Chapter 1

A HISTORY OF STEM CELL RESEARCH

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Chapter 1 outlines and describes the maturation of stem cell research, from early contemplations on the power of cell fate to cutting-edge clinical trials involving human embryonic stem cell (hESCs). A multitude of different stem cell types are described as they make their chronological appearance on the research front and key researchers as well as their findings and discoveries are highlighted throughout the chapter.

> "The possibility of obtaining a strain of cells in tissue culture which may become determined to differentiate in a variety of alternative ways is very attractive."
>
> Martin Evans, PhD (1972)—2007 Nobel Prize Winner in Physiology or Medicine

EARLY STUDIES

The existence of **stem cells**, which are defined as biological cells capable of self-renewal and the capacity to differentiate into a variety of cell types and are present within most if not all multicell organisms, has been contemplated for greater than 100 years. In fact, the concept of "stemness" can be traced back as far as ~300 BC when Aristotle disagreed with the generationally accepted hypothesis of spontaneous generation (Figure 1.1).

Russian-born medical doctor Alexander A. Maximow first coined the term "stem cell" in 1908, while addressing a hematologic society congress in Berlin (see Focus Box 1.1). Maximow was a scientist and histologist who spent several years around the turn of the 20th century contemplating the existence of a unique cell type that would allow for generation of many differentiated, mature phenotypes. Maximow's main focus was on blood cell type identity and what drives the generation of the terminally differentiated cells in the hematopoietic system. It was as a professor at the Imperial Military Academy in Saint Petersburg, Russia from 1903 to 1922, where he refined his theories on the existence of a common hematopoietic precursor cell. He is generally credited with the formulation of the **theory of hematopoiesis**, which states that all blood cellular components are derived from a common precursor stem cell. Maximow finished his career as a professor of anatomy at the University of Chicago.

Stem Cells: A Short Course, First Edition. Rob Burgess.
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EVENT

Aristotle disagrees with the theory of spontaneous generation — circa 350 BC
Robert Hook discovers the cell — 1665
Francesco Redi disproves theory of spontaneous generation — 1668
Franz Leydig proposes *omne vivum ex vivo* — 1857
Rudolph Virchow proposes *omnis cellula e cellula* — 1858
Louis Pasteur disproves spontaneous generation — 1864
Ramon y Cajal proposes “no new neurous” hypothesis — 1928
Tadpoles cloned — 1952
McCulloch & Till discover stem cells in bone marrow — 1961
Altman and Das discover adult brain neural stem cells — 1967
First successful allogeneic human transplant — 1968
Stem cells discovered in cord blood; 1st IVF baby born — 1978
Mouse ES cells isolated independently by Martin, Evans and Kaufman — 1981
Luskin and Evans “rediscover” adult brain stem cells — 1993
1st ES cells isolated from non-human primate — 1995
Bonnet discovers cancer stem cells; Dolly the sheep is cloned — 1997
Thomson and Gearhart independently derive human ES cells — 1998
Yamanaka derives mouse iPS cells — 2006
Yamanaka and Thomson derive human iPS cells — 2007
Human blastocysts derived by SCNT — 2008
First human clinical trial with ES cells — 2010

YEAR

Figure 1.1 Timeline of historical advances in stem cell theory and research. (Adapted from Rob Burgess, *Stem Cells Handbook* (Humana Press), 2nd Edition, Chapter 1.)

After the initial contemplation of the existence of hematopoietic stem cells (HSCs) in 1908, the field was relatively silent for more than 50 years. It was not until the early sixties when true scientific advancements in the area of stem cell research began to take place. Specifically, in 1963 researchers Ernest McCulloch and James Till (Altman and Das, 1967) of the University of Toronto demonstrated the existence of stem cells in the bone marrow (Figure 1.2). This was accomplished by injecting bone marrow cells into irradiated immune-deficient mice, which resulted in the growth of visible lumps termed **spleen colonies.** It was postulated that these colonies were the result of bone marrow-derived stem cells, and their clonal origin was confirmed. Published in the journal *Nature* that year, this finding is considered to be one of the most seminal discoveries in the field of stem cell research, laying the groundwork for virtually every major breakthrough in the discipline since.

Focus Box 1.1: Alexander A. Maximow and the Theory of Stem Cells

Alexander A. Maximow (1874–1928) was a Russian-born medical doctor and histologist and the first person to contemplate the existence of stem cells. His “theory of hematopoiesis” and histological textbook, which has been suggested to be the world’s most respected textbook in histology, laid the groundwork for many of the stem cell discoveries impacting medicine today. (Photo courtesy Wikimedia Commons; reprinted with permission.)

Figure 1.2 The late Ernest McCulloch and James Till after accepting the 2005 Lasker Award for their studies on bone marrow-derived stem cells. Ernest McCulloch is at left. (Photograph courtesy Environmental Protection Agency; reprinted with permission.)

The lymphatic system was not the only area of hot pursuit for the identification and characterization of stem cells. In 1967, a key demonstration of **neurogenesis**, defined as the generation of neurons and glial cells, occurring in the adult brain was accomplished by Drs. Joseph Altman and Gopal Das of Massachusetts Institute of Technology (Prindull et al., 1978) (Figure 1.3). In these studies, an autoradiographic technique was employed to measure both mitotic activity and tag cells for tracking at later time points. To accomplish this, tritiated (^{3}H) thymidine was injected intraperitonially into 6-day-old guinea pigs and then monitored for incorporation into the cells of the cerebellar external germinal and cortical subependymal layers of the brain. Tritiated thymidine will incorporate into the DNA of mitotically active cells, thus marking cell division. In addition, it will remain in these cells long term as a tag for subsequent cell marking and characterization. Dr. Altman's group used this technique to reveal active mitosis in the brains of adult guinea pigs followed by confirmation that the tagged cells differentiate into identifiable small-caliber mature interneurons he termed "**microneurons.** " The findings of Altman and Das went against the **no new neurons** central dogma of leading neuroscientist Santiago Ramon y Cajal, and thus were largely dismissed by the scientific community (Altman and Das, 1967). It was only in the 1990s when adult neurogenesis was "rediscovered" that Altman's theories on adult brain neurogenesis were accepted by the scientific community. Dr. Altman and

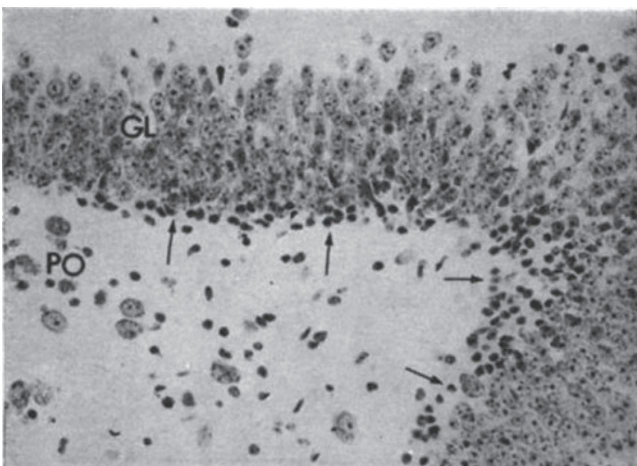


Figure 1.3 Discovery of active neurogenesis in the adult brain. The arrows denote ^{3}H-thymidine uptake in glial cells in rodent brain regions associated with trauma. Neurons and neuroblasts also demonstrated some staining, confirming mitosis and corresponding neurogenesis. (Photo courtesy *Nature* (Altman and Das, 1967); reprinted with permission.)

Figure 1.4 Dr. Robert Alan Goodwith, President Richard Nixon, and colleagues at the White House Conquest of Cancer Program in 1973. Dr. Goodwith is circled; President Nixon is second from the left. Also pictured is Dr. Robert L. Clark of the University of Texas M.D. Anderson Cancer Center. (Photo courtesy Nixon archives; reprinted with permission.)

his wife and colleague, researcher Shirley Altman-Bayer, still actively promote their research theories today and have a forthcoming book titled *MENTAL EVOLUTION: Origins of the Human Body, Brain, Behavior, Consciousness, and Culture*.

In 1968, a major therapeutic breakthrough based on the potential of stem cells present in bone marrow was realized when the first successful human bone marrow transplant was accomplished by the late American physician Dr. Robert Alan Goode while he was Professor in Pediatrics, Microbiology, and Pathology at the University of Minnesota Medical School (Figure 1.4). The transplant was performed between siblings for the treatment of **severe combined immunodeficiency syndrome (SCID)**, a genetic disorder in which both B and T cells of the immune system are severely compromised due to a defect in one of several possible genes. It was widely speculated at the time (and later confirmed) that bone marrow-derived stem cells from the healthy sibling aided in reconstructing the immune system of the recipient SCID patient. Dr. Good won the Albert Lasker Medical Research Award in 1970 and is generally accepted by the medical and research communities as the founder of modern immunology.

HEMATOPOIETIC STEM CELL DISCOVERY

Therapeutic advancements in bone marrow transplants set the groundwork for a related major discovery in 1978 when Dr. Gregor Prindull, Professor of Pediatric Hematology/Oncology in the Department of Paediatrics at the University of Gottingen in Gottingen, West Germany (now retired), and colleagues B. Prindull and N. Meulen discovered the presence of HSCs in human umbilical cord blood. In this study, the researchers extracted cord blood from newborn infants 8–10 days old. Cells from the blood samples were processed by sedimentation and clearing of nonlymphoid cells and subsequently cultured in a methylcellulose cell culture system. By the 10th day of cell culture the researchers identified a small subpopulation of cells (1 in 1,678 on average) in a sample of nonadherent

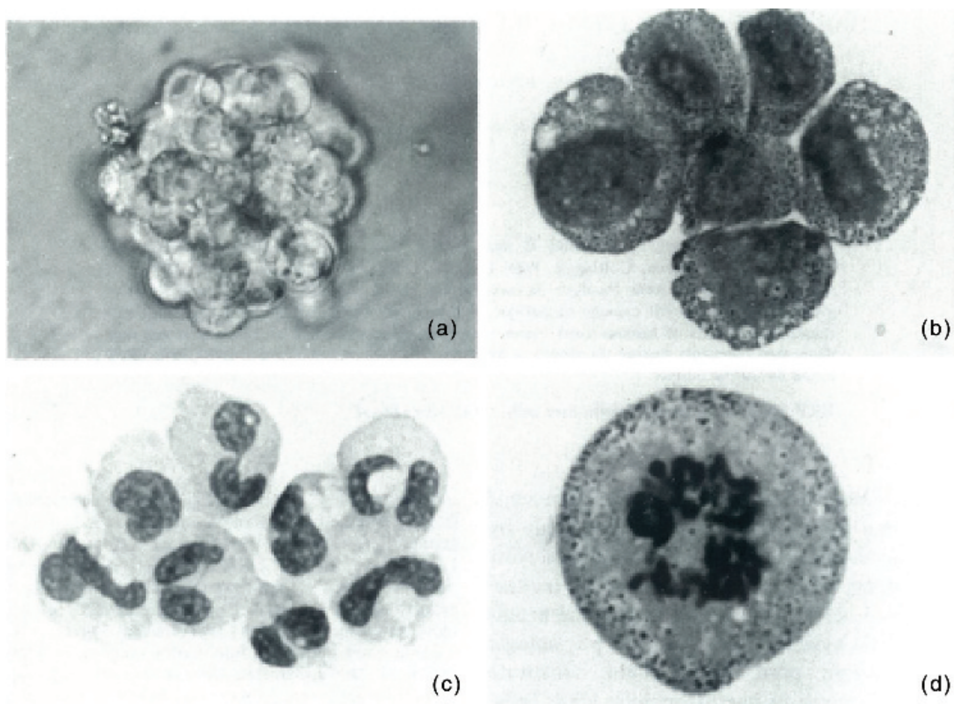


Figure 1.5 Hematopoietic stem cells isolated from human umbilical cord blood. (a) Colony cultured on methylcellulose. (b) Myelocytes and metamyelocytes. (c) Neutrophils. (d) Dividing myelocyte. (Photo courtesy Dr. G. Prindull and *Acta Paediatrica Scandinavica* (Prindull and Prindull, 1978); reprinted with permission.)

mononuclear cells that represented myelocytic **colony forming units (CFUs),** cells that have the ability to divide and form a clonal colony in tissue culture. These cells formed adherent colonies in a methylcellulose matrix (Prindull and Prindull, 1978). **Myelocytes** are of granulocytic origin and normally only present in bone marrow, yet given the high degree of proliferation during fetal development, as is evidenced by this study, they accumulate in the cord blood of newborn infants (Figure 1.5).

Focus Box 1.2: Gail R. Martin and the discovery of mouse embryonic stem cells

As a professor in the Department of Anatomy at the University of California, San Francisco, researcher Gail R. Martin is widely credited with the co-discovery of mouse embryonic stem cells, ushering in a new era of scientific research in embryonic development and the study of gene function. She is a member of the American Academy of Arts and Sciences, the National Academy of Sciences, and currently runs the Program in Biological Sciences at UCSF.

Source: Reproduced with permission from G. R. Martin.

MOUSE EMBRYONIC STEM CELL DISCOVERY

In 1981, the term “embryonic stem cell” was coined by University of California, San Francisco researcher Gail R. Martin, when she derived cells from the inner cell mass (ICM) (defined below) of 3.5-day-old mouse embryos and confirmed that these cells could give rise to a variety of mature, differentiated cell types. These cells also highly resembled embryonal teratocarcinoma cells which are known to be multipotent in nature (Bonnet and Dick, 1997) (see Focus Box 1.2 and Case Study 1.1). This seminal finding was simultaneously and independently accomplished by University of Cambridge professor Sir Martin Evans and researcher Matthew Kaufman. Evans later went on to receive the 2007 Nobel Prize in Physiology or Medicine for his contribution to rodent-based gene targeting technologies along with the University of Utah’s Mario Capecchi and the University of Wisconsin’s Oliver Smithies. The discovery of embryonic stem cells in mice and the development of corresponding gene targeting technologies are discussed in detail in the Chapter 7, section Embryonic Stem Cells and Animal Models of Gene Function.

Case Study 1.1: Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells

Gail R. Martin

In this study, Professor Gail R. Martin and colleagues at UCSF successfully isolated and cultured a clonal population of mitotically active pluripotent cells from the ICM of mouse 3.5 days post coitum (dpc) embryos. This was accomplished utilizing a special **“conditioned medium”** removed and concentrated from the culture of PSA-1 embryonal carcinoma (EC) cells. Given the inherent capacity of EC cells to differentiate into numerous cell types the medium was speculated to contain a growth factor or growth factors secreted by these cells capable of driving cell division and/or inhibiting differentiation. The embryo-derived stem cells exhibited a striking resemblance to EC cells and were demonstrated to have the capacity to differentiate into a wide variety of cell types in tissue culture (Martin, 1981 and Figure 1.6). As a final proof of pluripotency, Martin and colleagues showed that these cells could form teratocarcinomas when injected into mice (Martin, 1981).

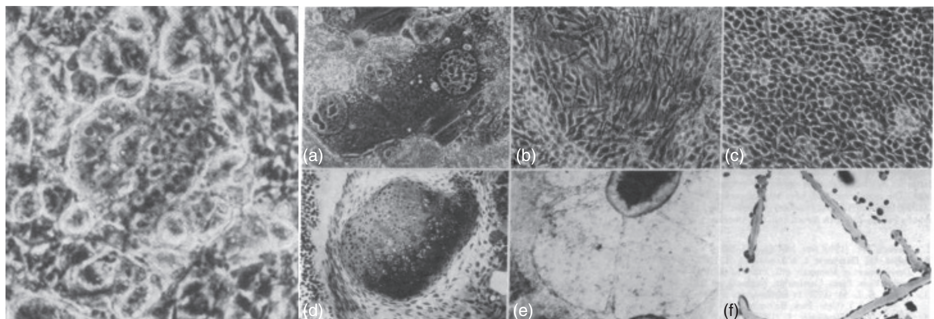


Figure 1.6 The discovery of mouse embryonic stem cells. (Left) The first published photo documentation of a mouse embryonic stem cell colony. (Right) Embryoid bodies demonstrating a variety of different cell types including (a) giant cells, (b) neuron-like cells, (c) endodermal cells, (d) cartilage, and (e) cells forming tubules. Source: Martin, 1981. Reproduced with permission from G. R. Martin.)

SUCCESSFUL NEURAL STEM CELL CULTURE

Despite Joseph Altman's speculation and demonstration regarding the existence of neural stem cells in the adult brain in 1967, it was only in 1992 when Brent A. Reynolds and Samuel Weiss in the Department of Pathology at the University of Calgary School of Medicine first successfully isolated neural progenitor and stem cells from the **subventricular zone** (a neurogenic region) of adult mouse brain tissue that their existence was accepted by the scientific community. In that same year researchers in the Department of Genetics at Harvard Medical School, led by Constance Cepko, isolated a multipotent cell line from adult mouse brain tissue and transformed it with **v-myc** —the viral homolog of c-myc—which is capable of cellular transformation, to create a stable indefinitely dividing neural stem cell population. Characterization of clonal cell populations revealed a common viral integration site, suggesting that individual lines actually originated from a common infected progenitor cell. Two cell lines exhibited extensive **process**-bearing morphology (a process with respect to neural or neuronal cell culture refers to either axon or dendrite-like protrusions). Three cell lines were demonstrated to have the capacity to differentiate into mature neurons and glia, and further subcloning of each line revealed the same morphological and molecular characteristics across these lines. For *in vivo* studies, the cells were marked for identification with a β-galactosidase (LacZ) genetic tag that allows for tracing of individual cell fate. **β-galactosidase** is an enzyme that catalyzes the hydrolysis of β-galactosides into monosaccharides. It also cleaves the organic compound X-gal to produce a characteristic blue dye for use in histology. When the researchers transplanted v-myc-transformed, LacZ-tagged neural cells into the cerebellum of newborn mice, the cells integrated in a non-tumorigenic fashion and properly differentiated into either neurons or glia depending upon the location of cerebellar integration (Snyder et al., 1992) (Figure 1.7).

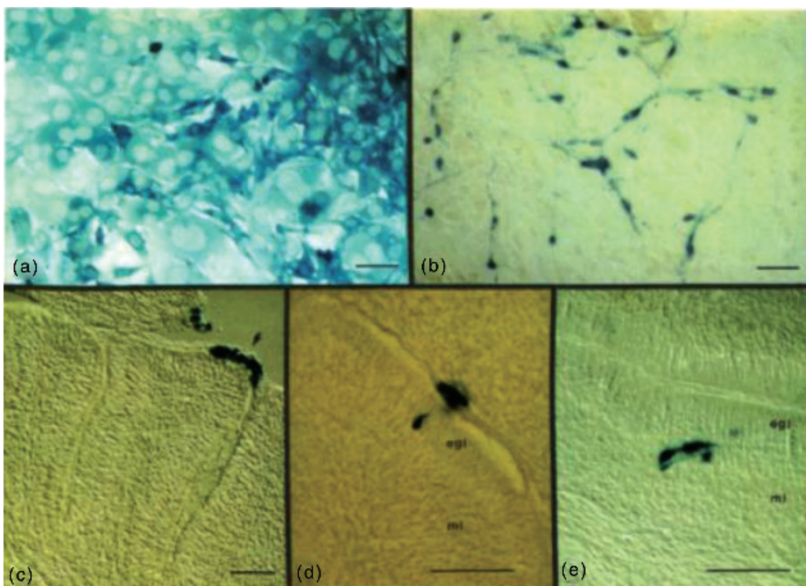


Figure 1.7 Generation and characterization of multipotent neural stem cells. (a) Non-cultured control and (b) 8-day coculture of transformed neural stem cells (stained in blue) with dissociated primary mouse cerebellum demonstrating process formation. (c–e) Sections of the cerebellar region of a mouse brain transplanted with LacZ tagged v-myc transformed neural stem cells. (c) Six hours post transplant; (d and e) 72 hours post transplant demonstrating proper migration into the molecular layer. (Photos courtesy Constance Cepko and *Cell* (Snyder et al., 1992); reprinted with permission.)

THE DISCOVERY OF CANCER STEM CELLS

Cancer can be defined as abnormal growth of cells that tend to proliferate in an uncontrolled way, and, in some cases, to **metastasize** (spread to other parts of the body). Perhaps the most intriguing key property of cancer cells is their ability to proliferate almost indefinitely. Cells exhibiting a cancerous phenotype have, in most cases, acquired multiple mutations resulting in stem cell-like properties such as active cell division, despite origins as mature, differentiated cell types. As such, it has been suggested that a subpopulation of cells, called cancer stem cells (CSCs—defined in more detail in Chapter 6), is not a prerequisite for tumorigenesis. Yet, given the stringent requirements of cancer cells to retain the genetics required for continuous mitotic activity, it has been widely speculated that CSCs exist within certain types of cancer for which relapse and metastasis are common. Initially in 1994 and then in a seminal research study in 1997, researchers in the Department of Genetics at the Hospital for Sick Children in Toronto, Canada led by John E. Dick identified a subpopulation of cells in a human acute myeloid leukemia (AML) sample that originated from a primitive HSC. In this study, a human cell type previously demonstrated as capable of initiating AML when introduced into NOD/SCID mice was characterized as having all the hallmarks of a primitive rather than committed progenitor cell. Termed a SCID-Leukemic Initiating Cell (SL-IC), the researchers confirmed the line's ability to indefinitely proliferate, self-renew, and differentiate into normal as well as cancerous leukemic cells of the immune system (Figure 1.8). Flow cytometric analyses revealed the SL-IC population to be exclusively $CD34^+/CD38^-$, which is a molecular hallmark of pluripotent, undifferentiated HSCs. Self-renewal properties were assessed through serial transplantation studies in which SL-IC cells were transplanted into primary and subsequent secondary recipients with no observable change in leukemic morphology or cell surface phenotype (Bonnet and Dick, 1997).

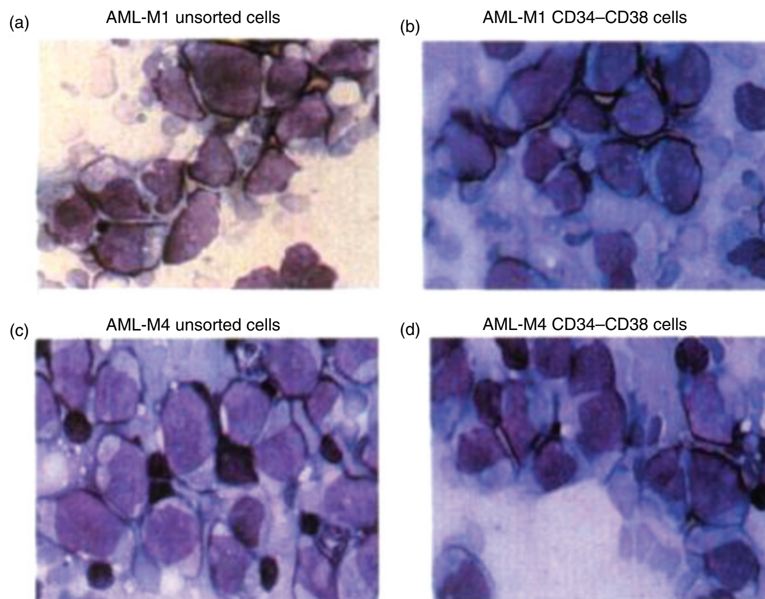
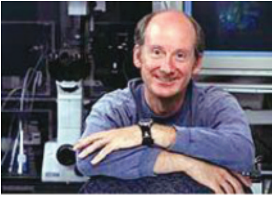


Figure 1.8 Differentiation capacity of SL-IC cancer stem cells. (a and c) Unsorted and (b and d) sorted CD34+/CD38- SL-ICs demonstrating colonization of the bone marrow of a recipient NOD/SCID mouse as assayed by the presence of the marker CD45 which is a transmembrane glycoprotein present on the cell surface of all cells of hematopoietic origin. (Photos courtesy John Dick and *Nature Medicine* (Bonnet and Dick, 1997); reprinted with permission.)

Focus Box 1.3: James Thomson and the discovery of hESCs



Dr. James A. Thomson of the University of Wisconsin is an American developmental biologist credited with deriving the first clonal hESC line in 1998. He later expanded upon this work in 2007 when he derived induced pluripotency stem (iPS) cells. He is currently Director of Regenerative Biology at the Morgridge Institute for Research in Madison, Wisconsin and a professor in the Department of Cell and Regenerative Biology at the University of Wisconsin School of Medicine and Public Health. He is a member of the National Academy of Sciences and was named one of *Time* magazine's 100 most influential people in 2008. (Photo courtesy UW; reprinted with permission.)

HUMAN EMBRYONIC STEM CELL DISCOVERY

The co-discovery of mouse embryonic stem cells by Gail Martin's group at UCSF and Martin Evan's team at the University of Cambridge immediately raised the speculation that a similar cell type might exist as a component of the ICM of developing human embryos. The existence of a hESC would have huge implications for medical research. For example, specific terminally differentiated cell types could be generated from hES cells to be used in drug screening assays or directly as cell-based therapeutics. The ability to derive embryonic stem cells from discarded human embryos was no easy feat, however, and it took a full 17 years of cell culture optimization before this was accomplished. Dr. James A. Thomson (see Focus Box 1.3) was the first to accomplish the isolation and characterization of a hESC line, which was published in the November 6, 1998 issue of *Scienc*e magazine. In 1999, this discovery was featured again in *Science*'s "Breakthrough of the Year" article. In this study, Dr. Thomson's group obtained **cleavage-stage** (2-, 4-, 8-, and 16-cell stages containing blastomeres) human embryos produced by *in vitro* fertilization for clinical purposes. Embryos were cultured until fully formed blastocysts had developed and ICMs were further isolated for culture and characterization. Specific clonal isolates expressed high levels of telomerase activity (see Figure 1.9) and were designated as H1, H7, H9, H13, and H14 line with H7 and H9 of the female XX **karyotype** (the number and appearance of chromosomes in the nucleus of a cell) and the other lines male (XY) in origin. The **H9 cell line** is perhaps the most popular and widely studied of the original hES cell isolates, given its stable karyotype over extended passages and long periods of cell culture (Figure 1.9) (Thomson et al., 1998). Interestingly, the embryonic stem cell lines isolated in this study were not clonal (from a single cell), but were expanded from heterogeneous, uniform, undifferentiated colonies. Thomson's group went on to perform a number of analyses to determine the stability of each line and its capacity for differentiation. **Telomerase** is an enzymatic ribonucleoprotein which functions to add telomeric repeats to chromosomal ends and plays a critical role in extending the lifespan of a cell. Its expression and presence within a cell is directly correlated with cellular immortality. Each cell line isolate was shown to express high levels of telomerase activity. In addition, numerous cell surface markers previously identified in other embryonic stem cell lines such as those of mouse origin as defining pluripotency were expressed at high levels in each H line. These include SSEA3, SSEA4, Tra-1-60, and Tra-1-81. These markers will be discussed in detail in Chapter 3. Finally, in an experiment similar to that conducted by Gail Martin's group for the

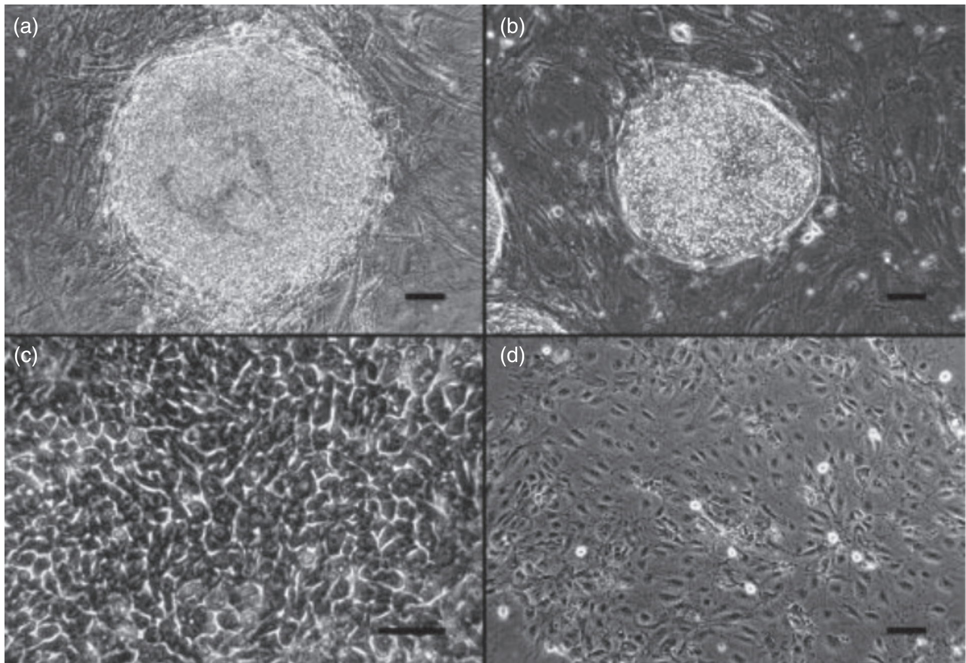


Figure 1.9 Derivation of the 1st clonal human embryonic stem cell line. (a) First inner cell mass colony cultured on a mouse feeder layer. (b) H9 clonal undifferentiated human ES cell colony. (c) High magnification of individual human ES cells. (d) Differentiated human ES cells cultured in the absence of a mouse feeder layer. (Photos courtesy Dr. James A. Thomson and *Science* (Thomson et al., 1998); reprinted with permission.)

characterization of mouse ES cells, cells from each isolate were confirmed to form teratomas in SCID-beige mice, a hallmark of pluripotency.

So how did Thomson's group successfully isolate true embryonic stem cells from human blastocysts when others had failed? First, it is speculated that the heterogeneity of each cell line population contribute to its pluripotent state, with hES cells secreting factors that keep neighboring hES cells undifferentiated. Second, the cell culture conditions implemented to isolate hES cells were notably different than that for mouse or other embryonic stem cells, such as those of primate origin. A conditioned medium prepared from feeder cells was not used for hES cell derivation and it can be speculated that differentiation inducing factors present in conditioned media were not present in the simpler formulation used by Thomson's group. Third, it is evident from the successful isolation of the H9, H13, and H14 lines that mechanical dissociation of cellular clumps, rather than the use of enzymes such as trypsin, may result in less stress on the individual cells and thus promote the undifferentiated state. Subsequent use of a less harsh enzyme, crab collagenase, also appears to be a factor in maintaining cell pluripotency.

It should be noted that in 1998 John Gearhart and his research team at Johns Hopkins published the first report on the derivation of pluripotent stem cells from germ cells of the human embryo (Shamblott et al., 1998). In addition, serious ethical and moral issues have surrounded the derivation and study of hESCs. This topic will be discussed in detail in Chapter 3.

STEM CELLS AND CLONING

Cloning is defined as the process of creating genetically identical individuals from a single donor. In order to grasp the impact cloning has had on stem cell research it is important to understand the basic mechanistic procedure behind cloning. **Somatic cells** are defined as any biological cell forming the body of an organism other than a germ cell, gamete, gametocyte, or undifferentiated stem cell. **Somatic cell nuclear transfer (SCNT)** is defined as a technique for creating a clonal embryo by combining an ovum (egg) with a donor nucleus. In SCNT a donor nucleus is removed from a cell for which cloning is desired and inserted microsurgically into an enucleated (nucleus has been removed) egg. The "clonal" resulting cell may be either propagated in tissue culture or allowed to develop into an embryo and transplanted into a surrogate mother, a process known as **reproductive cloning** (Figure 1.10). It is important to note that, while reproductive cloning has not been carried out on humans, it has been successfully accomplished in other species such as mice, sheep, monkeys, and dogs, to name a few. **Therapeutic cloning** can be defined as reproduction of a genetically identical cell for purposes of cell therapy such as cellular replacement. Numerous research studies are currently underway to provide clonal populations of embryonic stem cells for either therapeutic or drug discovery initiatives.

Researchers at the Rosland Institute in Scotland, led by Sir Ian Wilmut, an English embryologist who is currently Director of the Medical Research Council Centre for Regenerative Medicine at the University of Edinburgh, successfully cloned the first mammal from an adult somatic cell in 1996. **Dolly the sheep** was cloned by combining the nucleus of a mammary cell from a Finn Dorset sheep with an enucleated egg from a Scottish Blackface ewe (Figure 1.11).

So if cloning technologies are based on the manipulation of somatic cell nuclei, why is cloning relevant from a stem cell perspective? Why would there be a desire to gen-

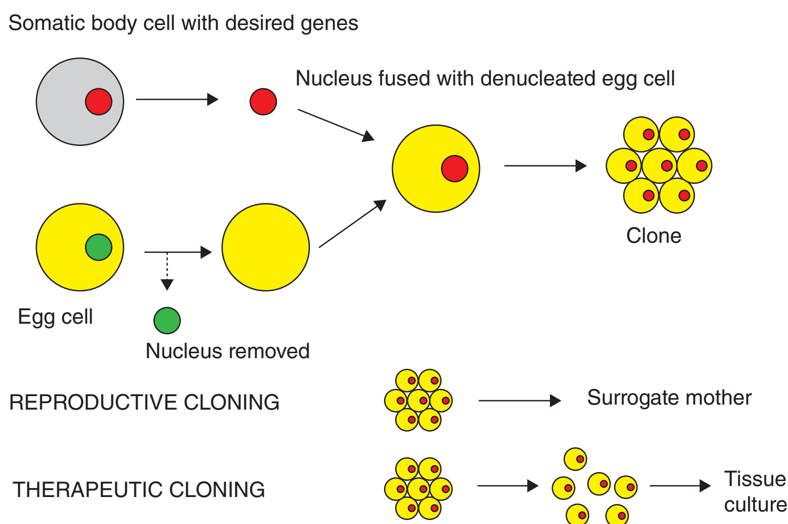


Figure 1.10 Diagrammatic illustration of Somatic Cell Nuclear Transfer (SCNT). See text for a detailed description. (Diagram courtesy Wikipedia.org; reprinted with permission.)

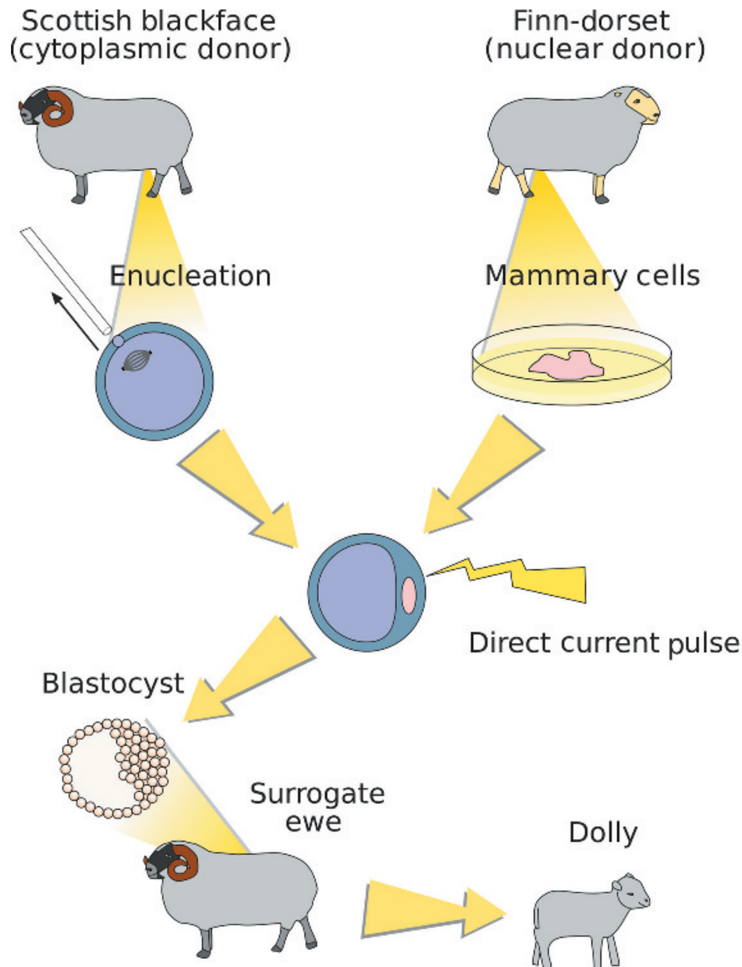


Figure 1.11 Diagram of the procedure undertaken for cloning Dolly the sheep. (Diagram courtesy Wikimedia Commons; reprinted with permission.)

erate a clonal population of stem cells from a stem cell? In a manner similar to that for somatic cells, cloning utilizing embryonic stem cells could allow for the generation of a virtually limitless supply of stem cells that are genetically identical, thus providing an extremely valuable therapeutic or perhaps even drug discovery platform as the population of cells would be genetically and phenotypically of an identical origin. Yet, having originated from adult cells, the population of cells would still have a composition of genetic material that has undergone the rigors of environmental influence and the aging process that so significantly damages that of adult somatic cells. As an example, there is much speculation that Dolly's premature death (she died in 2003 after only 7 years of life) may have been due to genetic abnormalities inherent in the aged mammary cell used as a nuclear donor. Scientific evidence indeed backs this theory as analysis of Dolly's chromosomal makeup revealed abnormally shortened **telomeres**, a

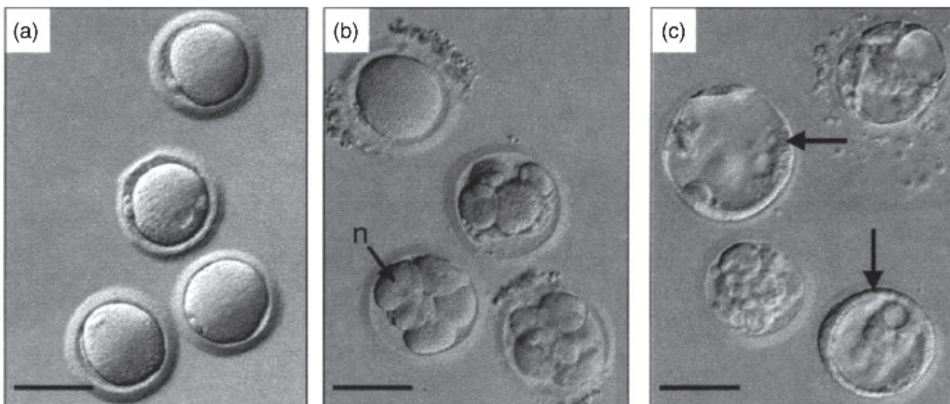


Figure 1.12 Advanced Cell Technology's parthenogenetically activated human embryos. (a) **Isolated** unfertilized eggs. (b) 4–6 cell embryos 48 hours after activation of parthenogenesis. (c) Day 6 revealing blastocoele cavities indicated by arrows. (Photos courtesy Jose B. Cibelli and *Scientific American* (Cibelli et al., 2002); reprinted with permission.)

hallmark of aging, although the authors of this study deny that aging played a role in Dolly's demise.

In 2001, the Worcester, Massachusetts-based company Advanced Cell Technology (ACT) published a study focused on the generation of genetically identical hESCs. In this study, researchers led by Jose B. Cibelli, who is now a Professor of Animal Biotechnology in the Departments of Animal Science and Physiology at Michigan State University, created autologous embryos by two methods. First, they exploited a process known as parthenogenesis to produce the world's first cloned human embryos from eggs. **Parthenogenesis** is defined as a form of asexual reproduction in females where no fertilization from a male is required in order to reproduce. It is prevalent in the plant kingdom and numerous natural examples exist in animals. In ACT's study, parthenogenesis was artificially induced in 22 unfertilized eggs by various chemicals, which resulted in a change in ion concentrations thus influencing parthenogenic development of single cell unfertilized eggs. Specific chemicals utilized included a calcium ionophore and either puromycin or 6-dimethylaminopurine (DMAP) which have been previously demonstrated to trigger not only **pronucleus** (the nucleus of an egg cell during the process of fertilization) formation but early stage cleavage as well. A small number of the eggs divided and developed into embryos containing a **blastocoele**, which is defined as a cleavage cavity or segmentation cavity present in a developing embryo (Figure 1.12) (Cibelli et al., 2002). Unfortunately these embryos did not develop beyond the blastocoele stage, thus no ICMs were observed preventing the isolation of embryonic stem cells.

The second method pursued in this study for the generation of autologously derived embryonic stem cells involved SCNT as discussed above. Nuclei from both human fibroblasts and **cumulus cells**, which are specialized granulosa cells that surround and nourish a developing egg, were utilized in separate experiments to reconstitute human embryos via SCNT. Some of these embryos developed to the 6-cell stage but did not reach the blastocyst level required for isolation of embryonic stem cells (Figure 1.13).

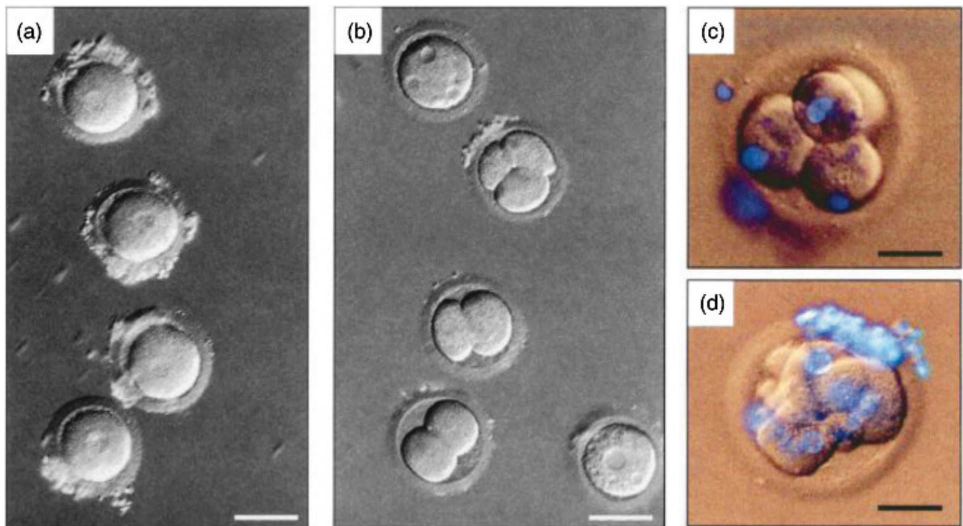


Figure 1.13 Somatic cell nuclear transfer cumulus cell-derived human embryos. (a) **12 hours**, (b) 36 hours (2 cell stage), (c) 72 hours (4-cell stage), and (d) 72 hours (6-cell stage) after nuclear transfer. (c) and (d) indicate nuclei stained with the fluorescent label bisbenzimide. (Photos courtesy Jose B. Cibelli and *Scientific American* (Cibelli et al., 2002); reprinted with permission.)

CORD BLOOD EMBRYONIC-LIKE STEM CELLS—AN ALTERNATIVE TO ES AND ADULT STEM CELLS

Given the controversial nature of utilizing hESCs for therapeutic purposes and their as yet unproven nature with respect to possible side effects such as tumorigenicity, many researchers have realized that commonly accepted use of hES cells in the clinic will not occur anytime soon. In addition, the large-scale production of a homogeneous population of hES cells or desired terminally differentiated lineages without the use of cell feeder layers represents a significant current technological hurdle. This issue, coupled with the near universal immunological compatibility most recipients need, could hinder near-future clinical use. As such, numerous scientists have turned their attentions to the isolation and characterization of other ES cell-like cells that have similar multipotency capabilities but have not yet been isolated from developing embryos. For example, in 2005 researchers in the Stem Cell Therapy Programme at Kingston University in Surrey, United Kingdom led by Colin P. McGuckin isolated a population of stem cells—termed cord blood-derived embryonic-like stem cells (CBEs and defined in Chapter 4)—from human umbilical cord blood that bore striking resemblance to hES cells and possessed the capacity to differentiate into a variety of lineages. To accomplish CBE isolation, the researchers employed an immunomagnetic process to remove unwanted granulocytes, erythrocytes, and hematopoietic/myeloid/lymphoid progenitors from umbilical cord blood isolated by elective Cesarean section. This allowed for a concentration of the CBE population, given that typically only a limited number of all cell types exist in umbilical cord blood. Individual colonies were cultured for a period of 6 weeks followed by sub-cloning and an additional culture period for a minimum of 13 weeks. In addition, the cells were cultured in a liquid suspension environment and exhibited similar patterns of exponential growth as hESCs under these

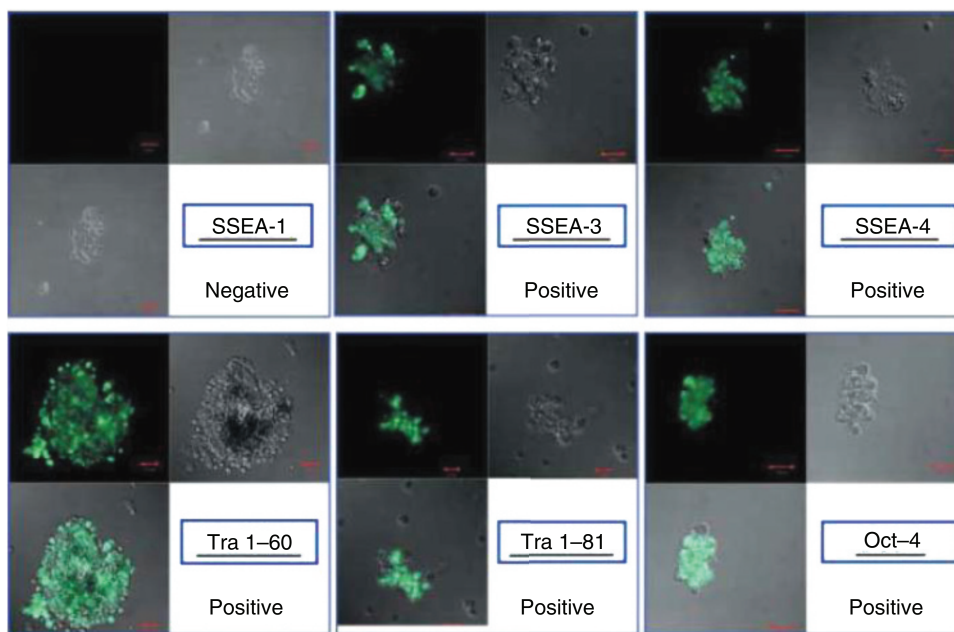


Figure 1.14 Marker characterization of cord blood embryonic-like stem cells. Cells were positive for the classical ES markers SSEA-3, SSEA-4, Tra 1-60, Tra 1-81 and Oct-4 yet, as is characteristic of embryonic stem cells, the CBEs did not express SSEA-1. (Photos courtesy Colin P. McGuckin and *Cell Proliferation* (McGuckin et al., 2005); reprinted with permission.)

conditions. Marker analysis was performed by immunofluorescence with antibodies specific for antigens well characterized in hESCs, revealing a similar profile to most pluripotent hES cell lines (Figure 1.14) (McGuckin et al., 2005).

McGuckin and colleagues also assessed the differentiation capacity of CBEs in a three-dimensional rotating cell culture microbioreactor. Single cells were introduced into the system and differentiation was initiated through the addition of hepatic cues for a period of 1 month. Three-dimensional clusters were immuno-phenotyped, revealing the presence of hepatic-specific antigens including cytokeratin-18, α-fetoprotein, and albumin, suggesting effective directed termination differentiation. The studies by McGuckin and colleagues laid the foundation for CBEs as a possible autologous stem cell-based therapeutic platform for a number of anomalies.

BREAKTHROUGH IN SPINAL CORD INJURY REPAIR

The promise of applying stem cells for real-world therapeutic intervention clearly is the primary focus of the widespread research and development efforts in this field. The adult central nervous system (CNS) has a very limited capacity to regenerate itself and, as a consequence, injuries to the spinal cord often result in partial or complete irreversible paralysis. Secondary degeneration of the CNS post injury also aggravates this scenario. Neural stem and progenitor cells have long been speculated as a potential source of therapeutic intervention by promoting tissue survival, growth, and the replacement of cells lost as a result of trauma or disease. In 2005, the first significant *in vivo* evidence of direct

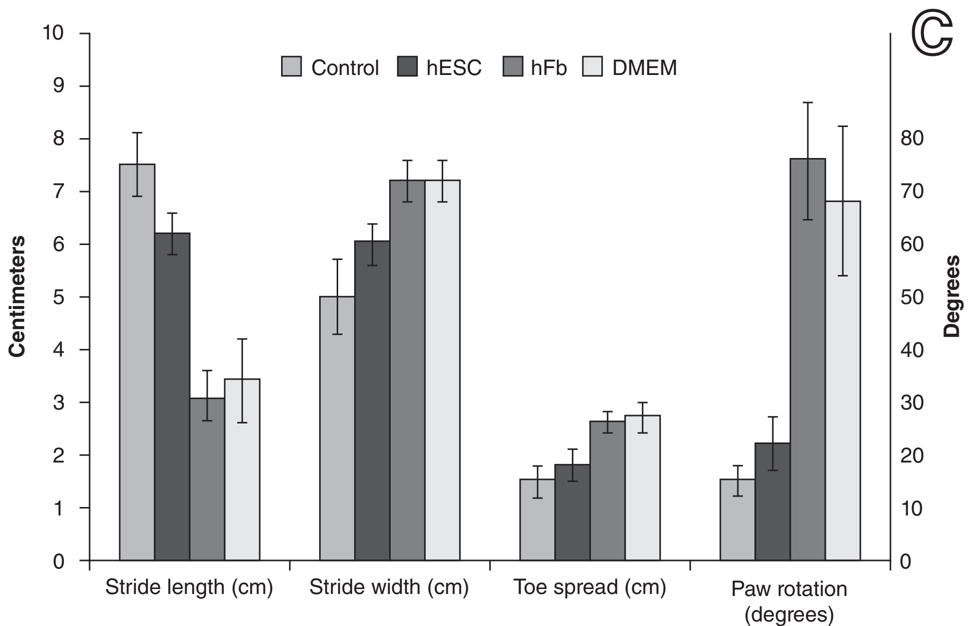


Figure 1.15 Human ES cell-derived OPCs improve locomotor recovery in rats. Note: decreased rear paw stride length and increased rear paw stride width, rear paw toe spread, and rear paw rotation are typical deficits in injured rats. These were largely corrected with hES-derived OPC transplants. (Graph courtesy Hans S. Keirstead and the *Journal of Neuroscience* (Keirstead et al., 2005); reprinted with permission.)

neural stem cell-based therapeutic applications came to light in a research study headed by Hans S. Keirstead, an Assistant Professor in the Department of Anatomy and Neurobiology, Reeve-Irvine Research Center at the University of California Medical School in Irvine. Dr. Keirstead's primary research focus is on the degeneration and regeneration of the spinal cord and efforts to repair spinal cord injuries. His team utilized induced rat models of human spinal cord injury, specializing in the study of **demyelination**, which is loss of the myelin sheath insulating the nerves. One particular study demonstrated an enhancement in myelination and improvement of locomotor recovery in injured rats upon intraspinal cord transplantation of hESC-derived **oligodendrocyte progenitor cells (OPCs)**. OPCs are defined as immature myelin-producing stem cells. When transplanted, the cells drove partial reconstitution of the myelin sheath through remyelination, which is speculated to have partially restored neuronal cell function (Figure 1.15) (Keirstead et al., 2005). These findings illustrate that it is possible to at least partially restore locomotor function in spinal cord injured vertebrates using stem cell-based transplant technologies.

THE GENERATION OF IPS CELLS

The controversy surrounding the use of embryonic stem cells as well as the restricted multi-lineage differentiation capacity of adult stem cells has driven researchers to search for yet other alternative cell-based therapeutics platforms. It has long been known by cell and developmental biologists that in certain instances adult differentiated cells may be

driven to lose their morphological and molecular identities and transform into other cell types. The process is referred to as **transdifferentiation**, which is defined as a non-stem cell transforming into a different cell type, or when a differentiated stem cell generates other cell types outside of its normal realm of multipotency. If it can be harnessed and controlled, the phenomenon of transdifferentiation provides an exciting alternative to the use of embryonic or adult stem cells to produce the mature cell phenotypes needed for cell replacement therapy. In addition, in an artificial setting, differentiated cells can be reprogrammed to an embryonic-like state, a process known as **dedifferentiation**, which can be accomplished by either nuclear transfer into oocytes or fusion with other ES cells. Until 2006, it was not possible to drive this process with individual factors. It was in this year that researchers in the Department of Stem Cell Biology at the Institute for Frontier Medical Sciences in Kyoto, Japan led by Shinya Yamanaka managed to induce adult mouse fibroblast cells to dedifferentiate into stem cells and become pluripotent (See Case Study 1.2). The same researchers followed this groundbreaking study in 2007 with a demonstration of pluripotency induction of human fibroblasts. That same year, James Thomson‘s group at the University of Wisconsin published a similar finding separately and independently (see Figure 1.18).

The groundbreaking studies by Yamanaka's group in 2006 on the induction of pluripotent properties in mouse adult fibroblasts (see Case Study 1.2) provided a solid framework for similar efforts on human cells. From a clinical perspective, the production of iPS stem cells from human adult cell types would be of enormous benefit for several reasons. First, as the cells could be derived from a patient's own cell sample—for example, a skin punch biopsy—it would allow for the generation of patient-specific stem cells, thus eliminating any possibilities of immunorejection. Second, due to their pluripotent properties, it would allow for the development of individual cell types that could be valuable for the treatment of specific diseases. In 2007, two groups separately and independently accomplished the creation of human iPS cells from adult human fibroblasts. In a manner similar to that for the induction of iPS properties in mouse fibroblasts in 2006 (see Case Study 1.2), the introduction of four key transcription factors into human fibroblasts proved critical to drive dedifferentiation and the induction of pluripotency. Yamanaka's group employed retroviral transduction to introduce genes encoding the four key transcription factors, Oct 3/4, Sox2, Klf4, and c-Myc to human dermal fibroblasts (HDFs). They demonstrated a striking resemblance of the iPS cells to hESCs with respect to a number of characteristics including morphology, proliferation properties, cell surface markers, telomere length/telomerase activity, and differentiation capacities (Figure 1.17) (Takashi and Yamanaka, 2006).

Interestingly, in a study headed by James Thomson and colleagues at the Genome Center of Wisconsin and the Wisconsin National Primate Research Center at the University of Wisconsin in Madison the four factors Oct3/4, Sox2, Nanog, and Lin28 were sufficient for the induction of pluripotency in human somatic cells. The difference in the identity of transcription factors utilized for iPS induction in comparison to the research of Yamanaka and colleagues is intriguing, and suggests that there is not one universal transcription factor code necessary for dedifferentiation of somatic cells to an embryonic-like state. Thomson's group implemented a lentiviral transduction system to introduce genes encoding these four transcription factors into human mesenchymal cells. While Nanog could be removed from the system and iPS clones successfully derived, this transcription factor was shown to improve clonal recovery, resulting in an over 200-fold increase in reprogramming efficiency (Figure 1.18) (Thomson et al., 1998). iPS induction utilizing these four transcription factors was also performed on human adult somatic cell fibroblasts with similar results.

Case Study 1.2: Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors

Kazutoshi Takahashi and Shinya Yamanaka

In 2006 Shinya Yamanaka and colleagues at the Institute for Frontier Medical Sciences focused their efforts on the introduction of four key transcription factors via retroviral transduction methods into adult mouse fibroblasts to drive reprogramming of these cells into an embryonic stem cell-like state (iPS). The factors Oct 3/4, Sox2, c-Myc and Klf4 were demonstrated to be necessary and sufficient for fibroblast dedifferentiation and reprogramming. Interestingly, the transcription factor Nanog, which has been suggested by many researchers to be required for the pluripotent properties of embryonic stem cells, was dispensable in this study. The researchers confirmed the pluripotency of reprogrammed, GFP tagged iPS cells by introducing them into host blastocyst stage embryos and monitoring iPS cell contribution to the three primary germ layers of the host embryos (Figure 1.16) (Takashi and Yamanaka, 2006). The key transcription factors noted in this study will be discussed in more detail in Chapter 5.

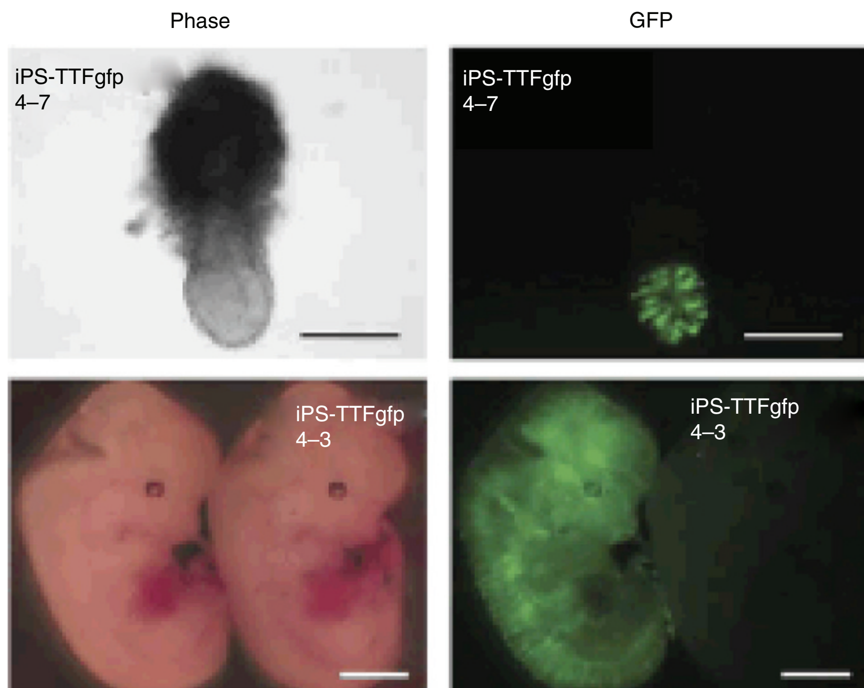


Figure 1.16 Contribution of iPS cells to mouse embryonic development.

iPS cells tagged with green fluorescent protein (GFP) were microinjected into mouse blastocysts. Embryos were characterized at either E7.5 (upper panels) or E13.5 (lower panels) for iPS cell contribution to the embryo proper. (Photos courtesy Shinya Yamanaka and *Cell* (Takashi and Yamanaka, 2006); reprinted with permission.)

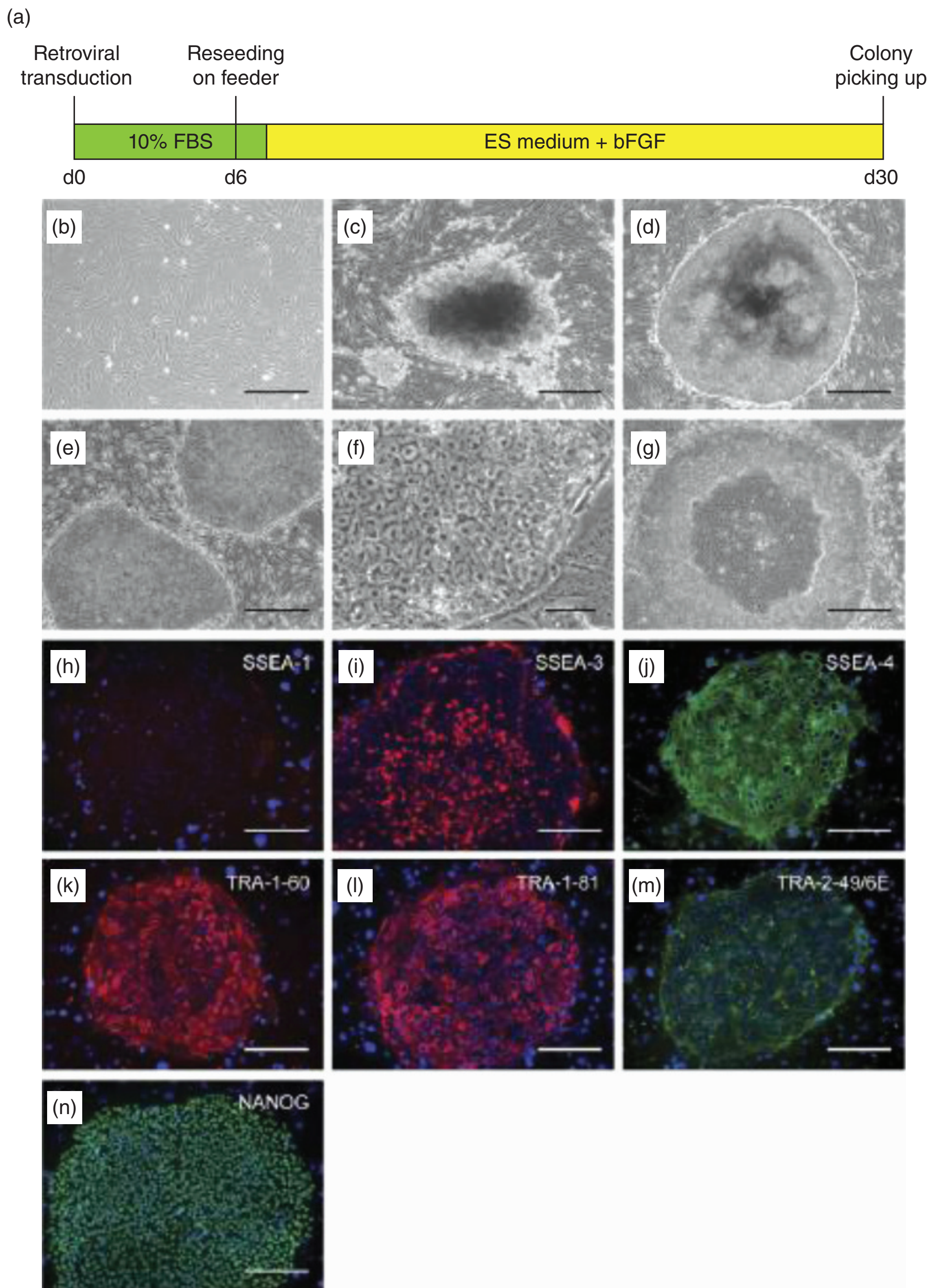


Figure 1.17 Induction of pluripotency in human adult dermal fibroblasts. (a) Chronology of induction strategy; (b) HDF morphology before induction; (c) HDF colony before induction; (d) Example of human ES cell colony; (e) P6 iPS HDF colony; (f) Same under higher magnification; (g) Spontaneous differentiation in the center of the iPS HDF colony; (h–n) Immunocytochemistry for the noted markers demonstrating a similar expression patter in iPS HDFs to that of human embryonic stem cells. (Photos courtesy Shinya Yamanaka and *Cell* (Takashi and Yamanaka, 2006); reprinted with permission.)

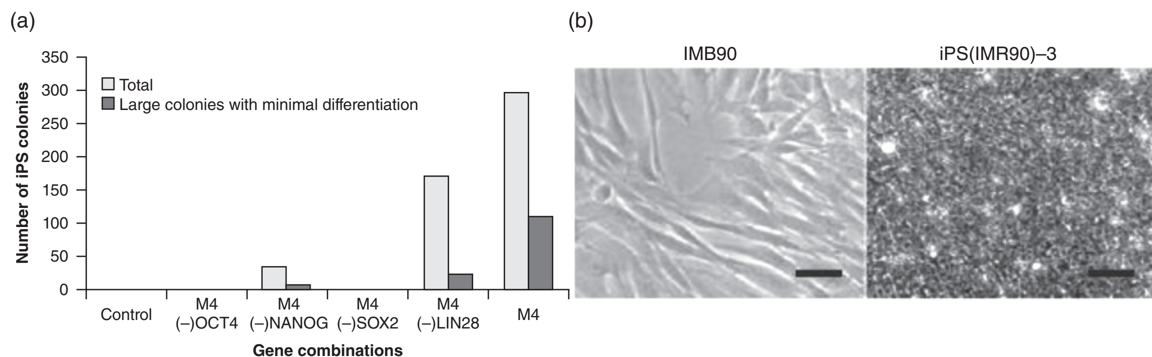


Figure 1.18 Gene combinations driving induced pluripotency reprogramming in human adult somatic cells. (a) Comparison of gene combinations for generating iPS colony numbers and sizes. When individual genes are removed from the mixture (M4) colony numbers and sizes drop. (b) Brightfield images of p18 human somatic cell fibroblasts before induction (left) and p18 iPS cells after induction of pluripotency. (Figure courtesy James Thomson and *Science* (Thomson et al., 1998); reprinted with permission.)

iPS Cells Derived from Keratinocytes

The ability to utilize somatic cells that are readily available as a source for the generation of iPS cells is a critical factor for ultimately generating banks of autologous cell lines to be used for therapy. While skin fibroblasts are accessible, there is perhaps no more readily available somatic cell source than that of a human hair. Painless collection of samples and a virtually endless supply for almost every individual make hair follicles an ideal adult somatic cell source for iPS cell generation. In October 2008, a team of scientists led by Juan Carlos Izpisúa Belmonte, a professor in the Gene Expression Laboratory at the Salk Institute for Biological Studies in La Jolla, California, successfully reprogrammed keratinocytes isolated from human foreskin into iPS cells. In a manner similar to that for the Yamanaka studies mentioned above, iPS was accomplished via retroviral transduction of genes encoding the key transcription factors Oct4, Sox2 Klf4, and c-Myc. Interestingly, the researchers demonstrated that iPS reprogramming of keratinocytes was 100-fold more efficient and at least 2-fold faster than that for fibroblast conversion. The resulting iPS cell population was dubbed **KiPS cells,** and exhibited striking morphological and molecular similarities to hESCs. The researchers also confirmed the cells' ability to differentiate into lineages representing the three primary germ layers. This study was followed by the induction of KiPS cells from a single plucked human hair. To retrieve keratinocytes needed for iPS induction, the hair was cultured in mouse embryonic fibroblast conditioned hES cell medium promoting the proliferation of keratinocytes out of the hair outer root sheath. Following expansion, retroviral transduction was performed in a manner similar to that for foreskin keratinocytes. The resulting KiPS cells resembled those derived from human foreskin keratinocytes and expressed the classical hES cell pluripotency marker **alkaline phosphatase** (Figure 1.19) (Aasen et al., 2008).

iPS Induction Without the Use of Viruses

The utilization of viruses to induce iPS in cells to be used in regenerative medicine applications results in the inherent risk of transformation of these cells toward a cancerous

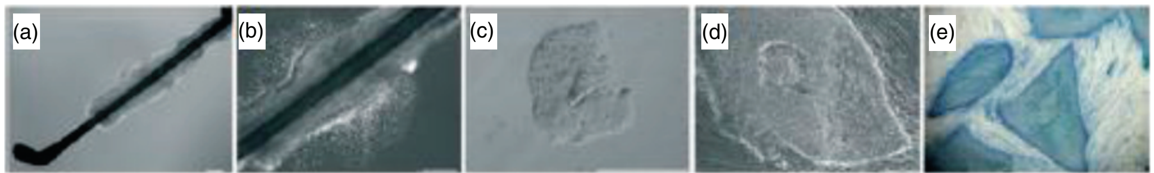


Figure 1.19 Induction of pluripotency in keratinocytes isolated from a single plucked human hair. (a) Portion of follicle cultured in hES medium. (b) Keratinocyte outgrowth from the outer root sheath 5 days after initiation of culture. (c) Colony of hair keratinocytes after reprogramming exhibiting typical hES cell morphology. (d) P1 iPS colony 10 days after picking. (e) High magnification of alkaline phosphatase -stained KiPS colony. (Photos courtesy Juan Carlos Izpisúa Belmonte and *Nature Biotechnology* (Aasen et al., 2008); reprinted with permission.)

phenotype. This is due to the mechanism of viral transduction whereby viral genomic material randomly integrates into the host cell's genome. These random integrations could occur in proto-oncogenes and thus transform the cells. Therefore mechanisms for induction of pluripotency that do not modify the host genome would be of great value and possibly safer to implement. The following sections outline two methods for accomplishing non-viral-mediated induction of pluripotency.

Transposon-Mediated iPS As of 2009 most efforts at inducing adult somatic cells to dedifferentiate and take on pluripotent characteristics had been focused on invasive intracellular viral or plasmid-based introduction of the four key transcription factors: Oct4, Sox2, Klf4, and c-Myc. Strategies involving retroviral, lentiviral, adenoviral, and plasmid transfection had the desired effect of resulting in high levels of genetic material and corresponding protein products present within the cells to promote pluripotency. The utilization of retroviral and lentiviral methods to introduce the key genes relies on stable incorporation into the host cell's genome. This is a major issue and consideration as integration into the wrong loci could promote tumorigenesis. It is only adenoviral and plasmid transfection that represented transitional, non-stable introduction of key genes into the cells. However, an obvious diminished capacity to drive and maintain iPS long-term is a key issue with these methods. This is due to the fact that over time and through multiple cell divisions the concentration of genetic material encoded by the transient presence of adenoviral or plasmid vectors becomes low or even non-existent. In addition, there are no guarantees of transient presence, and molecular incorporation into the host genome of viral genetic products may occur in rare instances. In April 2009, researchers at the Samuel Lunenfeld Research Institute, Mount Sinai Hospital, and Department of Molecular Genetics, University of Toronto, Toronto, Canada implemented a novel non-integrative approach to drive gene expression and the formation of key proteins necessary for iPS induction in mouse somatic cell fibroblasts. They employed a transposon/transposase-based system known as ***piggyback*** **(PB)** which allows for the stable but transient integration of genetic material into the host cell genome and drives the maintenance of its expression long-term. *Piggyback* is host factor-independent, making it suitable for transduction of a variety of somatic cell types. The genomic insertion may be seamlessly, efficiently, and precisely excised at will, thus leaving the iPS cell genetically unaltered following excision. The researchers implemented the *piggyback* system to introduce genetic material encoding Oct4, Sox2, Klf4, and c-Myc into mouse fibroblasts. They subsequently demonstrated clean excision of the integrants and confirmed iPS in whole embryos and adult chimeras (Figure 1.20) (Woltjen et al., 2009).

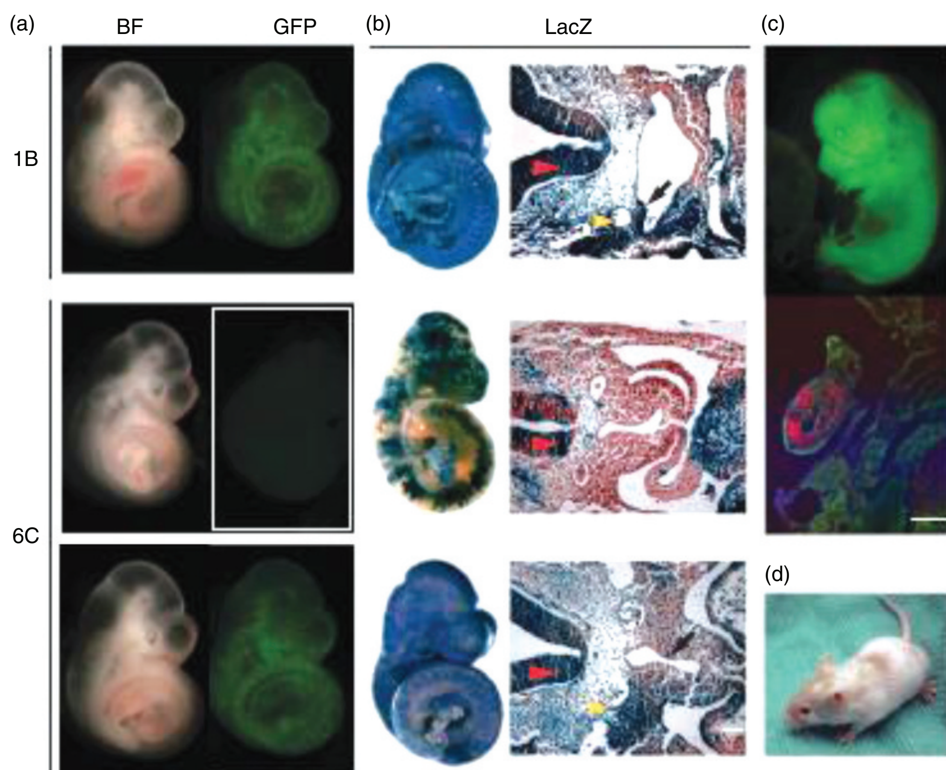


Figure 1.20 *Piggyback* (PB)-mediated factor transposition results in fibroblast reprogrammed to an iPS state. (a) 10.5dpc demonstrating high-percentage contribution of PB GFP labeled iPS cells to the embryo proper. (b) PB iPS cells contribute to all three primary germ layers as assayed by LacZ staining. (c) Tetraploid complementation using iPS cells results in complete derivation of the embryo from iPS cells as assayed by GFP presence. (d) Adult mouse chimera generated by co-culture of PB iPS cells with diploid 8-cell stage albino embryo. (Photos courtesy Knut Woltjen and *Nature* (Woltjen et al., 2009); reprinted with permission.)

Protein-Based iPS Up to 2009 all studies on induced-pluripotency reprogramming involved either the stable or transient introduction of genetic material encoding key factors required for dedifferentiation of somatic cells and promotion of the pluripotent phenotype. The application, for example, of viral-based systems often result in multiple viral integrants within the genome of the host cell, the location of which can, for the most part, not be controlled. As such, one cannot rule out the effect of insertional mutagenesis resulting in tumor-promoting integrants and other unpredictable genetic dysfunctions. From a therapeutic perspective, a system is therefore needed that allows for the safe production of patient-specific stem cells without genetic alteration to produce such cells. In June 2009, a technique was perfected for the introduction of key transcription factor proteins directly into human somatic cell newborn fibroblasts (HNFs). Led by Kwang-Soo Kim, Associate Professor of Psychiatry and Neuroscience at the McLean Hospital, Harvard Medical School, Harvard Stem Cell Institute, Boston, Massachusetts and CHA Stem Cell Institute in Seoul, South Korea, researchers focused

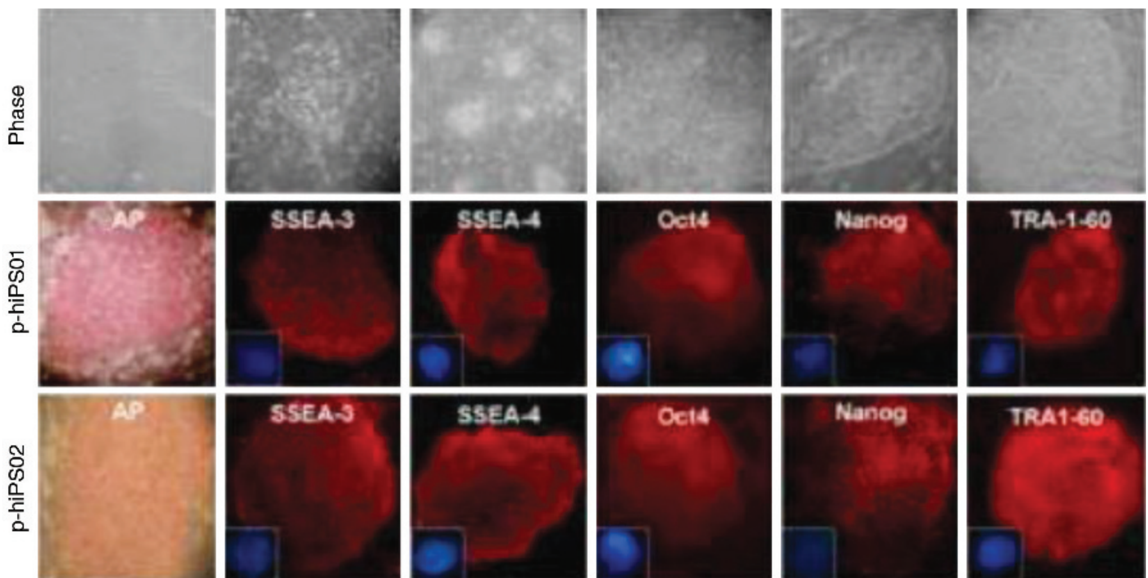


Figure 1.21 Marker expression of protein-induced human iPS cells. Two independent lines were analyzed by immunofluorescence for the classical markers of pluripotency after cycled introduction of CPP-anchored reprogramming factors. Both morphological and marker analysis reveal similarities to iPS lines generated by genetics-based approaches. (Photos courtesy Kwang-Soo Kim and *Cell Stem Cell* (Kim et al., 2009); reprinted with permission.)

on devising a strategy to efficiently introduce the four key reprogramming proteins Oct4, Sox2, Klf4, and c-Myc directly into cells. Proteins and other macromolecules have only a limited ability to cross the cell membrane, thus a technique was needed to drive active transport across the lipid bilayer and into the cytoplasm. Certain proteins and short peptides, referred to as cell penetrating peptides (CPP), have been shown to actively cross the cell membrane, and can carry other macromolecules along with them during this process. Kim's team anchored each of the reprogramming proteins to CPP. After several failures performing single introduction experiments, the researchers attempted repeated protein treatment cycles and observed iPS-like morphology and alkaline phosphatase expression, a key marker of the embryonic stem cell-like phenotype. Dubbed protein-induced human iPS (p-hiPS) cells, they were demonstrated to express all the classical markers of pluripotency and could contribute to the three primary germ layers in murine teratoma studies (Figure 1.21) (Kim et al., 2009). The technique resulted in the induction of pluripotency without the need for viral transduction or plasmid transfection of genetic material encoding these proteins. The authors noted that the process of CPP-anchored protein transduction was inefficient and required further optimization, but this study is an important first step in developing a non-genetics-based approach to pluripotency induction in somatic cells. It opens the door to a safer non-genetics-based alternative to viral transduction or plasmid introduction of iPS induction factors.

THE DISCOVERY OF HUMAN AMNIOTIC STEM CELLS

The derivation of embryonic stem cell lines from human embryos and the induction of pluripotency in adult cells are both technologies that hold much promise in the field of stem cell therapeutics. By creating a source for the generation of terminally differentiated cells of various types, many medical disorders such as diabetes and cardiac hypertrophy could theoretically be addressed. However, a more readily available source of pluripotent stem cells that eliminates the controversy or technical challenges of hES cell derivation/embryo destruction and iPS technology would be advantageous for the treatment of a number of disorders. Sources such as cord blood embryonic-like cells mentioned above eliminate the need to destroy embryos for ES cell derivation or induce pluripotency in tissue culture. They also may provide a readily abundant autologous cell source for some specific desired lineages. In early 2007, a research group led by Anthony Atala, the W.H. Boyce Professor and Director of the Wake Forest Institute for Regenerative Medicine at Wake Forest University School of Medicine in Winston-Salem, North Carolina successfully isolated both human and rodent **amniotic fluid-derived stem (AFS) cells** and demonstrated striking properties inherent in these cells that could make them a valuable source for stem cell therapeutics initiatives. To efficiently isolate the cells from other cell types in the amniotic fluid, the researchers employed **immunoselection**—the isolation of an antigen using antibody specificity—and magnetic isolation to separate cells expressing c-Kit from others in the heterogeneous population. **C-Kit**, also referred to as **CD117**, is a tyrosine kinase cell surface receptor known to be a marker for progenitor and stem cell lineages such as those of the prostate, thymus, and of hematopoietic origin. The isolated cells exhibited striking properties that could make them a valuable cell-based therapeutics source. For example, after more than 250 population doublings, the stem cell lines maintained a normal karyotype and retained long telomeres, a sign of genomic stability. In addition, the cell lines could be directed to differentiate into multiple adult lineages including those of the adipogenic, osteogenic, myogenic, endothelial, neuronal, and hepatic phenotypes; thus they were classified as multipotent in nature (Figure 1.22) (De Coppi et al., 2007) (See Chapter 2).

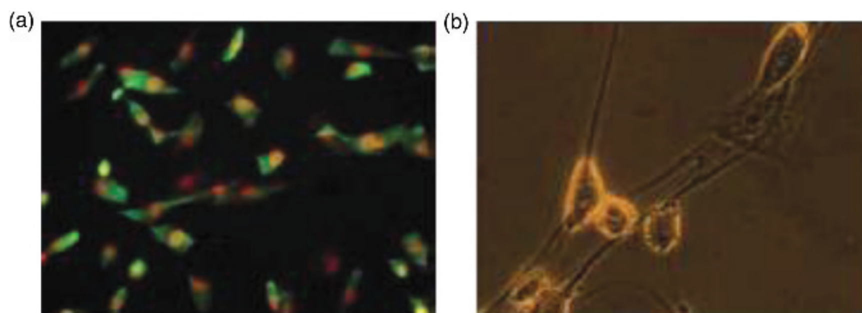


Figure 1.22 Amniotic fluid-derived stem cell differentiation. (a) Immunofluorescence staining for nestin; (b) phase contrast microscopy of dopaminergic neurons. Both were directed to differentiate from AFS cells. (Photos courtesy Anthony Atala and *Nature Biotechnology* (De Coppi et al., 2007); reprinted with permission.)

HUMAN EMBRYONIC STEM CELLS GENERATED WITHOUT EMBRYO DESTRUCTION

The destruction of human embryos for the derivation of embryonic stem cells is perhaps the most controversial aspect of hES cell research. Although embryonic stem cell isolation typically occurs at the very earliest stages of embryonic development, given that it is carried out post fertilization, many feel that this is truly a destruction of human life. This poses ethical concerns and in fact is formally outlawed in a number of countries. Specifically in the United States, federal funding may not be utilized for research that involves the destruction of human embryos. As such, a great deal of effort has been focused on deriving embryonic stem cells or creating embryonic-like stem cells without the need for embryo destruction. Examples of this include the application of iPS technology, which generates embryonic-like stem cells as discussed above, but these are not true ES cells. For the derivation of authentic hESCs, to avoid controversy and, some would say, for the preservation of human life, a method is needed that does not destroy nor harm the developing human embryo. In January of 2008, scientists at Advanced Cell Technology in Worcester, Massachusetts published a scientific article outlining that they had accomplished this by implementing a technique similar to **preimplantation genetic diagnosis (PGD)**, which is an embryonic manipulation procedure employed to profile the genetics of an embryo prior to implantation. In PGD, controlled ovarian stimulation is employed for the release of oocytes, which are subsequently fertilized, most often by intracytoplasmic sperm injection. Cells from embryos are isolated and biopsied at specific stages of development and assessed for genetic defects that might result in a termination of pregnancy. The stage and mechanism for cell isolation in most cases does not damage the developing embryo. Researchers at Advanced Cell Technology isolated **blastomeres**, which are defined as cells resulting from the cleavage of a fertilized ovum during early embryonic development, from fertilized eggs (8 cell stage; 1 or 2 blastomeres isolated per egg) and cultured them via a modified PGD approach which was designed to reconstitute the "inner cell mass niche". To accomplish this goal, a microdrop co-culture system was implemented in the presence of hESCs, which are thought to provide support and secrete factors promoting pluripotency. To distinguish between hES cells used for co-culture and blastomeres, the hES cells were labeled by stable transfection of a gene encoding green fluorescent protein (GFP). Medium was supplemented with laminin and fibronectin and the cultures transferred to a mouse embryonic fibroblast layer for expansion and characterization. This allowed for an improvement in successful ES cell derivation rates, which were comparable to that of derivations utilizing whole embryos. Five independent lines were derived that maintained normal karyotypes and marker expression after more than 50 passages. In addition, it was shown that the lines could differentiate into lineages representing all three germ layers (Figure 1.23) (Chung et al., 2008).

HUMAN CLONING

In the realm of cell biology, **cloning** can be defined as the identical reproduction of another organism at the molecular and cellular level. From the perspective of humans, it is highly controversial and raises ethical issues on individual identity and even on religious grounds. In addition, serious safety concerns are associated with human cloning. In 2008, despite the fact that significant progress had been made with respect to both iPS reprogramming and

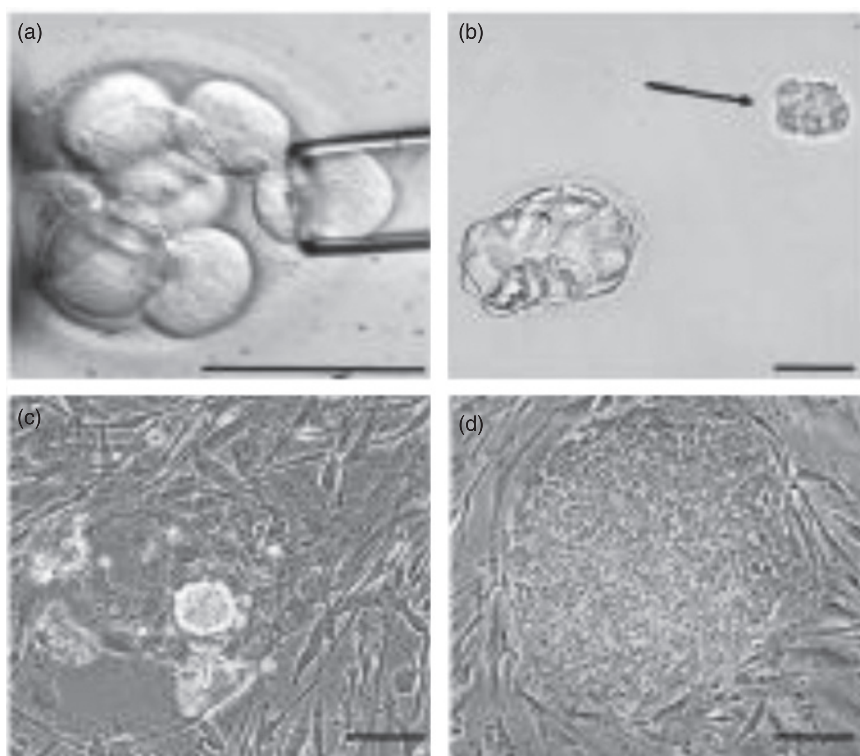


Figure 1.23 Derivation of hES cells without embryo destruction. Brightfield microscopy of (a) Actual biopsy of a blastomere from a human embryo. (b) Biopsied blastomere (denoted by arrow) growing alongside the embryo from which it was derived. (c) Outgrowth of a blastomere culture on mouse embryonic fibroblasts (MEFs). (d) Colony of blastomere-derived human embryonic stem cells. (Photos courtesy Robert Lanza and *Cell Stem Cell* (Chung et al., 2008); reprinted with permission.)

hESC isolation without damage to the embryo, many research groups remained focused on SCNT, also referred to as cloning, as an alternative way to generate hESCs. Andrew J. French and colleagues at Stemagen Corporation and the Reproductive Sciences Center in La Jolla, California published a research article in the journal *Stem Cells Express* that delineated the first recorded example of successful cloning of human blastocysts through SCNT of nuclei from adult fibroblasts into enucleated oocytes. In this study, mature oocytes obtained from donors were enucleated either via extrusion or aspiration and nuclei transferred into these oocytes from adult male fibroblasts exhibiting normal karyotypes. The team observed high rates of **pronucleus** formation (66%), cleavage (47%), and blastocyst development (23%) following SCNT. In addition, the morphology of the embryos during early development post SCNT appeared normal (Figure 1.24) (French et al., 2008). Perhaps in order to deflect from the controversial field of embryonic stem cell research, French's team coined the term **nuclear transfer stem cells (NTSC)**, which are defined as stem cells derived from a SCNT-generated embryo, to distinguish stem cells created and derived through SCNT procedures from other sources. The cloning of humans remains a highly controversial topic and raises many ethical, religious, and moral issues that are beyond the scope of this text.

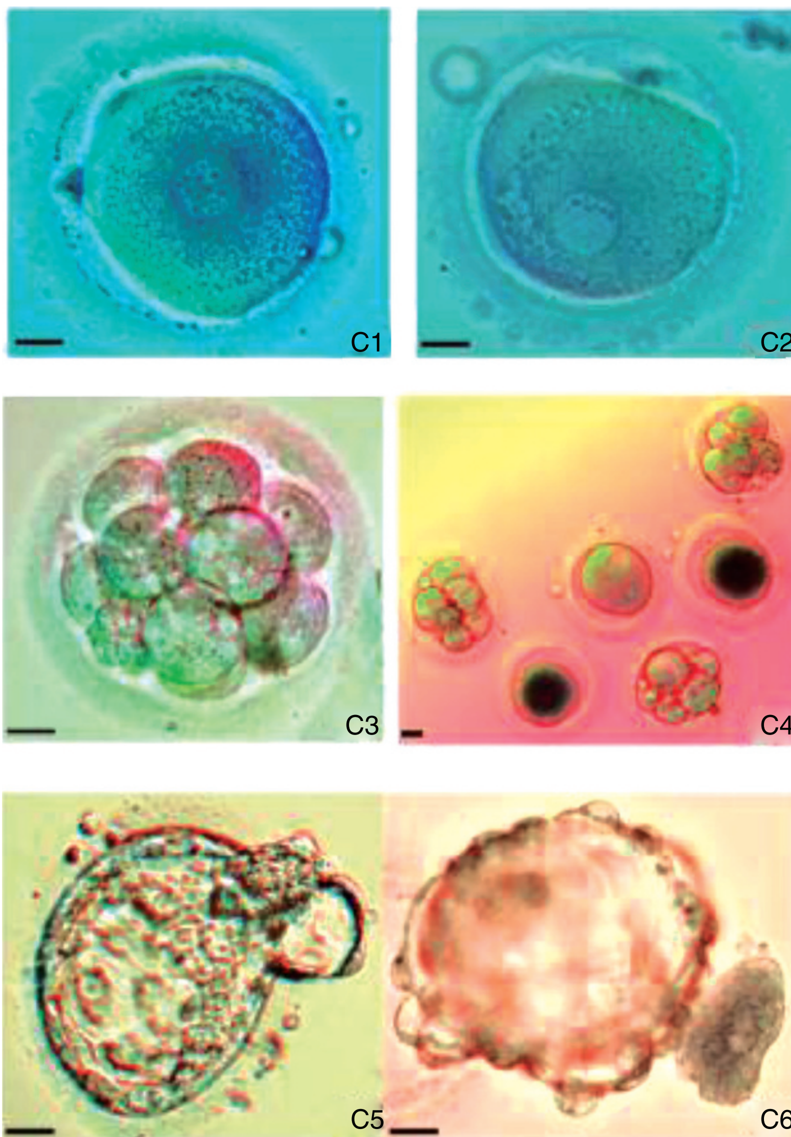


Figure 1.24 Development of cloned human embryos. Brightfield images following somatic cell nuclear transfer of an adult fibroblast nucleus into an enucleated oocyte. (C1) Pronuclear phase. (C2) Pronuclear phase (separate donor fibroblast donor). (C3) Late day 3. (C4) Late day 3 (separate fibroblast donor). (C5) Late day 5. (C6) Early day 6 (separate fibroblast donor). (Images courtesy Andrew J. French and *Stem Cells Express* (French et al., 2008); reprinted with permission.)

MESENCHYMAL STEM CELL-DERIVED HUMAN KNEE CARTILAGE

Regenerative medicine can be defined as the ability to regenerate tissue rather than surgically extracting or altering that tissue. It can be classified into three unique categories, including platelet augmentation, recombinant growth factor amplification, and stem cell isolates. As platelet augmentation methods, including platelet-rich plasma (PRP) implantation, and growth factor injection are beyond the scope of this text, the focus of this initial overview section will be on the application of stem cell isolates for cartilage regeneration as it is in this area where stem cell-based regenerative medicine has had the largest impact clinically. For example, mesenchymal stem cells (MSCs)

TABLE 1.1 Cartilage volume analysis after mesenchymal stem cell injection. Measurements were taken in mm^3.

Image	Area of measurement	Volume ($n = 3$)	STDEV	SE	Change from pre-injection (%)
Pre-injection	Cartilage surface	4,020	12.1	6.99	
	Meniscus	5,178	164.57	95.13	
1 month	Cartilage surface	4,924	149.01	86.13	22.49
	Meniscus	5,647	453.57	262.18	9.06
3 month	Cartilage surface	4,795	113.5	65.61	19.28
	Meniscus	6,661	146.47	84.67	28.64

Source: Centeno et al., 2008. Reproduced with permission from C. Centeno.

(defined in Chapter 4) have been demonstrated to differentiate into bone marrow, as well as synovial and adipose tissues. In June 2008, researchers at Regenerative Sciences, Inc. and Centeno-Schultz Clinic in Westminster, Colorado conducted a clinical study in which mesenchymal stem cells were isolated from the iliac crest of patients, cultured, and subsequently reintroduced **autologously** (transplant from one part of the body into another of the same patient) into the subject's knee to combat degenerative joint disease previously diagnosed by magnetic resonance imaging (MRI). The goal of the study was to drive the growth and development of new cartilage in the degenerated area. To prepare the MSCs for implantation, whole bone marrow was isolated and centrifuged for removal of red blood cells. Plasma was subsequently removed and nucleated cells were cultured for expansion purposes through five passages. **Percutaneous** (under the skin) implantation of cultured, expanded MSCs was performed in an autologous fashion in combination with dexamethasone to induce differentiation of the stem cells into cartilage precursors and ultimately functional cartilage. Table 1.1 outlines the results of this study, revealing considerable new cartilage surface and meniscus growth 1 month and 3 months post injection. This is the first published study of successful cartilage regeneration in a human knee via autologous stem cell therapy (Centeno et al., 2008).

THE FIRST CLINICAL TRIAL USING HUMAN EMBRYONIC STEM CELLS

2010 was a pivotal year for embryonic stem cell research at the clinical level with the launch of a clinical trial utilizing an embryonic stem cell derived therapy termed GRNOPC1 by **Geron Corporation**. Geron is a biotechnology company based in Menlo Park, California that has developed proprietary technologies for the growth, maintenance, and scaling of hESCs and other cell types for therapeutic purposes. Spinal cord injury is among the most devastating medical problems, and affects

over 250,000 individuals in the United States alone. It is both painful and debilitative, causing immeasurable suffering for those unfortunate enough to experience it. As of this publication, Geron has spent a total of $170M USD developing a stem cell treatment platform for spinal cord injury. The company utilized feeder- and serum-free fully defined growth conditions to culture and propagate a variety of stem cell-based therapeutics. The defined growth environment coupled with an absence of any animal components has enabled Geron to obtain approval by the Food and Drug Administration (FDA) for the initiation of a clinical trial of an embryonic stem cell-based platform to treat spinal cord injuries. Initial findings from the clinical study, which focused on the application of ES cell-derived oligodendrocytes progenitor cells (the OPC in GRNOPC1), demonstrated high tolerance without any serious side effects. Unfortunately, the company halted the clinical trial as of November 2011, citing the need to conserve funds. At the time of this publication, Geron was actively seeking partners who “have the technical and financial resources to advance its stem cell programs.” It is clear that studies like this must proceed if stem cell-based therapeutics are to become a reality. This and other clinical trials implementation a variety of different stem cell types are discussed in more detail in Chapter 8.

MITOCHONDRIAL DNA: A BARRIER TO AUTOLOGOUS CELL THERAPEUTICS

Much of the research on the generation of embryonic stem cells from SCNT or the production of iPS cells has been based on the premise that by using host nuclear material immune system rejection of the resulting cells would not be an issue post transplantation. Yet it must be noted that these cells are not 100% identical to that of the donor host. Specifically, the **mitochondria**, which are defined as spherical or elongated organelles in the cytoplasm of mostly eukaryotic cells containing genetic material and many enzymes important for cell metabolism, are derived from donor ES cells or oocytes in SCNT. The genetic material of mitochondria (mtDNA) has accumulated mutations over the life of the cell, which results in a unique and potentially lethal difference in the cellular makeup compared to that of the host. In the case of iPS cells generated from host donor cells also have accumulated mitochondrial DNA mutations throughout the aging process. These mutations result in desired differentiated lineages that are different from the host donor, which may contribute to immune rejection by the host recipient of any derived cell therapy. Researchers at the Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan studied the effects of **allogeneically -** (taken from different individuals of the same species) introduced mtDNA into various mouse strains. These cells shared the same nuclear genetic material and background, but differed in mtDNA makeup. Theoretically, since the nuclear material from these cells was the same as the host strain, no immune response should have been observed. However, the researchers observed that transplants with mtDNA from the same murine strain as the host strain were rejected, most likely due to inherent mutations (Figure 1.25) (Ishikawa et al., 2010). Interestingly, it was confirmed that the rejection was due to innate rather than acquired immune response. **Innate immunity** is naturally or inherently present and is not due to sensitization of the immune system by an antigen. Thus, differences in mtDNA between a donor cell and host recipient for either SCNT- or iPS-based cell therapeutics will be a factor in immunorejection, and must be addressed for purposes of safety.

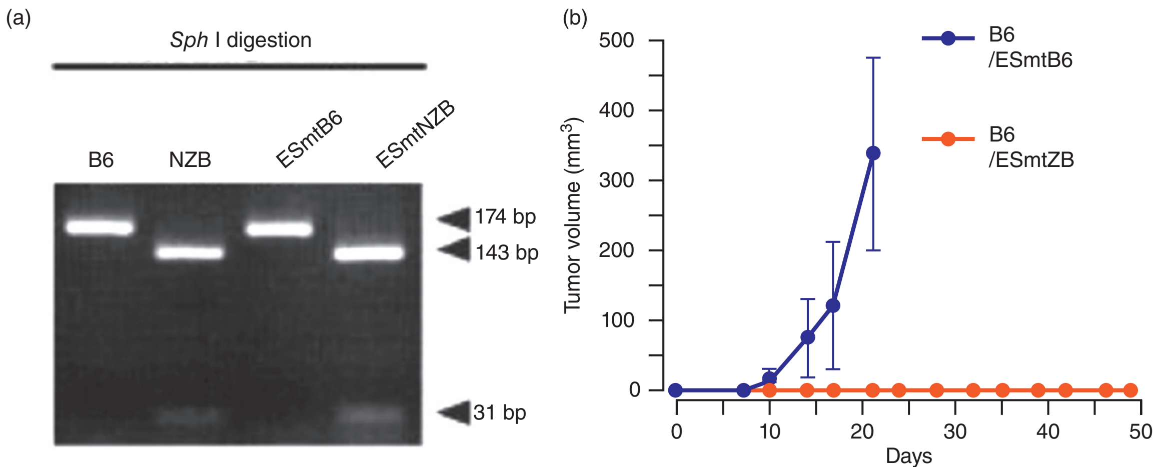


Figure 1.25 Rejection of cells containing allogeneic mtDNA by host mice. (a) Confirmation of mtDNA genotypes by PCR. (b) Analysis of tumor formation size after ES inoculation into host mice. The blue line demonstrates significant tumor growth in allogeneic transplants of B6 mtDNA with a B6 host. The red line reveals suppressed tumor growth in allogeneic transplants of NZB strain mitochondrial DNA with a B6 host suggesting mutations in the mtDNA trigger host rejection due to the innate immune system. (Figures courtesy Jun-Ichi Hayashi and the *Journal of Experimental Medicine* (Ishikawa et al., 2010); reprinted with permission.)

INDUCED PLURIPOTENCY AND THE POTENTIAL TO SAVE ENDANGERED SPECIES

The development and application of technologies for the production of pluripotent stem cells is not just useful in the therapeutic realm. Manufacturing cells capable of producing multiple differentiated lineages or even entire organisms has enormous implications in zoology. Species preservation has been a top priority for zoologists and conservation biologists for literally hundreds of years. In many examples of highly endangered species there are simply too few animals capable of the reproductive capacity necessary to maintain species numbers. In other cases the species

has been declared officially extinct, with no known surviving examples. Yet in both cases SCNT and iPS technologies may allow for a rescue and perhaps even a reintroduction of the species into the ecosystem. In September 2011, Jeanne Loring, Professor and Director of the Center for Regenerative Medicine at the Scripps Research Institute in La Jolla, California, and her team focused their studies on the induction of pluripotency in adult somatic cells to address the problem of species endangerment and extinction. Specifically, they focused on two endangered species: a primate, the drill, ***Mandrillus leucophaeus*** and the nearly extinct northern white rhinoceros (NWR), ***Ceratotherium simum cottoni***. The drill is considered one of the most endangered species on the African continent and its numbers have drastically declined over the past 20 years due to both the destruction of its native habitat and illegal poaching. Current captive populations of drills are sustained by small reproductive colonies, which poses a considerable risk of inbreeding and therefore genetic issues for the entire population. The horns of the northern white rhinoceros make it a target for hunting and illegal poaching—only seven confirmed living rhinos exist today. The goal of these studies was to generate iPS cells from frozen somatic cell fibroblasts corresponding to each of these species, which might later be utilized to generate fully mature, adults capable of reproduction. To accomplish this, the Loring team retrovirally transduced drill and northern white rhinoceros fibroblasts with human genes encoding Oct4, Klf4, Sox2, and c-Myc. Frozen fibroblast samples were obtained from the Frozen Zoo of the San Diego Zoo Institute for Conservation Research. Following thaw, expansion, and transduction of the fibroblasts, putative reprogrammed lines were initially selected based upon morphological similarities to embryonic stem cells and iPS cells obtained from other species. Four and three independent, clonal iPS lines were derived from the drill and northern white rhinoceros, respectively, and further characterized for pluripotency properties. Figure 1.26 outlines the characterization of the northern white rhinoceros iPS cells illustrating ES cell-like morphology, normal karyotypes, expression of markers and the ability to generate lineages representative of the three primary germ layers (Ben-Nun et al., 2011)). In addition, the researchers performed **glycomic profiling**, which is the analysis of the complete repertoire of glycans and glycoconjugates that cells produce under specified conditions, of each iPS line. The profiling revealed a clustering and close correlation of the drill and NWR iPS lines with those from other species. While complementing SCNT, these studies set the stage for the possibility of a reintroduction of genetic material into endangered species breeding populations for purposes of species survival and the elimination of extinction risks. In addition, it may provide an avenue for the rescue of already extinct species should viable cell samples for these species exist.

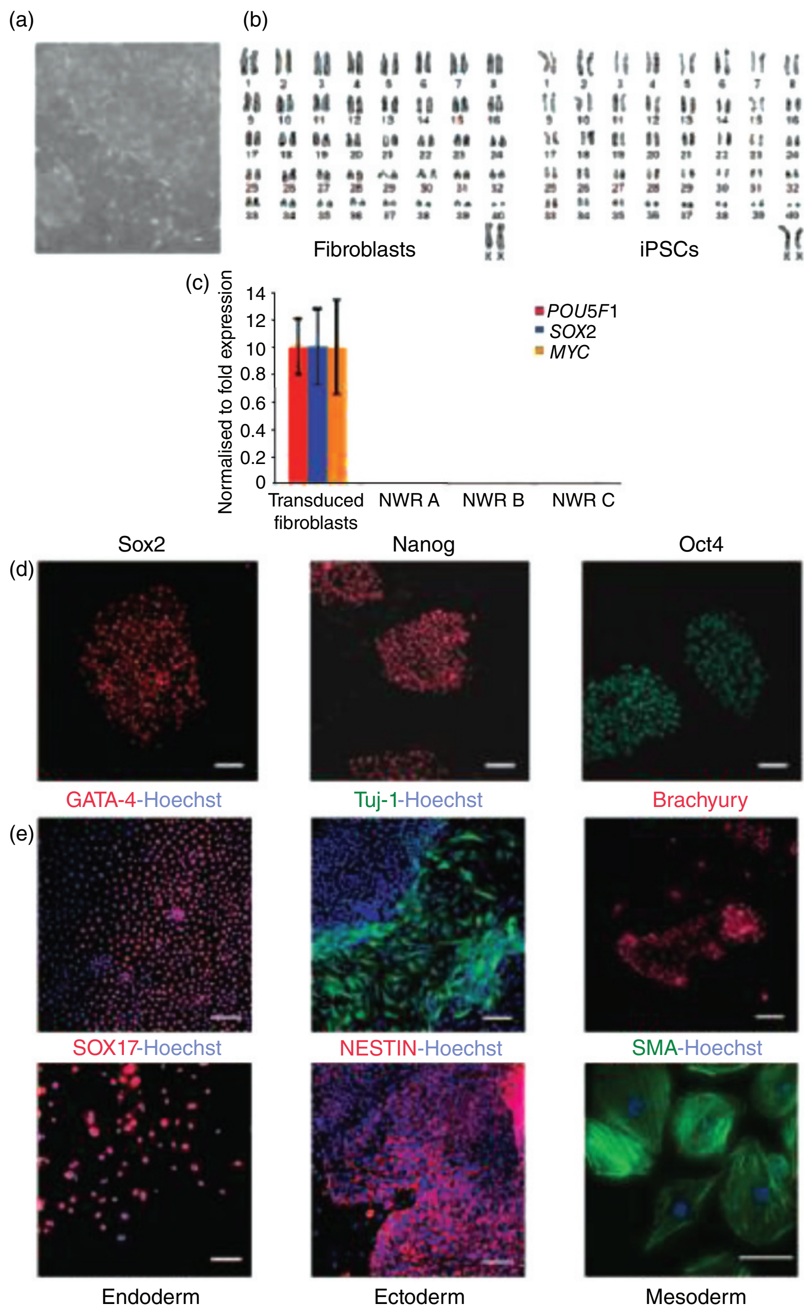


Figure 1.26 Characterization of northern white rhinoceros iPS cells. (a) iPS cell morphology. (b) Normal karyotype of pre-programmed fibroblasts and resulting iPS cells. (c) Quantitative RT-PCR of three of the four key genes involved in reprogramming, (d) Immunocytochemistry of pluripotency markers. (e) Immunocytochemistry of markers for the three primary germ layers. (Data and photos courtesy Jeanne Loring and *Nature Methods* (Ben-Nun et al., 2011); reprinted with permission.)

CHAPTER SUMMARY

Early Studies

1. The existence of stem cells has been contemplated for over 100 years, their presence first recognized by Alexander A. Maximow in his theory of hematopoiesis.
2. Ernest McCulloch and James Till demonstrated the existence of bone marrow-derived stem cells.
3. Joseph Altman and Gopal Das were the first to demonstrate neurogenesis in the adult brain, going against the “no new neurons” central dogma.
4. Robert Alan Good performed the first successful bone marrow transplant.

Hematopoietic Stem Cell Discovery

1. Gregor Prindull and his colleagues discovered the presence of HSCs (HSCs) in human umbilical cord blood.
2. HSCs can form myelocytic CFUs in tissue culture.

Mouse Embryonic Stem Cells

1. Gail R. Martin and Sir Martin Evans simultaneously and independently discovered mouse embryonic stem cells.
2. Mouse ES cells have been pivotal in the development of rodent-based gene targeting technologies.
3. Gail Martin used conditioned medium to derive and culture mouse ES cells.

Successful Neurosphere Culture

1. Brent A. Reynolds and Samuel Weiss were the first to isolate neural stem cells from the adult brain.
2. Constance Cepko generated a v-myc transformed neural stem cell line that could propagate in tissue culture and colonize the adult brain.
3. LacZ staining allows for tracing of individual cell fate.

The Discovery of Cancer Stem Cells

1. Cancer cells proliferate almost indefinitely.
2. Multiple mutations result in stem cell-like properties in CSCs.
3. John Dick identified a subpopulation of cells in a human AML sample that originated from a primitive HSC.

Human Embryonic Stem Cell Discovery

1. James A. Thomson discovered hESCs.
2. The H9 hES cell line is perhaps the most popular and widely studied of the original hES cell isolates given its stable karyotype over extended passages and long periods of cell culture.

3. Telomere lengths are an indication of a cell's lifespan.
4. hES cells may secrete factors that keep neighboring hES cells undifferentiated.
5. Mechanical dissociation of hES cell clumps is preferable to enzymatic digestion for reducing stress and differentiation of the cells.
6. Serious ethical and moral issues have surrounded the derivation and study of hESCs.

Stem Cells and Cloning

1. Sir Ian Wilmut successfully cloned the first mammal, Dolly the sheep.
2. Cloning utilizing embryonic stem cells could allow for the generation of a virtually limitless supply of stem cells that are genetically identical, thus providing an extremely valuable therapeutic or drug discovery platform, as the population of cells would be genetically and phenotypically of an identical origin.
3. Cloned adult somatic cells still have a composition of genetic material that has undergone the rigors of environmental influence and the aging process that so significantly damages that of adult cells.
4. Jose B. Cibelli and Advanced Cell Technology, Inc. created the world's first human cloned eggs via parthenogenesis and SCNT.

Cord Blood Embryonic-Like Stem Cells—An Alternative to ES and Adult Stem Cells

1. Colin P. McGuckin isolated a population of stem cells, termed CBEs, from human umbilical cord blood that bore striking resemblance to hES cells and possessed the capacity to differentiate into a variety of lineages.
2. CBEs may act as a possible autologous stem cell-based therapeutic platform for a number of anomalies.

Breakthrough in Spinal Cord Injury Repair

1. The adult CNS has a very limited capacity to regenerate itself, and, as a consequence, injuries to the spinal cord often result in partial or complete irreversible paralysis.
2. Hans S. Keirstead demonstrated an enhancement in myelination and improvement of locomotor recovery in injured rats upon intraspinal cord transplantation of hESC-derived oligodendrocyte progenitor cells (OPCs).

The Generation of iPS Cells

1. Adult differentiated cells may be driven to lose their morphological and molecular identities and transform into other cell types.
2. In an artificial setting, differentiated cells can be reprogrammed to an embryonic-like state.
3. Shinya Yamanaka and James Thomson were the first to induce adult mouse fibroblast cells to dedifferentiate into stem cells and become pluripotent.

4. The factors Oct 3/4, Sox2, c-Myc, and Klf4 were demonstrated to be necessary and sufficient for fibroblast dedifferentiation and reprogramming.
5. iPS cells can contribute to the three primary germ layers of the host embryos.

The Discovery of Human Amniotic Stem Cells

1. Anthony Atala successfully isolated both human and rodent amniotic fluid-derived stem (AFS) cells and demonstrated striking properties inherent in these cells that could make them a valuable source for stem cell therapeutics initiatives.
2. To efficiently isolate the cells from other cell types in the amniotic fluid the researchers employed immunoselection for CD117.
3. Human amniotic stem cells can maintain a normal karyotype over many population doublings and differentiate into a variety of lineages.

Generation of Human iPS Cells

1. Human iPS cells could provide a valuable source for autologous cell therapy to treat a variety of diseases and disorders.
2. Shinya Yamanaka and James Thomson were the first to generate human iPS cells, but utilized genes encoding different transcription factors to accomplish this.
3. The transcription factor Nanog was shown to improve iPS clonal recovery, resulting in an over 200-fold increase in reprogramming efficiency.

Human Embryonic Stem Cells Generated Without Embryo Destruction

1. The destruction of human embryos for the derivation of embryonic stem cells is perhaps the most controversial aspect of hES cell research.
2. The company Advanced Cell Technology generated embryonic stem cells without embryonic destruction using a modified version of preimplantation genetic diagnosis (PGD) to remove single blastomeres.

Human Cloning

1. Andrew J. French at Stemagen Corporation reported the first example of successful cloning of human blastocysts through SCNT of nuclei from adult fibroblasts into enucleated oocytes.
2. The cloning of humans remains a highly controversial topic and raises many ethical, religious and moral issues that are beyond the scope of this text.

Mesenchymal Stem Cell-Derived Human Knee Cartilage

1. Regenerative medicine can be classified into three unique categories including platelet augmentation, recombinant growth factor amplification and stem cell isolates.
2. Mesenchymal stem cells (MSCs) have been demonstrated to differentiate into bone marrow as well as synovial and adipose tissues.

3. Researchers at Regenerative Sciences, Inc. and Centeno-Schultz Clinic conducted the first successful study of cartilage regeneration in a human knee via autologous stem cell therapy.

iPS Cells Derived from Keratinocytes

1. Painless collection of samples and a virtually endless supply for almost every individual make hair follicles an ideal adult somatic cell source for iPS cell generation.
2. Juan Carlos Izpisúa Belmonte successfully reprogrammed keratinocytes isolated from both human foreskin and hair follicles into iPS cells.

iPS Induction Without the Use of Viruses

1. The utilization of retroviral and lentiviral methods to introduce the key genes relies on stable incorporation into the host cell's genome.
2. Integration into the wrong loci could promote tumorigenesis.
3. Researchers at the Samuel Lunenfeld Research Institute implemented the *piggyback* system to introduce genetic material encoding Oct4, Sox2, Klf4 and c-Myc into mouse fibroblasts that could later be cleanly and precisely excised.

Protein-Based iPS Reprogramming

1. A system is needed that allows for the safe production of patient-specific stem cells without genetic alteration to produce such cells.
2. Kwang Soo Kim and colleagues at the Harvard Stem Cell Institute devised a strategy to efficiently introduce the four key reprogramming proteins Oct4, Sox2, Klf4, and c-Myc directly into cells using cell penetrating peptides (CPP).
3. Protein-induced human iPS (p-hiPS) cells were confirmed to be pluripotent and had no genetic alterations due to protein introduction.

The First Clinical Trial Using Human Embryonic Stem Cells

1. Geron Corporation developed a stem cell therapy called GRNOPC1 to aid in spinal cord injury.
2. Geron used feeder- and serum-free fully defined growth conditions to culture and propagate a variety of stem cell-based therapeutics.
3. Despite solid initial clinical trial findings, Geron halted the trials in November 2011.

Mitochondrial DNA: A Barrier to Autologous Cell Therapeutics

1. Mitochondria are derived from donor ES cells or oocytes in SCNT, carrying with them unique genetic material that has acquired mutations over time.
2. Age-related mitochondrial mutations may contribute to immune rejection by the host recipient of any derived cell therapeutic.
3. Researchers at the University of Tsukuba observed that transplants with mtDNA from the same murine strain as the host strain were rejected—most likely due to inherent mutations—and this rejection was due to innate immunity.

Induced Pluripotency and the Potential to Save Endangered Species

1. The ability to produce cells that may be capable of producing multiple differentiated lineages or perhaps even entire organisms has enormous implications for species preservation.
2. Jeanne Loring and her team at the Scripps Research Institute generated drill and northern white rhinoceros iPS cells through retroviral transduction of the human genes encoding Oct4, Klf4, Sox2, and c-Myc into corresponding fibroblasts from each species.
3. iPS technology may provide an avenue for the rescue of already extinct species should viable cell samples for these species exist.

KEY TERMS

(Key terms are in the order as they appear in the text.)

- **Stem cells—**biological cells capable of self-renewal and that have the capacity to differentiate into a variety of cell types; present within most, if not all, multi-cell organisms.
- **Theory of hematopoiesis—**all blood cellular components are derived from a common precursor stem cell.
- **Spleen colonies—**visible lumps present within irradiated immune-deficient mice due to the injection of bone marrow cells.
- **Neurogenesis—**the generation of neurons and glial cells.
- **Microneurons—**small-caliber mature interneurons.
- **"No new neurons" central dogma—**Ramon and Cajal's hypothesis that mammals are born with a preset number of neurons and that there is no growth or generation of new neurons after birth.
- **Severe combined immunodeficiency syndrome (SCID)—**a genetic disorder in which both B and T cells of the immune system are severely compromised due to a defect in one of several possible genes.
- **Colony forming units (CFUs)—**cells that have the ability to divide and form a clonal colony in tissue culture.
- **Myelocytes—**cells of granulocytic origin present in bone marrow that proliferate during fetal development and accumulate in the cord blood of newborn infants.
- **Conditioned medium—**cell culture media prepared in the presence of live cells that secrete key growth factors needed for cell survival.
- **Subventricular zone—**a paired brain structure situated throughout the lateral walls of the lateral ventricles.
- **v-myc—the viral homolog of c-myc which is capable of cellular transformation.**
- **Process (in neural cells)—**either axon or dendrite-like protrusions.
- **β-galactosidase—**an enzyme that catalyzes the hydrolysis of β-galactosides into monosaccharides.
- **Cancer—**abnormal growth of cells that tend to proliferate in an uncontrolled way and, in some cases, to metastasize.
- **Metastasis—**the spread of cancer cells to other parts of the body.
- **Cleavage-stage—**2-, 4-, 8-, and 16-cell embryonic stages containing blastomeres.
- **Karyotype—**the number and appearance of chromosomes in the nucleus of a cell.

- **H9 cell line—**the most popular and widely studied of the original hES cell isolates given its stable karyotype over extended passages and long periods of cell culture.
- **Telomerase—**an enzymatic ribonucleoprotein which functions to add telomeric repeats to chromosomal ends and plays a critical role in extending the lifespan of a cell.
- **Cloning—**the process of creating genetically identical individuals from a single donor.
- **Somatic cells—**any biological cell forming the body of an organism other than a germ cell, gamete, gametocyte, or undifferentiated stem cell.
- S**omatic cell nuclear transfer (SCNT)—**a technique for creating a clonal embryo by combining an ovum (egg) with a donor nucleus.
- **Reproductive cloning—**propagation in tissue culture of an SCNT-derived cell or its allowance to develop into an embryo and be transplanted into a surrogate mother.
- **Therapeutic cloning—**reproduction of a genetically identical cell for purposes of cell therapy such as cellular replacement.
- **Dolly the sheep—**the first mammal and sheep to be cloned by combining the nucleus of a mammary cell from a Fin-Dorset sheep with an enucleated egg from a Scottish Blackface ewe.
- **Parthenogenesis—**a form of asexual reproduction in females where no fertilization from a male is required in order to reproduce.
- **Pronucleus—**the nucleus of an egg cell during fertilization.
- **Blastocoele—**a cleavage cavity or segmentation cavity present in a developing embryo.
- **Cumulus cells—**specialized granulosa cells that surround and nourish a developing egg.
- **Demyelination—**loss of the myelin sheath insulting the nerves.
- **Oligodendrocyte progenitor cells (OPCs)—**immature myelin-producing stem cells.
- **Transdifferentiation—**a non-stem cell transforming into a different cell type, or when a differentiated stem cell generates other cell types outside of its normal realm of multipotency.
- **Dedifferentiation—**reprogramming of cells to an embryonic-like state.
- **KiPS Cells** - keratinocyte-derived induced pluripotency (iPS) cells.
- **Alkaline phosphatase—**an enzyme that is considered a classical pluripotency marker for embryonic stem cells derived from a variety of species.
- ***piggyback*** **(PB)—**a host factor-independent transposon/transposase system that allows for the stable but transient integration of genetic material into the host cell genome and drives the maintenance of its expression long-term.
- **Cell penetrating peptides (CPP)—**short peptides that actively cross the cell membrane and can carry other macromolecules along with them during this process.
- **Protein-induced human iPS (p-hiPS)—**iPS cells generated using repeated protein treatment cycles.
- **Amniotic fluid stem (AFS) cells—**multipotent stem cells derived from amniotic fluid.
- **Immunoselection—**the isolation of an antigen using antibody specificity.
- **C-Kit (CD117)—**a tyrosine kinase cell surface receptor known to be a marker for progenitor and stem cell lineages such as those of the prostate, thymus, and of hematopoietic origin.
- **Preimplantation genetic diagnosis (PGD)—**an embryonic manipulation procedure employed to profile the genetics of an embryo prior to implantation.
- **Blastomeres—**cells resulting from the cleavage of a fertilized ovum during early embryonic development, from fertilized eggs (8 cell stage; 1 or 2 blastomeres isolated per egg).

- **Cloning—**the identical reproduction of another organism at the molecular and cellular level.
- **Pronucleus—**the nucleus of a sperm or egg cell during fertilization.
- **Nuclear transfer stem cells (NTSC)—**stem cells derived from a SCNT-generated embryo.
- **Regenerative medicine—**the ability to regenerate tissue rather than surgically extracting or altering that tissue.
- **Autologous—**transplant from one part of the body into another of the same patient.
- **Percutaneous—**under the skin.
- **Geron Corporation—**a biotechnology company based in Menlo Park, California that has developed proprietary technologies for the growth, maintenance, and scaling of human embryonic stem cells and other cell types for therapeutic purposes.
- **Mitochondria—**spherical or elongated organelles in the cytoplasm of nearly all eukaryotic cells containing genetic material and many enzymes important for cell metabolism.
- **Allogeneic—**taken from different individuals of the same species.
- **Innate immunity—**naturally or inherently present and is not due to sensitization of the immune system by an antigen.
- ***Mandrillus leucophaeus*—**an endangered primate.
- ***Ceratotherium simum cottoni*—**the northern white rhinoceros.
- **Glycomic profiling—**the analysis of the complete repertoire of glycans and glycoconjugates that cells produce under specified conditions.

REVIEW QUESTIONS

(Answers to select review questions can be found at www.stemcelltextbook.com.)

1. Who first coined the term “stem cell” and what was his background?
2. What happened in 1963 that changed stem cell research forever?
3. What contribution did Joseph Altman and Gopal Das make to the field of stem cell research?
4. Describe the transplant that occurred in 1968 that set the stage for stem cell therapeutics.
5. How were hematopoietic stem cells discovered?
6. How were mouse embryonic stem cells derived and what researchers independently contributed to this discovery?
7. From what region of the brain were the first neural progenitor and stem cells isolated and who accomplished this?
8. Describe a method for tagging stem cells to monitor their *in vivo* presence?
9. What led to the hypothesis that cancer stem cells exist?
10. Who discovered human embryonic stem cells and what changes in cell culture methodology over that of mES cells were necessary to accomplish this?
11. Describe the process of somatic cell nuclear transfer.
12. What is the difference between therapeutic and reproductive cloning?
13. How was Dolly the sheep cloned?

14. Describe the two methods used by Jose Cibelli's group to produce autologous embryos.
15. How did Colin McGuckin's group isolate cord blood stem cells?
16. What hepatic-specific antigens were expressed in differentiated cord blood stem cells?
17. What two factors aggravate spinal cord injury repair?
18. How did Hans Keirstead and colleagues partially restore locomotion in rat models of spinal cord injury?
19. What is the difference between transdifferentiation and dedifferentiation?
20. How did Shinya Yamanaka and colleagues induce pluripotency in adult mouse fibroblasts?
21. What method did Anthony Atala's group employ to isolate human amniotic stem cells?
22. What is the difference in transcription factor identity for human iPS cell creation by Yamanaka's vs. Thomson's groups?
23. Describe preimplantation genetic diagnosis.
24. How did Andrew French's group successfully clone human embryos?
25. How were mesenchymal stem cells prepared for autologous replacement therapy?
26. Why is *piggyback* considered a superior way to introduce genes encoding transcription factors into cells for reprogramming purposes?
27. How did Kwang-Soo Kim and his group efficiently introduce transcription factor proteins into fibroblasts for induction of pluripotency?
28. What was Geron Corporation's strategy for treating spinal cord-injured patients?
29. Why does the mitochondria drive innate immunity rejection?
30. From what two endangered species did Jeanne Loring's group produce iPS cells?

THOUGHT QUESTION

All chapters throughout this book include at least one thought-provoking question designed to test your knowledge of the material and also to give you the opportunity to think critically about the chapter's content and how it might be applied in a real-world setting. Note that there are not necessarily any definitive or correct answers to these questions. They are merely meant to prod your intellect.

How would you go about deriving human embryonic stem cells without embryo destruction and how might you optimize both technique and equipment to increase clonal yield?

SUGGESTED READINGS

Books, Compilations, and Lectures

Altman, J. (2012). *MENTAL EVOLUTION: Origins of the Human Body, Brain, Behavior, Consciousness, and Culture*. In Press, 2012. Independent Publisher.

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