

Cloning Facts and Fictions

BY JAMES A. MARCUM

The heated debate in our society over reproductive cloning, as well as therapeutic cloning to obtain embryonic stem cells, has been fueled by misconceptions and hyperbole on both sides. We need to separate the facts from the popular fictions about human cloning.

Cloning is revolutionizing and reshaping our understanding of human nature: just as Darwinian evolution challenged the belief in humans as a special creation of God, so cloning is challenging the belief in the uniqueness of human identity and individuality. And just as human sanctity was defended from the perceived attacks of Darwinian theorists, so today human dignity is defended from the perceived threat of cloning scientists.

The possibility that a person's genome can be cloned repetitively strikes fear into our collective consciousness and causes us to question our identity. Who or what exactly are we? Are we reducible simply to genes? In other words, is genetic material responsible for our individual uniqueness and identity, or are there dimensions of our existence not reducible to the genome? These types of questions are at the center of the debate over human cloning, especially reproductive cloning. In addition, there is an equally contentious debate over therapeutic cloning. What is the moral status of the blastocyst or embryo? Is its dissection to obtain embryonic stem cells morally justifiable?

Although animal cloning was first conducted successfully in the 1950s—the first animal cloned by nuclear transfer was a tadpole—biologists commonly held that mammals could not be cloned. They believed that the adult mammalian body cell's nucleus is too specialized or differentiated to provide the genetic information needed to direct an organism's development. That is, the information necessary to guide an organism's growth is

locked up too securely to be accessed or, simply stated, the cell's nucleus is just too old to be born anew. Of course, that position changed with the cloning of the sheep "Dolly," who was born on July 5, 1996. Since then other mammalian species, such as cows, pigs, mice, and cats, have been cloned successfully. Some biologists believe it is only a matter of time until humans are cloned.

Biologists also knew about stem cells for decades, but their therapeutic potential was not generally appreciated until human stem cells were isolated in 1998. Stem cells are immature cells found both within embryos and adults. Some of these cells, especially from the embryo, have the potential to form any cell within the body. For example, embryonic stem cells can form nervous and heart tissue. But the use of embryonic stem cells has met with severe criticism.

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Since the late 1990s, then, reproductive and therapeutic cloning has engendered a heated debate in our society. But the debate has at times been fueled by misconceptions and hyperbole on both sides. We need to separate the facts and from the popular fictions about human cloning.

TYPES OF CLONING

Two types of cloning are prevalent today in the biomedical sciences. The first is *reproductive cloning* in which an adult organism is duplicated by removing a nucleus from one of its body cells and transferring it to an egg in which the nucleus has been removed. The cloned cell is totipotent, in that it gives rise to all the cells needed for development of a new organism. In the case of mammals, the resulting embryo from the cloned cell is then transferred to a womb for the remaining period of gestation. This type of cloning is asexual and the cloned organism is genetically similar to the organism donating the nucleus. These clones are usually called "delayed genetic" or "spaced" twins, in contrast to identical twins.

The second type is *therapeutic cloning*. The initial process is similar to that described above for reproductive cloning, except the clone is dissected at an early development stage to harvest its stem cells. The embryonic stem cells are pluripotent, in that they can form any one of the roughly 210 specialized cells types that make up the human body. Scientists claim that these stem cells represent the potential for curing degenerative diseases such as Alzheimer's disease, Parkinson's disease, diabetes, heart disease, and cystic fibrosis, among many other diseases.

EARLY STAGES OF HUMAN DEVELOPMENT

Human development normally begins with fertilization, that is, when a sperm enters an egg. Upon entry the sperm is dissolved and absorbed by the egg, except for its chromosomes—structures that contain the genes. These chromosomes couple with their mates from the egg to form a new, genetically unique individual organism. But before chromosome coupling can occur, the egg must eject its excess chromosomes. After fertilization, which occurs in the fallopian tubes and takes about one day, the fertilized egg or zygote then begins the journey to the womb or uterus and, along the way, undergoes cell division to form a multicellular morula—a solid ball of cells resembling a mulberry. The morula enters the uterus and continues to undergo cellular division, until a cavity appears in its center called the blastocele.

After the formation of the blastocele, the morula or zygote is now called a blastocyst and is composed of two cell types. The first type is the cell present in the interior of the embryo called the inner mass cell or embryoblast. This is the pluripotent cell that gives rise to the cells that make up the developing organism and thereby represents its potential or presumptive tissues. It is the cell harvested as embryonic stem cells. The second cell type is the trophoblast, which is the embryo's outer lining of cells and forms the placenta. The trophoblast cells are also responsible for implantation of the developing zygote into the lining of the uterus. The blastocyst continues to undergo cell division and differentiation—the process by which cells eventually become different tissues and organs of the organism.

Around two weeks later, the blastocyst transforms into the gastrula, with the appearance of the primitive streak—the structure that eventually becomes the nervous system. The gastrula is composed of three cell layers—ectoderm, endoderm, and mesoderm—that give rise to the various tissues and organs of the developing organism. For example, the ectoderm gives rise to the skin, nerves, and brain, while the mesoderm gives rise to muscles, bones, heart, and blood and the endoderm to respiratory and digestive tissues. These various stages of human development are complete around three weeks after fertilization.

CLONING TECHNOLOGY

The basic cloning technology, called somatic cell nuclear transfer, is rather simple conceptually. Begin with an egg and remove its nucleus, the cell structure that contains the chromosomes, being careful not to damage the egg's viability or capability to divide and develop after introduction of another nucleus. This process is called enucleation. A donor nucleus—generally from an adult body or somatic cell—is then transferred to the enucleated egg. Two ways are commonly used to achieve the transfer: one is by promoting union between the enucleated egg and the intact body or

somatic cell; the other way is to remove the nucleus from the donor cell and place it directly inside the enucleated egg.

The reconstituted or nucleated egg is then activated by a specific chemical or an electric shock in order to start cell division. The zygote is now placed in an artificial environment that contains a chemical medium to stimulate growth through cell division and other chemical substances to promote differentiation associated with the early stages of human development. For reproductive cloning the embryo is placed next into the womb, while for therapeutic cloning the embryo remains in a Petri dish or test tube until it reaches the blastula stage, after which it is dissected for the embryonic stem cells.

PROBLEMS WITH CLONING

Although the process of cloning is easy conceptually, it faces a number of technical difficulties and challenges. The technique for reproductive cloning is terribly inefficient in that the success rate is very small. To clone "Dolly," for example, required 277 cloned embryos and thirteen pregnancies before success was achieved. Overall, more than 90% of attempts to clone an organism are unsuccessful. Besides the poor efficiency of the process, successfully cloned organisms usually do not live terribly long. "Dolly" lived only one-half of her expected life-span of twelve years (interestingly, she was cloned from a six year-old sheep), and around a third of the cloned bovine calves die prematurely. Unfortunately, many cloned organisms do not survive long enough to evaluate their aging process—although studies show that the ends of the chromosomes, which are indi cators of an organism's longevity, are usually much shorter in cloned than non-cloned organisms.

Most cloned organisms live unhealthy and poor quality lives like "Dolly," who developed a host of chronic diseases prematurely, including obesity, arthritis, and lung cancer. Many cloned organisms have poorly functioning immune systems that lead to high rates of infection. Although clones may appear to be healthy at birth, they often have subtle defects and die prematurely for no apparent reason. Interestingly, studies show that cloned bovine calves score lower on average than non-cloned calves in behavioral tests for attentiveness and intelligence. Finally, the genomes of many cloned organisms are compromised or defective; for instance, cells from cloned monkeys do not contain nuclei, so that the chromosomes (which normally are located within the nucleus) are scattered throughout the cells.

These problems may result from the enormous stress placed upon both the cell and nucleus during the cloning process. Enucleating an egg involves suctioning out its nucleus, which often removes more than the nu- cleus. The process may also remove the fluid filling—the cytoplasm—of the egg, which contains many important substances for directing the

early stages of development. Biologists believe that disruption or removal of these substances leads to many of the cloning problems. Meanwhile, other problems connected with cloning may result from asking a mature or differentiated nucleus, which has been regulating cellular activity within a specific somatic or adult cell, suddenly to direct the development of an embryo. The genetic material of an adult cell is programmed to maintain the viability of that cell type only. Cloning demands that the genetic material of a differentiated cell be reprogrammed, and by a process that is poorly understood.

Similar challenges and problems face the techniques of therapeutic cloning to obtain embryonic stem cells. The efficiency of therapeutic cloning is almost as dismal as it is for reproductive cloning, for only about one out of ten embryos can be used to provide embryonic stem cells. Moreover, many embryonic stem cells simply do not grow under artificial conditions. It is well known that the microenvironment of the developing embryo provides the cues needed for cell growth and differentiation. These cues are lost in an artificial environment, unless supplied externally. Many of the cues are not known and it is hard to induce the pluripotent stem cell to differentiate into the specialized cell required to address the patient's need. Often the stem cells produce an unwelcome mixture of specialized cell types under artificial conditions.

Two other problems concern the fate of embryonic stems cells after they are placed in the patient. The first is that the cells do not effectively relieve the symptoms of the disease. For example, stem cells induced to produce insulin under artificial conditions were injected into mice, but the level of insulin production was insufficient to prevent the mice from dying from the complications of diabetes. In another example, only half of rats suffering from Parkinson's disease were modestly relieved of symptoms using embryonic stem cells. Recently, however, paralyzed mice and rats regained partial function of their limbs after an infusion of human embryonic stem cells.

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The second problem with the fate of embryonic stem cells involves the production of tumors within the host. Although many embryonic stem cells do not grow under artificial conditions, there is a small number that grow uncontrollably. When embryonic stem cells engineered to produce insulin

were transplanted into mice to treat diabetes, some cells did not reverse the diabetes but formed tumors. Moreover, not only do these rogue cells produce a tumor but also a wide range of other specialized cells. For example, transplantation of fetal tissue into the brain of a person suffering from Parkinson's disease resulted in the formation of non-brain tissue such as bone, skin, and hair. Of course, the patient did not survive.

Another technical problem involves the timing for the expression of the genes in embryonic stem cells. During development genes are turned on at precise times and are responsible for the synthesis of a protein, which often influences the next stage of development. Stem cells when placed in a foreign environment do not control the expression of their genes in a reliable fashion, which may lead to problems associated with an unstable genome. For example, studies with mice demonstrate that embryonic stems cells express genes in a highly variable way—around 4% of the roughly 10,000 genes from liver and placental cells of cloned mice were expressed abnormally. If the genes expressed abnormally are critical for development, often grotesque morphological anomalies result.

The last of the challenges is not technical but involves the supply of eggs. For therapeutic cloning to be beneficial for the thousands of patients with degenerative diseases, the number of eggs needed would be enormous. Some researchers would need hundreds of eggs per day, compared to the handful available today. The problem is where to obtain that supply. One solution is to use eggs from other species, such as cow. However, this raises the technical problem of patient's rejection of the hybrid-species embryonic stem cells—not to mention the ethical issues involving the generation of hybrid-species organisms.

FACTS AND FICTIONS

The debate over human cloning involves a tremendous amount of hype. Today companies like Clonaid claim that human cloning is just around the corner and that it represents the first step towards immortality by creating "identical twins" of ourselves or deceased family members. Unfortunately, "Dolly" or any other animal created using nuclear transfer technology, is not truly an identical clone of the donor animal. Only the clone's chromosomal or nuclear DNA is the same as the donor. Some of the clone's genetic materials come from the mitochondria in the cytoplasm of the enucleated egg. Mitochondria, which are subcellular structures called organelles and serve as power sources to the cell, contain their own short segments of DNA. Moreover, there are important factors within the cytoplasm of the egg that dictate gene expression during development. Change that pattern of expression and the result is a different individual.

Can we clone our loved ones? No. We are historical, contingent beings. Human personhood is not reducible to a genetic code—as natural identical twins so vividly demonstrate. Clones cannot be nurtured under the same

conditions or environment as the nuclear donor. The only way to clone an individual is to produce the body from a donor's nucleus and download the donor's personality, including memories, cognitive patterns, intelligence, etc., into the clone. Of course, the obvious problem, besides the technology to achieve such a feat, is the more fundamental issue of what constitutes an individual's personality. Is it memories, cognitive patterns, intelligence, etc.?

In regard merely to protecting human identity, then, there is no reason in principle why humans should not be cloned. Since we are not reducible to our genome, clones would certainly be unique individuals. The only problem would be a reduction in genetic variability in the human population, if reproductive cloning were conducted on a grand scale. But this does not consider the technical or ethical issues now facing reproductive cloning, which prohibit any attempt currently at human cloning.

The reductive sword, however, cuts the opposite way with therapeutic cloning. Just as we are not reducible to our genetic material, neither are we reducible to a ball of cells such as skin cells. Therapeutic cloning is often justified by claiming the embryo is nothing more than a ball of cells. The justification of therapeutic cloning based on this reductive move is at best blind to the importance of the cells that make up the embryo. Each cell represents the tissues that will eventually comprise the adult organism. Thus, embryonic cells are not comparable to adult somatic cells.

Importantly, there is a viable alternative to embryonic stem cells. Adult tissue from almost every organ in the body, umbilical cord blood, and the placenta contain stem cells. These stem cells are generally called adult stem cells, although this is misleading since they are also present in fetal tissue. They were originally thought to be multipotent in their ability to form specific tissues—in other words, adult stem cells would only form tissue similar to the organ from which they were harvested. For example, stem cells from bone marrow responsible for the production of blood cells were thought only to form these cells. However, recent studies demonstrate that adult stem cells may rival the pluripotency of embryonic stem cells. Adult bone marrow stem cells can form skeletal and cardiac muscle cells. Some researchers believe that adult stem cells are better suited for therapeutic purposes, since they avoid problems associ-

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ated with embryonic stem cells. Currently a dozen companies, like Osiris Therapeutics of Baltimore, are developing therapies using adult stem cells.

CONCLUSION

The fact is that cloning today is more art than science. All else is fiction. Success often depends more on the tacit skills of the investigator, like riding a bike, than on the techniques employed. We understand little about the processes involved in the development of an organism, and without an answer sheet on the subject we cannot gauge our progress in mastering those processes. Until we can efficiently and effectively control cloning, the results will be hit or miss as they are now, often with unfortunate results. Human cloning, whether reproductive or therapeutic (to obtain embryonic stem cells), is certainly out of the question until we better understand the underlying developmental mechanisms.

JAMES A. MARCUM
is Associate Professor of Philosophy and Director of the Medical Humanities Program at Baylor University in Waco, Texas.