labeled cells from the prostate epithelium are combined with embryonic mesenchyme from the embryonic urogenital sinus and engrafted under the kidney capsule of immunodeficient mice. A small subfraction of the grafted prostate epithelial cells is able to give rise to tubules containing differentiated cells and resembling normal prostate. Taking an alternative approach, Michael Shen's lab used lineage labeling to follow the behavior of different epithelial cell populations during androgen-dependent serial regression and regeneration of normal mouse prostate. In this model, cells are first lineage labeled, the mice castrated to shrink the prostate, and then androgens are given back to promote regrowth. Using this model, the investigators have obtained evidence that a rare population of $NKX3.1^+$ luminal cells can give rise to both luminal and basal cells during regeneration. They speculate that mouse prostate, which normally turns over very slowly, contains more than one population of progenitor cell for regeneration—basal cells and $NKX3.1^+$ luminal cells (Shen et al.).

In the long run, it will be important to replace in vivo assays with culture methods in which the behavior of stem cells can be followed in real-time and high-throughput screens performed to test compounds for their effect on stem cell behavior. Hans Clevers described the development of an in vitro assay for culturing isolated crypts and associated villi in Matrigel with the goal of following the

behavior of stem cells and their progeny in real time. The Witte lab reported using an assay in which dissociated epithelial cells from the mouse prostate are combined with mesenchyme and embedded in Matrigel for in vitro culture rather than grafting (Lukacs et al.). In the future, more of these in vitro assays must be developed to quickly identify and test mechanism regulating stem cell behavior.

THE STEM CELL NICHE: DYNAMIC PERSPECTIVES AND PRACTICAL APPLICATIONS

"Classical" stem cells are anchored in a highly regulated microenvironment or niche (Ohlstein et al. 2004; Xie and Li 2007). Polarized stem cells may be anchored in such a way that when they undergo asymmetric division, one cell (the mother stem cell) remains tightly bound to the niche, whereas the other daughter is displaced from the niche and behaves as a differentiating progenitor (Fig. 1). The niche functions to integrate the different signals regulating the behavior of stem cells. The critical word here is "integrate." In his Symposium talk, Alan Spradling beautifully articulated the concept of the niche and stem cell as a dynamic, integrative unit (Spradling et al.). He stressed that the interaction between the niche and the stem cells is two-way. The niche transmits up-to-date information (through local factors and cytokines, hormones, nutrients, mechanical stress, nerve activity, blood flow, etc.) about the needs of the organism. In response, the stem cells modify their behavior (proliferation rate, specification, fate of daughters, etc.) to maintain homeostasis, meet physiological demands, or repair the effects of injury. In return, the stem cells provide the niche with important information about their behavior so that both positive and negative signals can be ramped up or down accordingly. As stressed many times in the Symposium, the two-way nature of the conversation between stem cells and their niche has important implications in all organisms—not only in animals but also in plants.

The niche is composed of a variety of cells, including in some cases specialized cells that make direct contact with the stem cells, such as the cap cells of the *Drosophila* ovary (Xie et al.). In addition, the niche can include extracellular matrix molecules, blood vessels, and nerves. Two of the best-studied niches are those harboring the *Drosophila* male and female germ lines. The insights that can be gained from straightforward anatomical studies of the stem cell niche were highlighted by work on the organization of stem and ependymal cells of the mammalian SVZ (Alvarez-Buylla et al.). Future challenges will be to define the niche for stem cells in the hair follicle bulge, muscle satellite cells, crypt of the intestine, and plant root and shoot. Significant advances are being made in real-time imaging of stem cells in relation to their niche. For example, in mouse testis Yoshida provided evidence that the vasculature contributes to the niche in this tissue (Yoshida). A major challenge for the future is to identify the different extrinsic signals from the niche to the stem cells, their range of action and localization (Nusse et al.), and how they interact with intrinsic mechanisms functioning within the stem cells to control proliferation and developmental potential. Two outstanding questions, for example, are whether conserved mechanisms such as the Wnt signaling pathway function in all niches (Nusse et al.), and what is the relative importance of positive signals versus inhibitors and antagonists in stem cell regulation.

During the past few years, the niche has been found to be a ruthlessly competitive environment as well as a nurturing one. Studies have shown that if stem cells are destroyed, daughter cells that have begun to differentiate can replace the stem cell and acquire their phenotypic characteristics, including the ability to self-renew over the long term. It was suggested that competition for access to the niche among stem cells as well as among their daughters serves an important quality control process during homeostasis (Xie et al.). In addition, several talks in the Symposium considered the aging of stem cells and whether this affects the niche, stem cells, or both (Xie et al.).

Understanding the stem cell niche and how it regulates stem cell behavior is likely to have important practical applications as stem cell research moves forward. For example, replicating or reconstructing critical aspects of the niche ex vivo may enable us to expand populations of rare and highly valuable stem cells that can be used for transplantation. In vitro culture will allow their detailed phenotypic analysis and the visualization of stem cell–niche interactions in real time. In the long-term, these studies will help us to bioengineer replacement organs. They may also help us to understand how stromal cells in epithelial tumors promote or restrict tumor growth and/or metastasis.

Finally, it is important to understand how the niche changes with age and how this influences the proliferation and differentiation of stem cells as they, too, age. Studies have shown that satellite cells in old mice are less able to regenerate muscle than those in young animals (Levi and Morrison). However, the activity of old satellite cells can be restored by factors circulating in young animals. Age-related changes in the behavior of other tissue stem cells were also described. Identification of the different pathways involved in aging is an exciting area of future research. Progress may allow us to promote the proliferation of young stem cells, if their numbers are rate limiting for tissue growth, for example, in premature babies. In addition, the possibility needs to be considered that changes in stem cells as they age may affect the class of oncogenic mutations that will promote their self-renewal (Levi and Morrison).

STEM CELLS AND ASYMMETRIC CELL DIVISION

As discussed earlier, some polarized stem cells are anchored to their niche in such as way that after division the mother cell remains in the niche and maintains the stem cell phenotype, whereas the differentiating daughter is displaced and proceeds to give rise to postmitotic progeny. There is intense interest in the mechanisms regulating such asymmetric division and, in particular, how specific determinants are segregated into one daughter versus the other. In the male germ line of *Drosophila*, the mother cell (the GSCs) is attached to the hub (niche) cell.

When asymmetric division takes place, the old centrosome always remains anchored to the hub–GSC interface and the differentiating daughter inherits the new centrosome. It has been argued that cell polarity and asymmetric division with the differential inheritance of cell intrinsic factors are ancient mechanisms that evolved to cope with the problem of aging in single-celled organisms (Macara and Mili 2008). In budding yeast, for example, aging factors remain in the mother cell and are excluded from the young bud. Consequently, there was considerable interest shown during the Symposium in modeling stem cell asymmetry in yeast, which is a superb tool for both cell biology and genetics (Thorpe et al.). In addition, basic mechanisms of asymmetric cell division are also being studied in single animal cells such as the T cell (Chang and Reiner). Naïve T cells are not polarized. However, they associate transiently with partners (the antigen-presenting cell) and use specialized contacts to initiate cell polarization. This leads to asymmetric cell division, with the segregation of determinants into the two daughter cells that then uncouple from their association.

MECHANISMS REGULATING THE SELF-RENEWAL AND POTENCY OF EMBRYONIC AND TISSUE STEM CELLS

Two distinguishing features of multipotent stem cells are their ability to self-renew and give rise to daughter cells that differentiate into specialized cell types. This potential to give rise to different lineages is most dramatic in pluripotential embryonic stem (ES) cells that can generate most cells of the embryo and adult. For this reason, there has been an enormous amount of hard work and ingenuity geared to defining the trancriptional network that controls the phenotypes of mouse and human ES cells (Jaenisch and Young 2008). The consensus that has emerged from several laboratories is that the pluripotential state is maintained by the combined activity of multiple components—transcription factors (TFs), chromatin regulatory factors, signaling pathways, and noncoding RNAs—that cooperate as part of a metastable self-regulating circuit (Cole and Young; Orkin et al.; Chen et al.; Zwaka; Kagalwala et al.) Work has focused on a small group of "key regulators," for example, Oct4 (Pou5f1), Sox2, nanog, and various chromatin-remodeling complexes, that form the core of the network. At present, it is unclear just how many components comprise this core group, but one goal for the future is to devise high-throughput screens to identify additional members. A recurring theme seems to be that the key TF regulators do not work individually. Rather, they bind in combinations, and sometimes even in association, to multi-input regulatory motifs ("hot spots") in the promoters of target genes. Moreover, among these target genes are the genes encoding the TFs themselves. Consequently, the circuits maintaining pluripotency have built into them feedforward and feedback loops, so that under steady state conditions they are self-sustaining.

One of the major challenges for the future is to build similar metastable transcriptional networks for multipotent cells that can give rise to a smaller range of cell types compared to ES cells, for example, endoderm or mesoderm. Hand in hand with this challenge is the need to make connections among the different circuits and identify the switches or gates that allow cells to pass from one state to the next. These gates function during both embryonic development and adulthood. In the embryo, they control how cells within an organ primordium become increasingly restricted in their developmental potential. In adult tissues, they control the flow of progenitor cells down the hierarchy from multipotent stem cell to differentiated cell type (Fig. 1). Examples were given of the exciting progress being made in defining the genetic circuitry of multipotent myogenic lineages and muscle satellite cells (Lagha et al.; Deato and Tjian) and the TF circuitry in early endoderm (Zaret et al.) and neural progenitors (Elkabetz and Studer). One quite unexpected revelation from the Symposium is that the strategies used by stem and progenitor cells to regulate pluripotency and the switch to other states are similar, in principle, between animals and plants (Kornet and Scheres; Lohda et al.).

TURNING LEAD INTO GOLD: REPROGRAMMING DIFFERENTIATED CELLS INTO THE PLURIPOTENT STATE

One of the most unexpected and energizing advances in stem cell research, and indeed in modern biology, has been the discovery that differentiated somatic cells can be induced to become pluripotential embryonic stem-cell-like cells (iPS cells). This process, known as "direct reprogramming," was first achieved by the forced expression in fibroblasts of four TFs (Sox2, Oct4, c-Myc, and Klf4) under the control of retroviral vectors (Takahashi and Yamanaka 2006). However, more recent studies have shown that the proto-oncogene c-*myc* can be omitted from the cocktail, although this reduces efficiency. Likewise, if cells already express *Sox2*, this gene does not need to be added. Even under the best conditions, the efficiency of direct reprogramming is very low—in the range of 0.01–0.1% of transfected fibroblasts—and requires 2–3 weeks of continuous culture, during which stepwise changes in gene expression and epigenetic modification occur. The generation of iPS cells opens up many exciting vistas including the possibility of generating patient-specific pluripotential cells for basic studies into disease mechanisms and, ultimately, cell therapy. Several talks in the Symposium described advances in the derivation of iPS cells. A recurring theme was the need for a careful analysis of the intermediate states that occur during the first 6–12 days of the complex reprogramming process (Hanna et al.; Maherali and Hochedlinger). A future challenge is to circumvent the use of viral or DNA vectors to deliver reprogramming genes. The goal will be to replace the vectors with small molecules or drugs that will substitute for the proteins or induce transient coordinated upregulation of the endogenous genes in order to start a reprogramming cascade.

Direct reprogramming of differentiated cells to pluripotency is a relatively new phenomenon. Other examples of induced global changes in the genetic program of cells that have been studied for longer amounts of time were

well represented in the Symposium. It is critical that these models of extreme plasticity continue to be studied in parallel with direct reprogramming because each has significant advantages for studying different aspects of the rewiring process. Examples that were discussed include the reprogramming to pluripotency of the nuclei of differentiated cells transfered into the oocyte (de Vries et al.) and the conversion of primordial germ cells (PGCs) to embryonic germ (EG) cell lines (Surani et al.) and of spermatogonial stem cells to pluripotent stem cells (Kanatsu-Shinohara et al.).

Another process involving extensive and controlled reprogramming of the genome is the formation of totipotent germ cells during embryogenesis (Rangan et al.). Two basic mechanisms appear to be used within the animal kingdom. The first involves the specific allocation of maternally supplied "determinants" (RNA and proteins) to the cytoplasm of the future germ cells. These determinants are initially incorporated into granular inclusions known as germ plasm but are released to function at the transcriptional level and, in particular, the posttranscriptional level. This mechanism of "determinate specification" of the germ line has been extensively studied in *Drosophila* and *Caenorhabditis elegans*. The alternative mechanism, known as "inductive specification," requires signaling among cells within the early embryo and occurs in mammals and a wide range of other invertebrate and vertebrate species. It also occurs during a fascinating phenomenon that captured the intense interest of the Symposium audience: the regeneration of Planaria. Even very small fragments of Planaria can regenerate a complete gonad with germ cells. The germ cells are derived from multipotent neoblasts, or somatic stem cells, within the fragment in response to inductive signals (Newmark et al.). Intense effort is being expended on identifying the components of the germ plasm, how these components function, and the intercellular signals and downstream pathways involved in germ-line induction. From these studies, it appears that both mechanisms share many evolutionarily conserved components, for example, nanos, a zinc finger RNA-binding protein. Thus, the mechanisms differ largely in the initial localization of the conserved factors and the timing at which they become active. At the Symposium, investigators working with *Drosophila* presented evidence of a role for a whole new family of noncoding RNAs, known as Piwi RNAs, in regulating totipotency (Lin and Yin). Precisely how these RNAs regulate gene expression and chromatin modifications is a hot topic for the future.

REPROGRAMMING ADULT CELLS FROM ONE LINEAGE TO ANOTHER: THE THERAPEUTIC POTENTIAL OF TRANSDIFFERENTIATION

As we have seen, a major focus of the stem cell field has been on the mechanisms regulating pluripotency and the switching of differentiated cells to the pluripotent state by experimental manipulation of gene expression. One of the most provocative ideas highlighted during the Symposium was that adult cells can, under certain conditions, be switched from one differentiated state to another, without going all the way back to pluripotency. This process is known as "transdifferentiation" or "transdetermination" if it occurs in embryonic tissues such as the imaginal disc of *Drosophila*. In fact, examples of transdifferentiation or metaplasia in adult tissues, usually under conditions of injury and repair, have been well documented, and the potential of harnessing this plasticity for therapeutic purposes has long been recognized (Slack 2007). Thus, it was very exciting to learn that Doug Melton's lab had been able to convert exocrine pancreas into cells with both the molecular and morphological phenotype of insulin-producing β cells (Zhou and Melton). This was achieved by forced expression of three TFs, each of which has a critical role in guiding the embryonic development of the endocrine pancreas. This unexpected but tremendously exciting finding has great clinical promise if it could be applied, for example, to deriving β cells from liver, which is more accessible than exocrine pancreas. This is because there are presumably fewer steps that could go wrong in the process of generating a β cell from a cell already committed to the endoderm lineage than from an iPS or ES cell. It would be premature, however, to abandon other strategies that are being used to generate more β cells for clinical use, for example, from a potential population of multipotent cells in pancreatic ducts (Xu et al. 2008).

CANCER STEM CELLS

About one third of the talks at the Symposium involved cancer-related research. In recent years, this field has been greatly influenced by the idea that stem cell biology can throw new light on the origin, progression, and treatment of human tumors (Bonnet and Dick 1997; Reya et al. 2001; Wang and Dick 2005). The idea that tumors are perpetuated and sustained by "cancer stem cells" is based on the finding that some cancers can be serially transplantated by grafting them into immunodeficient mice. Moreover, individual cells in the tumor differ in their efficiency to give rise to new tumors containing the same range of cell types as the first. A rare subpopulation of cells—the presumptive multipotent cancer stem cells—are significantly more efficient at giving rise to new tumors than are the majority. Because "classical" stem cells are relatively quiescent, it has also been argued that these tumor stem cells can escape the action of standard anticancer drugs designed to kill rapidly proliferating cells. In contrast, drugs designed to block pathways specifically required for stem cell self-renewal would, according to the model, be particularly effective in blocking the progression and recurrence of tumors.

The Symposium talks illustrated the fact that, over time, the "cancer stem cell model" has encompassed several very different ideas, often leading to confusion (Kelly et al. 2007; Visvader and Lindeman 2008). Thus, the original model has been extended to include the idea that cancers arise from stem cells in normal tissues. In other words, stem cells are likely to be the "cell of origin" of the original cancer because only individual tissue stem cells stay around long enough to accumulate the combination of oncogenic mutations that lead to malignant transfor-

mation. Defining the mechanisms regulating self-renewal and differentiation of normal tissue stem cells is therefore critical for understanding how cancers arise and progress (Williams and Sherr; Pierfelice et al.; Rich). However, strictly speaking, this model can only apply to tissues in which progenitor or TA populations (Fig. 1) normally have a strictly limited self-renewal capacity and a high probability of giving rise to terminally differentiated cells that are lost from the body. If the committed progenitor cells self-renew during long periods of time, presumably they too could accumulate multiple oncogenic mutations and become the "cell of origin" of a first cancer. An interesting idea that was brought up was that changes in the number or properties of tissue stem cells over time may account for changes in cancer risk in human populations, for example, the increased risk of breast cancer in nulliparous women (Asselin-Labat et al.).

Once a tumor has formed, there is likely to be intense competition within the tumor population, favoring the survival and expansion of cells with high self-renewal and low differentiation potential. According to what is known as the "clonal expansion model" of cancer progression, cells within the tumor that normally behave like short-lived progenitors will have a selective advantage if they acquire mutations that promote a more stem-cell-like phenotype. According to this idea, "cancer stem cells" or "cancer-sustaining cells" from early tumors will have different properties than cells with the same behavior that evolve from progenitor cells in more advanced tumors.

Identifying cancer stem cells depends heavily on the assay of grafting dissociated tumor cells into immunodeficient mice and obtaining serial transplantable tumors. The point was made that this assay may only measure the ability of human cells to grow in mouse, rather than a cancer stem cell phenotype per se (Adams et al.). This caveat gains credibility as we understand more about the complex reciprocal interactions between epithelial tumors and their support environment or niche that includes mesenchymal stroma, blood vessels, and immune cells. Several speakers addressed this issue. For example, a very elegant series of experiments was reported by Sean Morrrison (Levi and Morrison), who took a systematic approach to developing an assay for propagating human mammary tumors in mice. In these and other assays, the tumor environment in severely immunocompromised mice is "humanized" by the addition of human mesenchymal cells that provide a "niche" for the tumor cells. In this way, the percentage of transplanted cells that can give rise to a tumor can be greatly increased (Quintana et al. 2008).

Other assays, most notably the "neurosphere assay" for neural stem cells, have been adapted to identify stem cells in cancers. This assay depends on neural stem cells grown in suspension that then form floating spheres containing undifferentiated and differentiated cell types that can be propagated over multiple rounds of dissociation and culture. Here again, the sphere-forming assay appears to work for some solid tumors but not for others. Owen Witte described the development of an in vitro sphere-forming assay for normal and tumor-derived prostate epithelial cells. In this assay, cells are combined with mesenchymal cells and grown embedded in Matrigel where they give rise to spheres that can be propagated over multiple passages (Lukacs et al.).

Finally, speakers pointed out that testing the idea that stem cells are the cells of origin of certain cancers will require the identification of more promoters to drive genetic recombination and the expression of different oncogenes in specific cell populations (stem cells and TA cells) in normal tissue (Alcantara Llaguno et al.). In addition, more surface markers are needed to sort subpopulations of tumor cells. Of course, these challenges are not confined to cancer-related research but confront all investigators taking a genetic approach to stem cell biology with animal models. In conclusion, one take-home message from the Symposium for cancer researchers was that enthusiasm for the "cancer stem cell" model needs to be tempered by a rigorous and critical analysis of the data and methods.

CONCLUSIONS

In this summary, I have highlighted some of the topics covered during the Symposium and the challenges raised by the new ideas and data that were presented. I apologize to speakers whose work I did not refer to specifically. In the few months since the meeting, there have been many impressive advances and already some of the challenges I outlined have been met. Yet, stem cell research remains one of the most influential areas of science, in part because it brings together people from many different backgrounds—those working in cell biology, developmental biology, genomics, immunology, bioengineering, chemistry, drug design, and medicine—to work together to achieve goals benefiting us all. Although it may take longer than we hope for advances in basic research to reach the clinic, there is no doubt that they eventually will. We can only imagine the topics of future Cold Spring Harbor Symposia as regenerative medicine and stem cell research come to maturity and fruition.

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