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Appendix A
The Science of Embryonic Stem Cell Research

This appendix provides a brief overview of the following questions: What are embryonic stem cells and how are they obtained? What benefits are expected from the study and use of embryonic stem cells? And what alternative methods are available to procure cells functionally similar or identical to embryonic stem cells, without destroying embryos?

Embryonic Stem Cells: What They Are

A notable feature of the genetic constitution of a living organism is the fact that the same genomic structure, found from cell to cell throughout the organism's body, plays a different functional role in each type of cell, tissue, and organ.[1] Consider the differences between, for example, the cells in the pancreas that are responsible for the production of the hormone insulin—which is necessary for regulating glucose levels in the blood—and the cells of the liver, which are responsible for, among other things, transforming glucose into glycogen in response to insulin produced in the pancreas.[2] Each of these types of cells does different work for the organism and is thus functionally different from the other types. Yet each type of cell (in human beings there are approximately 200 basic types and thousands of subtypes[3]) contains the full genetic complement of more than 20,000 genes; that is, different types of cells contain the same genotype.[4] What accounts for this difference in form and function if it is not due to a difference in genes?

The answer is that there *is* a difference between these cells—not in the genes possessed, but in the genes activated, or expressed.[5] Biologists estimate that most human cells only express about 20 percent of the genes they possess at any one time.[6] In different cell types, different genes are active or inactive, or are expressed at different rates; the resulting pattern of gene expression, in conjunction with other factors such as cell position, determines the nature and function of each cell in the organism's body.[7]

The full differentiation of cells (into, say, liver cells or blood platelets) is the result of a process that begins when an organism is an embryo and continues throughout its life. A human embryo develops two structures

within its first five days: an inner cell mass (ICM) of tightly compacted cells and an outer boundary called the trophoblast.[8] In each structure, cells are relatively unspecialized, or undifferentiated.[9] But in the course of the developing life of the embryo, fetus, and eventually newborn, those initial cells will divide through a process called mitosis and give rise to increasingly specialized families of cells. Generally speaking, cells from the trophoblast will give rise to cells that form part of the placenta (the organic support system of the fetus while it is in the womb), while cells of the ICM will give rise to all of the different cell types of the mature organism.[10]

Stem cells are defined by two properties: first, the capacity for self-renewal, and second, the capacity to produce other cells that are more differentiated.[11] Stem cells vary in their potency—the number of differentiated cell types they can produce. Embryos at the single-celled stage (the stage called the "zygote," or fertilized egg) are *totipotent*—capable of differentiating into any cell type of the ICM or trophoblast.[12] Embryonic cells remain totipotent through the first few stages of cell division, and any totipotent cell is capable of becoming a whole new embryo and producing a developed organism.[13] (This is evident in the phenomenon of twinning: one way twinning can occur is when the two-cell embryo splits apart into two separate totipotent cells, each capable of developing into an adult.[14]) While the cells of the early embryo are totipotent, researchers have not been able to isolate cells from the embryo to grow totipotent stem cells *in vitro*.[15]

Meanwhile, certain cells of the ICM are *pluripotent*—capable of producing all of the differentiated cell types of the mature organism, but not of producing cells of the trophoblast (although researchers have been able to induce embryonic stem cells to produce trophoblast cells under certain conditions).[16]

As the organism develops and matures, the process of cell production remains essential to its survival. In order to keep pace with the organism's growth, and with the continual process of cell death and replacement, new fully-differentiated cells must be produced in different regions of the body. This is the work of somatic stem cells, also known as adult stem cells.[17] Adult stem cells are typically *multipotent*—capable of producing only cell types belonging to particular tissues.[18] Generally speaking, then, stem cells become more restricted in potency over the early development of the organism; put another way, stem cells become more determinate in the types of tissue they will produce, while still maintaining their capacity for self-renewal.

The term "adult stem cells" is somewhat misleading, since these stem cells can be found in children and even fetuses.[19] Adult stem cells have a remarkable ability to renew themselves—a property that allows them to sustain the growth and development of the body—although scientists have had difficulty sustaining adult stem cell self-renewal indefinitely *in vitro*.[20]

Different types of adult stem cells can be extracted from different tissues in the body. Blood-forming stem cells, called hematopoietic, reside in the bone marrow.[21] The marrow is also one of several places where mesenchymal stem cells, which form bone, cartilage, and other types of tissue, can be found.[22] They can also be found in body fat, also called adipose tissue (which requires less invasive procedures to reach).[23] The placenta[24] and the umbilical cord[25] are also rich sources of stem cells that have the potential to develop into a variety of tissue types. Other somatic tissues, including muscles and neural tissue, can be sources of specialized stem cells.[26]

A wide variety of potential therapeutic uses exists for adult stem cells, and their extraction and use generates little if any controversy. But a key practical drawback to the therapeutic applications of adult stem cells is their limited potency: stem cells from a particular tissue region can usually be coaxed only into generating further cells of that tissue type.[27]

Embryonic stem cells, on the other hand, have a much greater capacity. Within the life of a developing organism, pluripotent cells play a foundationally important role: they are the ancestor cells that will give rise to all the different cell types of the mature organism's body. Their open-ended potentiality also makes them extremely attractive for scientific research when extracted from the embryo, especially by contrast with adult stem cells.

How Embryonic Stem Cells Are Obtained

The extraction by scientists of cells from the developing embryo—a process that destroys the source embryo—is typically carried out as follows: An embryo four to five days old is immersed in a chemical solution that dissolves and destroys its trophoblast cells, which allows for the cells of the ICM, called blastomeres, to be extracted.[28] These cells can then be placed in specialized culture conditions designed to enable them to grow as colonies of stem cells.[29] The chains of cultured embryonic stem cells and their progeny are referred to as embryonic stem cell *lines*.

Scientists generally employ three tests to assess the pluripotency of stem cells. The stem cells can be injected into an animal with a compromised immune system in order to see if they develop into teratomas, a special type of relatively benign tumor consisting of cells from all three

germ layers of the embryonic body.[30] Because the different germ layers represent distinct developmental paths, the ability of a cell to differentiate into cells from each of the three layers indicates its ability to form all the cell types of the body, even if the teratoma does not consist of each and every cell type in the body.[31] A second test of pluripotency is the ability of the stem cell to contribute to the development of a chimera—an organism with some cells that are genetically distinct from the rest of the organism.[32] In this test, the stem cells are injected into an early embryo, where they contribute to the development of the fetus and adult organism, resulting in a chimera in which cells originating from the stem cells are found in all of the tissue types in the adult organism's body.[33] In the third test of pluripotency, stem cells are injected into an embryo that has been modified so as to make it capable of developing into placental tissues but not the cells of the embryo itself. When the stem cells are added to this special embryo—called a "tetraploid" embryo because the procedure for creating it involves fusing the two cells of the early embryo, resulting in a cell with four sets of chromosomes—the ability of the stem cells to develop into all of the different cell types of the embryo complements the ability of the tetraploid embryo to develop into the tissues of the placenta, thus allowing for normal embryonic development.[34] This procedure, called the "tetraploid complementation assay," is the most stringent test of pluripotency because it creates an organism that is entirely derived from the stem cells used in the procedure. (It is worth noting that, although scientists use all three of these tests in researching *animal* stem cells, they do not use the chimera formation test or the tetraploid complementation assay on *human* stem cells. For ethical and practical reasons, they rely only on the teratoma formation test, in which human embryonic stem cells are injected into immune-compromised mice.[35])

While research on adult stem cells can be traced back decades—indeed, hematopoietic stem cell transplants have been used to treat persons suffering from bone marrow diseases, including cancer, since the 1950s[36]—the key breakthrough for human ES cell research was achieved in 1998 when University of Wisconsin researcher James Thomson announced that he had derived ES cells from human embryos.[37]

Two related issues at this point are of interest because of the ethical questions to which they give rise. The first concerns the *origin of the embryos* from which ES cells are derived. The most practicable source of ES cells is embryos that have been created in fertility clinics through IVF but are "left over" from attempts to aid infertile couples in conceiving. In IVF, a sperm and an egg cell (oocyte) are joined in a petri dish. The result-

ing embryo is then allowed to grow for several days before it is either implanted in the woman or, if it is not to be used immediately, frozen and stored.[38] An American IVF clinic will typically produce more embryos than are used in each cycle of treatment, in order to have additional embryos available in case some turn out to be unusable or the implantation is unsuccessful. Therefore, some embryos usually remain after an IVF cycle has been successfully initiated; currently, there are several hundred thousand of such "spare" embryos frozen in IVF clinics in the United States.[39] The parents of these embryos may choose to donate them to be used in research if they do not wish to use them in a future IVF cycle.[40] Many supporters of ES cell research see these embryos as the most promising source of ES cells. However, as enticing as this sitting stockpile may be to interested researchers, most of the stored embryos have not been designated by the parents for research; they may be unsure if they wish to try to conceive again in the future, or may be uncomfortable donating their embryos to research for other reasons.[41] Further, even when the parents do consent, there are various logistical barriers to using these embryos for research, including possible degradations experienced in long storage, the hazards of transportation from clinic to laboratory, and reduced viability to begin with (the fertility clinicians will have selected the strongest-seeming embryos for the first round of implantation).

The same IVF procedure of creating embryos for fertility treatment could also be used to create embryos specifically for research purposes.

Another source of embryonic stem cells involves the process known as somatic cell nuclear transfer (SCNT), a kind of cloning. In this approach, which will be discussed further below, an enucleated oocyte (that is, an egg whose nucleus has been removed) is fused with the nucleus of a somatic cell (a cell containing the full complement of genetic material, unlike a gamete cell such as a sperm or egg, which contains only half). The oocyte "reprograms" the nucleus back to a totipotent (undifferentiated) state. This one-celled organism, which is genetically almost identical to the organism that provided the somatic cell, is now effectively a new embryo, and it begins the process of cellular division and growth. The embryo could be implanted in a womb; this is how Dolly, the cloned sheep, was created.[42] Or the embryo could be used as a source of ES cells.[43]

It is worth noting that the cloned embryo and the ES cells that result from SCNT are usually not *completely* genetically identical to the original somatic cell and the organism that provided it. The DNA in the new cells' nuclei would be identical to that in the original cells' nuclei. But DNA is also present outside the nucleus, in the mitochondria. Except in cases

where a female provides both the eggs and the somatic cell for the SCNT procedure, the mitochondrial DNA of the egg used in the SCNT process will be different from that of the donor cell, possibly leading to mitochondrial disorders, which have been observed in cloned mammals.[44]

An alternative version of SCNT that would not require the procurement of egg cells from women is called interspecies SCNT (iSCNT). In this process, the nucleus of a *human* somatic cell is transplanted into an enucleated *animal* oocyte in order to produce embryonic stem cells. Because the nucleus of the animal oocyte has been removed, most of the DNA in the resulting embryo will be human, although the small amounts of mitochondrial DNA present in the cytoplasm of the animal oocyte will be present in the resulting embryo. The organisms created via iSCNT have been dubbed "cybrids"—cytoplasmic hybrids—since they have human DNA placed in the cytoplasm of an animal oocyte. While this technique has been successfully used to clone certain mammals of species that were closely related to one another,[45] attempts to perform iSCNT with human nuclei have been so far unsuccessful. Some scientists have expressed doubts about whether iSCNT can work in humans at all, since SCNT relies on the ability of the oocyte to "reprogram" the genome of the nucleus into an embryonic state, but the somatic cell nucleus must be compatible with the oocyte in order for this "reprogramming" to be successful.[46]

Three other procedures also can, in practice or in theory, produce human embryonic stem cells. First, it is possible to reprogram somatic cells to a pluripotent state by fusing them with existing ES cells.[47] Second, blastomeres can be extracted from living embryos without destroying the embryos. This kind of blastomere extraction is already done now in a practice called preimplantation genetic diagnosis (PGD), which is used by IVF clinics to screen embryos before they are implanted. Blastomere extraction apparently does not always significantly interrupt the embryo's biological functioning, although some embryos are evidently lost as a result of this process, as the rate of successful pregnancies following PGD is lower than with other assisted reproduction technologies, and there is evidence that twins or triplets born following PGD have increased rates of birth defects and infant mortality.[48] Third, dead embryos maintained in culture often contain living cells, which might also provide a source of ES cells in the strict sense.[49]

These latter two procedures highlight the second important issue surrounding embryonic stem cells, namely, the *consequences for the embryo* of ES cell extraction. When blastomeres are extracted from an IVF embryo or an SCNT embryo by dissolving the trophoblast, the resulting stem

cells have been obtained at the cost of the embryo's life. By contrast, when blastomeres are extracted from living embryos without dissolving the trophoblast, or when blastomeres are extracted from dead embryos, the resulting stem cells will not have been obtained by destroying embryos. Although the long-term medical consequences to a living embryo brought to term after blastomere extraction are not yet known, these techniques suggest the possibility of attaining human embryonic stem cells without the destruction of living human embryos.

The Value of Embryonic Stem Cells

Since the first successful extraction of human embryonic stem cells in 1998, the field of ES cell research has been awash in grand expectations. The source of these expectations is the link between ES cells and the field of regenerative medicine.[50] Because ES cells are pluripotent, they have the capacity, in principle, to proceed down almost any path of cell differentiation we might wish, provided only that we know what cues are necessary to induce such differentiation.[51] Knowledge of these cues—which include the proteins that promote or block transcription of DNA into RNA in a cell, known as transcription factors, as well as other physical and chemical factors such as adhesion, pressures, and various other aspects of the cellular environment—could help make it possible to grow tissue cultures of any specific type from ES cell lines, and perhaps even to grow entire organs. Across a range of medical cases, such as neurological damage, heart disease, or the inability of the pancreas to produce insulin, the hope is that stem cell therapies could facilitate the regeneration of damaged tissues or organs, or the cure of diseased tissues and organs, by replacing or supplementing existing tissues and organs with healthy ones.[52] (For a more extensive discussion of the treatment potential of stem cell-derived therapies, see Appendix B.)

However, the possibility of applying stem cell research to regenerative medicine faces a number of hurdles, of which three are especially significant. The first is the tumorigenic (tumor-forming) character of embryonic stem cells.[53] As discussed earlier, ES cells have the characteristic ability to form teratomas, which are a relatively benign form of tumor. But malignant tumors called teratocarcinomas tend to result from ES cells that have an abnormal number of chromosomes, which sometimes occurs when ES cell lines are grown *in vitro*.[54] This trait of ES cells constitutes a further difference between ES cells in an embryonic stem cell line and ES cells in the ICM, where they contribute to the ordinary course of

embryological development instead of forming tumors. The processes causing this transition to tumorigenicity are not well understood. But safe therapies involving ES cells will be difficult to develop unless a way is found to restrain this aspect of their power.

The second hurdle to ES cell therapies concerns the problem of immune rejection. This is the same difficulty intrinsic to any transplant procedure: when an organ from one organism is transplanted into another organism, the recipient's immune system recognizes that the transplant is genetically different and attacks the alien cells.[55] This process can sometimes work in reverse as well, since transplanted immune cells can recognize the new host as alien, resulting in graft-versus-host disease.[56] In either case, similar consequences can be expected where ES cell-derived tissues and organs with a different genetic character are used in regenerative therapies. (By contrast, many adult stem cell therapies can avoid the problem of immune rejection by using stem cells that actually come *from* the recipient, which allows for the transplantation of stem cells that are genetically identical to the patient.)

The problem with immune rejection has led to increasing interest in SCNT (cloning) as a method of obtaining embryonic stem cells. For example, if SCNT-generated embryos were used instead of IVF embryos, the patient's own somatic cells could be used as the source of the cell nucleus inserted into the oocyte and reprogrammed back to a totipotent state.[57] Since the ES cells and any tissues derived from them would be genetically almost identical to the recipient's cells, the problem of immune rejection might be eliminated. (As mentioned above, the SCNT-generated cloned cells would not be *completely* genetically identical to the recipient's cells, because they would retain the mitochondrial DNA of the egg used in the SCNT process.[58])

A third major challenge facing embryonic stem cell therapy involves generating the right kinds of differentiated cells using pluripotent stem cells. While ES cells have the theoretical ability to differentiate into any type of cell in the body, coaxing ES cells to develop into specific, functional cell types in the laboratory will require a thorough understanding of the factors that control stem cell differentiation.[59] While scientists have made progress differentiating ES cells into specific cell types, a recent study published in *Cell Research* found that the differentiated progeny of ES cells tend to express genes associated with early fetal development, raising questions regarding their therapeutic usefulness for adults.[60]

Beyond the possibilities of ES cell-derived regenerative therapies, which are still largely speculative, there lie a number of more immediate scientific

and medical benefits to be gained from the study of ES cells. At the most basic level, ES cells give scientists the opportunity to learn more about cell differentiation and about the factors implicated in gene expression. Such knowledge is additionally helpful in our understanding of the development of the human organism from its zygotic stages on, and will come to be integrated into the broader understanding of genetics and epigenetics.[61]

A second expected benefit comes from the use of ES cells to learn more about the workings and natural histories of genetic diseases. By studying ES cells taken from embryos with particular genetic conditions—often identified through preimplantation genetic diagnosis—scientists can learn more about how deficiencies in gene expression arise, and thus how they might be prevented or cured.[62] ES cells provide a window into genetic disease not easily obtained in any other way.

Finally, stem cell cultures that have been differentiated into particular tissue types may be used to study the effects of certain drugs, or to test for the toxicity of various chemicals.[63] Such options could alleviate the need for at least some animal testing and could also provide a more fine-grained knowledge of the effects of environmental conditions on human biology.[64]

For all these reasons, embryonic stem cells are considered by many researchers to be of critical scientific value and medical importance. However, in order to avoid the ethical worries that arise from destroying or harming embryos, researchers have proposed a number of alternative techniques for procuring pluripotent stem cells that are the functional equivalent of embryonic stem cells—techniques not dependent upon human embryos. While many believe that these alternative approaches can mitigate the ethical concerns, some scientists claim that even the alternative techniques require some research into ES cells, for such cells are said to provide the "gold standard" for understanding pluripotent cells more generally.[65] On this view, ES cell research provides an important gauge for the inquiries of scientists investigating alternatives to ES cells. In the following section we turn to some of the key attempts to find alternatives to embryonic stem cell research.

Alternatives to Embryonic Stem Cells

In this section we look at two of the most prominent methods suggested for obtaining pluripotent stem cells without extracting them from embryos. The first approach is called *altered nuclear transfer* (ANT), or, sometimes, altered nuclear transfer with oocyte-assisted reprogramming

(ANT-OAR).[66] The second approach, developed independently by Shinya Yamanaka in Japan and James Thomson in Wisconsin, is called somatic cell dedifferentiation, but is typically referred to by the name of its product, *induced pluripotent stem cells* (iPS cells).[67]

Both approaches rely upon what we know about the factors affecting gene expression in order to create pluripotent stem cells without ever creating embryos. Recall that in the successful cloning attempt that produced Dolly the sheep, the nucleus of a somatic cell was inserted into an enucleated oocyte, and the resulting new cell was dedifferentiated back to a totipotent, not a pluripotent, state.[68] This is a critical point: had the resulting cell not been totipotent, essentially the equivalent of a zygote, it could not have developed as a complete organism and there would have been no Dolly. Likewise, a human stem cell in any state other than totipotency is not and cannot become a complete human organism. In the ANT procedure, unlike in the SCNT cloning procedure, the nucleus of the cell transferred to the oocyte, or the cytoplasm of the oocyte into which it is transferred, is altered in order to prevent the cell from going through the stage of totipotency that is characteristic of a true embryo. These alterations change the patterns of gene expression to cause the cell to express genes characteristic of pluripotent stem cells, rather than the totipotent cells of the early embryo. Proponents of the procedure argue that none of the three cells involved in the process of ANT—the somatic cell with the altered nucleus, the oocyte, and the new cell—is at any point a zygotic, totipotent cell. Thus, ANT appears to provide pluripotent but non-embryonic stem cells.[69]

Like the ANT approach, the induced pluripotent stem cell approach capitalizes both on the ability of the somatic cell's nucleus to be coaxed into a less differentiated state and on our knowledge of the genes whose forced expression alters a cell's identity. Yamanaka and Thomson determined that by inserting genes for transcription factors associated with pluripotency into somatic cells by means of retroviruses, they were able to induce dedifferentiation in those cells, bringing them back to a stage of pluripotency.[70] The pluripotent stem cells created using this technique appear to have the classic marks of embryonic stem cells: they can be indefinitely maintained in a lab culture, and they are capable of multiple types of differentiation.[71] There are some differences in gene expression patterns between iPS cells and ES cells, but the consequences of these differences are at present unknown.[72]

Induced pluripotent stem cells seem to solve two problems that have bedeviled researchers—one moral and one technical. Unlike cells

produced through SCNT, iPS cells at no point go through a stage of totipotency. Thus, no human embryos are created or destroyed in the formation and use of iPS cells, so that that moral controversy is sidestepped. Additionally, like the embryos produced by SCNT and cells produced by ANT, iPS cell technology seems to offer a solution to the threat of immune rejection, because, in the event that regenerative therapies prove feasible, iPS cells could be dedifferentiated from the somatic cells of the diseased patient himself, and would thus have the same genome as the patient.[73]

The iPS approach has been widely and rapidly adopted by the scientific community: Yamanaka's technique was announced to work on mice in 2006, and only a year later was shown to work with human cells.[74] While, as noted, there are small differences in gene expression between iPS cells and ES cells, scientists studying iPS cells have typically been impressed with the degree to which iPS cells are functionally equivalent to ES cells. For example, Ian Wilmut, the researcher who created Dolly the sheep, announced after Yamanaka's discovery that he was halting his own cloning research, since he viewed the iPS cell approach as having more potential.[75] Some scientists have even used techniques similar to the ones used by Yamanaka and Thomson to attempt to reprogram differentiated adult cells of one sort into differentiated cells of another sort, altogether eliminating the pluripotent or multipotent stage.[76] Reliable techniques for reprogramming cells directly from one cell type to another could offer an alternative to stem cell-based cell therapies, but while research in this area has produced exciting preliminary results, more work will need to be done before these techniques could replace stem cell-based cell therapies.[77]

Moreover, because of the relative ease and non-intrusiveness with which iPS cells can be generated, some of the research possibilities proposed using ES cells might be more readily achieved using iPS cells. The difficulties involved in producing ES cells from IVF embryos, including obtaining the parents' permission, do not apply to iPS cells, which can instead be produced in large numbers and from a highly genetically diverse set of donors with little inconvenience to them.[78] And unlike ES cells produced through SCNT, iPS techniques do not require a supply of human eggs, which can be difficult or even dangerous to procure: the hormonal treatments used in collecting eggs from women can lead to such health-threatening complications as ovarian hyperstimulation syndrome.[79]

Induced pluripotent stem cells thus seem to offer many practical advantages sought by scientific and biomedical researchers. Nevertheless, there are some concerns about the iPS approach. One involves the use of retroviruses to introduce the transcription factors into the somatic cells. The

retroviruses "integrate randomly into the host genome," and even though the integrated viral genes are silenced in the iPS cells, there is a risk that they will reactivate.[80] This worry was particularly acute in the cells reprogrammed using some of the earlier iPS methods, as the transferred genes included some that are known to cause tumors.[81] The viral insertion of these genes can also interfere with the genetic functioning of the cell, perhaps even undermining the original purpose of the iPS cell by disrupting the expression of genes involved in developing desired traits.[82] Other concerns include the possibility of mutations or chromosomal abnormalities that can result from the genetic modifications necessary for inducing pluripotency, including some mutations that may contribute to the development of cancer (as has been documented in the use of retroviruses for gene therapy).[83] Growing iPS cells in culture for extensive periods also increases the likelihood of chromosomal abnormalities, including some that may increase the cells' tumorigenicity.[84] Furthermore, the presence of abnormalities or mutations in the tissue of origin can contribute to the risk of cancer in iPS cells.[85] While it was hoped that the ability of iPS cells to provide patient-specific stem cells would overcome the problems of immune rejection, a recent study published in *Nature* has indicated that tissues formed by iPS cells may still be subject to those problems.[86] The study found that certain iPS cells could trigger an immune response in mice, although more research is required to better understand how iPS cells and tissues derived from iPS cells react with organisms' immune systems.[87] The reprogramming of adult cells into iPS cells is also often incomplete, which can cause iPS cells to retain certain gene expression patterns from their tissue of origin.[88]

Some of these initial worries about iPS cells seem surmountable. Research conducted since the creation of the original iPS cells has shown that the process need not use some of the genes known to cause tumors.[89] Other experiments have used approaches that do not require retroviruses at all: some introduce genes into the cell without integrating DNA into the cell's chromosomes;[90] others directly add the transcription factor proteins, rather than transcription factor genes;[91] and progress has been made in modifying patterns of gene expression through the use of chemical compounds, rather than transcription factors, in order to reprogram cells to a pluripotent state.[92]

Another concern about iPS cells is that early attempts to generate them have not been very efficient: only a small proportion of the cells successfully dedifferentiate, with most studies reporting reprogramming between 0.001 and 1 percent of cells.[93] The techniques that involve less drastic genetic modifications to induce pluripotency tend to be less efficient.[94]

Additionally, there are some safety concerns related to the tendency of iPS cells to form dangerous tumors. The genetic and epigenetic changes necessary for inducing pluripotency share many features of the genetic and epigenetic changes associated with cancer; more research is needed to determine how to induce pluripotency without modifying cells in such a way that will increase the likelihood of cancer.[95]

A final difficulty related to the use of iPS cells is that it may not obviate the need for ES cells; as noted above, some researchers argue that ES cells are still necessary at least to provide a standard against which the success of iPS cells can be measured.[96] One example of a clinically relevant difference between iPS cells and ES cells involves the study of Fragile X syndrome, a developmental disorder caused by an inability to express the FMR1 gene.[97] Scientists who study the disorder have found evidence that the gene is expressed while the embryo's cells are still undifferentiated but is silenced as the embryo develops.[98] In ES cells derived from embryos that have the Fragile X mutation, the FMR1 gene is still expressed.[99] But the gene is *not* reactivated in iPS cells derived from *adult* Fragile X patients—indicating that the reprogramming process does not simply restore the cells to the state of undifferentiated embryonic cells.[100] This has led researchers to question the reliability of iPS cells for modeling the earliest developmental stages of diseases.[101] However, while the iPS cells used in this procedure may not have captured the very earliest stages of development, they were still useful for deriving tissues affected by the disorder, such as neurons.[102] Furthermore, other scientists have created iPS cells that were able to reactivate gene expression in the X chromosome that had been silenced during development, indicating that it may someday be possible to create iPS cells that exhibit the same patterns of gene expression as undifferentiated cells.[103]

Recent work by scientists at the Sanger Institute in the United Kingdom has resulted in a new technique for creating iPS cells that appears to be safer and more efficient, and to produce cells even more useful for research and therapy than human ES cells.[104] Many scientists have observed that mouse ES cells seem to represent a more developmentally immature state than human ES cells; the former have been described as being in a "naïve pluripotent state" while the latter are in a "primed pluripotent state."[105] The new Sanger iPS cells have many of the biological properties typically associated with the naïve state, including the activation in female cells of both X chromosomes, as opposed to the usual inactivation of one X chromosome in all mammalian cells (including human ES cells) past an early stage of embryonic development. Naïve mouse cells have shown more reli-

ability and consistency in their developmental potential than human ES or iPS cells, and some scientists have speculated that creating naïve human pluripotent stem cells will facilitate research on the ability of stem cells to differentiate into various tissues.[106] Further, some have argued that it will be easier to perform genetic engineering techniques, which may facilitate the creation of genetically modified tissues for disease modeling and cellular therapies.[107] Additionally, the Sanger iPS technique could open the door to the creation of human-animal chimeras for research or for cross-species organ transplantation.[108]

In sum, induced pluripotent stem cells are a very promising avenue for procuring pluripotent stem cells without the destruction of human embryos, but a number of difficulties with the procedure still need to be addressed.

Conclusion

In this appendix we have given an account of stem cells, and more particularly, of embryonic stem cells and some techniques for producing cells with similar powers. Stem cells clearly hold great potential for scientific research and, hopefully, for new and improved therapies. In the next appendix, we offer a sketch of the state of the art in therapeutic uses of stem cells.

Notes

1. Neil A. Campbell and Jane Reece, *Biology*, 7th ed. (San Francisco: Pearson Education, 2005), 955.

2. *Ibid.*, 955-956, 418.

3. *Ibid.*, 362.

4. International Human Genome Sequencing Consortium, "Finishing the Euchromatic Sequence of the Human Genome," *Nature* 431, no. 7011 (2004): 931-945. See also Wayne M. Becker *et al.*, *The World of the Cell*, 7th ed. (San Francisco: Pearson Education, 2009), 525.

5. Campbell and Reece, *Biology*, 415.

6. *Ibid.*, 362.

7. *Ibid.*

8. *Ibid.*, 1000.

9. The term "undifferentiated" is perhaps somewhat misleading. While these cells have the capacity to develop into any different cell type in the body, they should not be thought of as simple raw material for human development; just like any "differentiated" adult cell, embryonic cells are fully functional cells, which are responsible for

contributing to embryological development.

10. Campbell and Reece, *Biology*, 979.

11. Douglas A. Melton and Chad Cowen, "'Stemness': Definitions, Criteria, and Standards," in *Essentials of Stem Cell Biology*, 2nd ed., ed. Robert Lanza *et al.* (Burlington, Mass.: Elsevier Academic Press, 2009), xxiii.

12. Campbell and Reece, *Biology*, 418.

13. *Ibid.*; President's Council on Bioethics (PCBE), *Alternative Sources of Pluripotent Stem Cells*, Washington, D.C., 2005, 99.

14. PCBE, *Alternative Sources of Pluripotent Stem Cells*, 29.

15. Melton and Cowen, "'Stemness,'" xxiv.

16. PCBE, "Appendix A—Notes on Early Human Development," in *Monitoring Stem Cell Research*, Washington, D.C., 2004; R. H. Xu *et al.*, "BMP4 Initiates Human Embryonic Stem Cell Differentiation to Trophoblast," *Nature Biotechnology* 20, no. 12 (2002): 1261-1264.

17. Campbell and Reece, *Biology*, 418.

18. *Ibid.*

19. PCBE, *Monitoring Stem Cell Research*, 10.

20. Melton and Cowen, "'Stemness,'" xxiii.

21. Becker *et al.*, *The World of the Cell*, 722.

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