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The Moral Case for ANT-Derived Pluripotent Stem Cell Lines

Rev. Nicanor Pier Giorgio Austriaco, O.P.

On May 5, 2006, Senators Rick Santorum and Arlen Specter, both of Pennsylvania, introduced bill S. 2754 in the U.S. Senate, titled the "Alternative Pluripotent Stem Cell Therapies Enhancement Act." This proposed legislation would require the National Institutes of Health to conduct and to support research for the development of techniques to create pluripotent stem cells (i.e., stem cells that have the properties of embryonic stem cells), without either the creation or the destruction of human embryos.[1] The bill stands as one attempt to move our society beyond the moral impasse over the permissibility of destroying human embryos in order to harvest embryonic stem cells. It makes specific reference to four alternative approaches outlined by the President's Council of Bioethics in its 2005 report, *Alternative Sources of Human Pluripotent Stem Cells*,[2] but notes that it will also support "any other appropriate techniques and research."

In this paper, I will make the case for the moral liceity of deriving pluripotent stem cells using one of the Council's techniques, known as altered nuclear transfer

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[1] For the full text of the bill, see the Thomas Web site of the Library of Congress, at http://thomas.loc.gov/cgi-bin/query/z?c109:S.2754.

[2] President's Council on Bioethics, *White Paper: Alternative Sources of Human Pluripotent Stem Cells* (Washington, D.C.: PCB, 2005).

(ANT), which was initially described in 2002.[3] ANT involves either the deletion of transcriptional repressors that act to turn off the pluripotent stem cell program (ANT-Cdx2) or the overexpression of pluripotent stem cell-specific transcription factors that turn on the pluripotent stem cell program (ANT-OAR). I will propose that there are scientific and philosophical reasons to think that ANT is a technically feasible and morally acceptable alternative that would generate embryonic-stem-cell-like pluripotent stem cell lines without creating or destroying embryos.

I will begin with a review of the biology of normal and cloned embryos. I will then continue with an exposition of the science of epigenetics to defend the claims made by proponents of ANT that epigenetic differences can be used, with reasonable certitude, to distinguish normal embryos from non-embryonic entities. I will provide a philosophical account for these claims grounded in the hylomorphic theory of the Aristotelian-Thomistic tradition. I will affirm that philosophically understood, ANT is a proposal to alter the material disposition of a somatic cell nucleus toward the form of a pluripotent stem cell by using fundamental genetic and epigenetic transformations.

Finally, once I have scientifically explained and philosophically defended the ANT proposal, I will address several prudential concerns raised by critics of ANT who are worried that ANT may lead to the exploitation and destruction of human beings. In doing so, I will suggest that ANT provides a conceptual approach that could lead to a practical and morally acceptable alternative to the embryo-destructive research currently required to obtain human embryonic stem cells. Based on current evidence from successful studies in mice, I suggest that ANT should now be tested in a range of model animal systems, including nonhuman primates, to evaluate the feasibility of extending this approach to work with human cells.

What Is a Human Embryo?
What Is a Cloned Embryo?

At its earliest stage of development, the human embryo is a single cell. However, it is unlike any other human cell in that it is uniquely able to develop into a mature human being if it implants itself into its mother's womb. To put it another way, the human embryo is a human cell that is uniquely disposed to undergo the species-specific sequence of cell divisions and cell specialization events that, if all goes well, will transform it into a multicellular baby. As a whole living member of the human species, this cell has the intrinsic potential to become the fully formed human being made up of trillions of human cells, which—with a few exceptions[4]—are genetically identical to each other and to the original single-celled embryo. This species-specific organization involves the ordered and sequential appearance of specialized cells and tissues described in any embryology textbook. Based on studies of

[3] President's Council on Bioethics, "Statement of Dr. Hurlbut," *Human Cloning and Human Dignity: An Ethical Inquiry* (Washington, D.C.: PCB, 2002): 267–276.

[4] A few cells in the body, including red blood cells, white blood cells, and those cells that generate sperm and egg, undergo genetic alterations as they mature. As such, they are genetically distinct from the trillions of other cells of the human body that are genetically identical to each other and to the original single-celled embryo.

mice as a model system, it appears that in mammalian embryos the initiation of this specialization is already established at the two-cell stage, where the two cells have different characteristics and different developmental capacities.[5] Because of this capability, the single-celled human embryo is not only a cell: it is also an organism. Philosophically, an organism can be defined as a complete living substance that has its own internal principle of motion and change directed toward its natural perfection; and scientifically, as a discrete unit of living matter that follows a self-driven, robust developmental pathway that manifests its species-specific self-organization.[6]

With the advent of cloning technology, also known as somatic cell nuclear transfer (SCNT) technology, each human cell that contains a complete genome can now, theoretically, be transformed into a human embryo. The process of cloning is relatively straightforward. The cloner extracts the nucleus of an adult human cell (the nucleus is the part of a cell that contains its genetic information encoded in its genes), and then inserts it into a cytoplasmic sac (a cytoplasmic sac is the remnant of a cell after its nucleus has been extracted) containing the molecular contents normally found in a human egg. In doing this, the research scientist reconstitutes a cell—a human cell—which is genetically indistinguishable from all the skin cells, liver cells, or kidney cells of the mature human being from which it was cloned. However, this reconstituted human cell can also be an embryo—a cloned embryo—which like every other human embryo is able to develop into a mature human if it is allowed to implant itself into a woman's womb. Experience with animal cloning has shown that in many cases, the products of somatic cell nuclear transfer fail to grow. However, in a small percentage of cases, what is created is clearly an organism—the reconstituted cell is able to self-organize and develop, becoming a mature animal that, in some cases, is even able to reproduce normally.

Identifying Embryos with Epigenetics: A Scientific Analysis

The question that lies at the heart of the current discussion of ANT is the following: How can we tell whether a cell is a human embryo or not? Some may suggest that we could use genetics to identify a human embryo. However, at the outset, it is important to emphasize that genetics cannot be used to accomplish this

[5] K. Piotrowska et al., "Blastomeres Arising from the First Cleavage Division Have Distinguishable Fates in Normal Mouse Development," *Development* 128.19 (October 2001): 3739–3748.

[6] For my philosophical definition of an organism, I am indebted to the following sources: Aristotle, *Physics* II, ch. 9, and *Metaphysics* V, ch. 4; and St. Thomas Aquinas, *Summa Contra Gentiles* III, ch. 69, 77. For my scientific definition, I am indebted to the following sources: B. Goodwin, "Development as a Robust Natural Process," *Thinking about Biology: An Invitation to Current Theoretical Biology,* eds. W. Stein and F. J. Varela (Reading, MA: Addison-Wesley Publishing, Co., 1993): 123–148; and Juan de Dios Vial Correa and Monica Dabike, "The Embryo as an Organism," *The Identity and Status of the Human Embryo,* ed. Juan de Dios Vial Correa and Elio Sgreccia (Vatican City: Libereria Editrice Vaticana, 1999): 317–331. I also thank Rev. Bro. Dominic Legge, O.P., for his help in formulating these definitions.

task. A single-celled cloned human embryo would be genetically indistinguishable from all the cells of the human being from which it was cloned. All of these cells, again with the few exceptions already noted above, contain a complete and identical human genome. Therefore, possessing an intact human genome could never be the definitive criterion for identifying an embryo.

The advocates of ANT have suggested that in order to correctly identify human cells, including human embryos, we have to move from an analysis of a cell's genetic state to an analysis of its epigenetic state, and the developmental process that manifests this state. The genetic state of a cell refers to its complete set of genes: Which genes does a cell have and which genes does it lack? In contrast, the epigenetic state of a cell refers to the subset of its genes that are switched on or off: Of all its genes, which of its genes is a cell expressing and which ones is it not? Epigenetic analysis is routinely used to distinguish a cell of one type from another—for example, a liver cell from a skin cell—in the same human being. Recall that these cells are genetically indistinguishable from each other because they develop from the same single-celled embryo. In other words, they contain the same set of genes and thus have identical genetic states. However, most reasonable persons would acknowledge that a liver cell is different from a skin cell and that both are different from a nerve cell. How do biologists account for the difference between these cells?

Recent studies have shown that different cell types from the same human being— again, with a few exceptions—have identical genetic states but different epigenetic states.[7] To put it another way, from the biologist's perspective, a human liver cell can be identified because it is a human cell in which a unique subset of human genes characteristic of liver cells is turned on. It has the epigenetic state associated with liver cells. In contrast, a human skin cell can be identified because it is a human cell in which another unique subset of human genes, this time characteristic of skin cells, is turned on. It has another epigenetic state, the state associated with skin cells. In sum, the identity of a particular human cell can be identified not by the genes that are present in or absent from that cell, but by the particular subset of its genes that are turned on or turned off. In light of these biological realities, the advocates of ANT have proposed that to see if an unknown human cell is a human embryo, we will have to know if its epigenetic state matches the epigenetic state associated with embryos. If it does, then we will have evidence that our unknown human cell is a human embryo.

Significantly, the Green Laboratory at the University of Otago in New Zealand has already described a set of 1,542 mouse genes with well-matched human homologs that are preferentially expressed in early embryos.[8] This is potentially the molecular

[7] For a comprehensive overview of epigenetics, see Bruce Stillman and David Stewart, eds., *Epigenetics*, Cold Spring Harbor Symposia on Quantitative Biology, LXIX (Cold Spring Harbor, NY: Cold Spring Harbor Press, 2005).

[8] J. L. Stanton and D. P. Green, "A Set of 1542 Mouse Blastocyst and Pre-Blastocyst Genes with Well-Matched Human Homologues," *Molecular Human Reproduction* 8.2 (2002): 149–166. For a review, see J. A. Stanton, A. B. Macgregor, and D. P. Green, "Gene Expression in the Mouse Preimplantation Embryo," *Reproduction* 125.4 (April 2003): 457–468.

signature of the embryonic epigenetic state that we need to identify bona fide embryos. In contrast, researchers at the Magnasco Laboratory at the Rockefeller University in the United States, working with data independently obtained by three other laboratories, have identified 111 genes that are turned on and 95 genes that are turned off in human embryonic stem cells.[9] Like others, they propose that this pattern of gene expression define "stemness," uniquely identifying a cell as a stem cell. This is potentially the molecular signature of the pluripotent stem cell epigenetic state. Finally, comparing the two reports suggests that the two epigenetic states do not overlap. As we will see below, exploiting this difference is a crucial part of the ANT proposal.

To confirm our epigenetic analysis, we would also need to observe the cell's behavior. A human cell that is an embryo, if it is implanted into a woman's uterus, would be able to develop along the human developmental trajectory toward a mature human organism. If it did, we would have definitive evidence that our unknown cell is an embryo. Note that these two criteria—possession of an embryonic epigenetic state and the ability to develop into a mature organism—are not unrelated. From the perspective of systems biology, there is a causal relationship between the organization—and hence, the epigenetic state of a cell—and its behavior. We will return to this connection in greater detail below.

Identifying Embryos with Epigenetics: A Philosophical Analysis

The debate over ANT is philosophical in nature. Therefore, at this point it is important to place the scientific analysis described above within a philosophical framework that can accommodate the reality of epigenetics and relate it to the ontological status of the cell. As I have argued elsewhere, the hylomorphic theory of the Aristotelian-Thomistic tradition remains a potent explanation for living things.[10] Here, I will begin by highlighting the salient points of this theory. Next, I will suggest that the emerging field of systems biology can help us update this theory and translate it into a contemporary idiom. Finally, I will use this updated hylomorphic theory to explain the relationship between the epigenetic state of the cell and its ontological status.

For the Aristotelian tradition, all substances—especially all living beings—are composed of both a formal and a material principle. The form, which is also called the soul in living beings, constitutes every being as a specific kind of thing with certain causal powers. In the biological realm, it gives the plant or the animal its stability, its unity, and its identity. It organizes the organism, determines its nature,

[9] M. Suarez-Farinas et al., "Comparing Independent Microarray Studies: The Case of Human Embryonic Stem Cells," *BMC Genomics* 6 (July 22, 005): 99.

[10] For details, see the following essays: Austriaco, "On Static Eggs and Dynamic Embryos: A Systems Perspective," *National Catholic Bioethics Quarterly* 2.4 (Winter 2002): 659–683, and "Immediate Hominization from the Systems Perspective," *National Catholic Bioethics Quarterly* 4.4 (Winter 2004): 719–738. For a contemporary philosophical defense of hylomorphism, see David S. Oderberg, "Hylemorphic Dualism," *Personal Identity*, ed. E. F. Paul, F. D. Miller, and J. Paul (Cambridge, UK: Cambridge University Press, 2005): 70–99, http://www.rdg.ac.uk/AcaDepts/ld/Philos/dso/dso.htm#hyledualism.

and specifies its end. The matter, on the other hand, is the "stuff" out of which the organism is made. According to the hylomorphic theory, both matter and form are inseparable—matter is recognizable only because it is organized by a form, and a form is identifiable only because it organizes some matter. Together, both constitute a stable substance.

How are we to talk about a human "soul" or human "matter" in a scientifically informed manner? In other words, how are we to translate classical Aristotelian hylomorphism into a modern idiom? As I have described in greater detail elsewhere, we can use the emerging science of systems biology to update Aristotelian-Thomist hylomorphic theory.[11] From the systems perspective, a snapshot of a human cell—including the single-celled human embryo—at any point in time would reveal an intricate net of molecular interactions distributed in three-dimensional space. Moreover, as I have described elsewhere, systems biologists have shown how these molecular interactions define the nature of the cell or the organism and determine its biological end.[12] Because of this, a systems theorist could easily envision the human cell or the human organism as informed matter, here defined as molecular matter organized in a species-specific configuration with its own pattern of dynamic activity. In the end, this particular pattern, this organization of the molecules, which, from the biologist's perspective, constitutes its identity and drives its developmental trajectory, would, from the philosopher's perspective, be one of the manifestations of its soul, in the same way that higher-level expressions, such as the sounds of a person's voice, his gestures, and the expression on his face, are manifestations of the joy or anger in his soul.[13]

Within this philosophical framework, how are we to understand the reality of epigenetics and its relationship to the ontological status of the cell? Or, to reformulate the question, given the dependence of the ontological status of the cell to its formal principle, how are we to relate the epigenetic state of a cell to its soul? To respond, I propose that the epigenetic state of a cell is one manifestation of its form. Recall that

[11] Austriaco, "On Static Eggs and Dynamic Embryos," 681–683.

[12] Ibid., 661–665.

[13] For now, I put aside the question of how the soul of an individual human cell cultured in the laboratory relates to the soul of the human organism from which it is derived. (Note that human cells can exist in culture long after their human donor has died. For instance, the HeLa cells used in laboratories throughout the world today were first isolated from Henrietta Lacks who died in 1951.) I will simply assume without argument that human cells in culture are informed by a formal principle that is the explanation for their life, their organization, and their behavior. Furthermore, I also will not discuss the philosophical basis for cell transformation—does the transformation of a skin cell into a muscle cell involve a substantial or an accidental change? For simplicity's sake, I will presuppose that the transformation of isolated cells in culture involves a substantial and not an accidental change. I believe that this presupposition rests on biological fact—the transformation of an isolated skin cell into a muscle cell involves a radical, a substantial, change in the cell's organization and its behavior. For more discussion, see the appendix to this essay, "Knowing Embryos, Princes, and Toads: A Further Response to *Communio*."

the epigenetic state of a cell refers to the subset of its genes that are switched on or off. Genes, however, encode the molecules that make up a cell. If gene A is turned on, then molecule A is present in the cell; if it is turned off, then molecule A is absent. Thus, a cell's epigenetic state is both a reflection of and a cause of its molecular composition: it is one of the manifestations of its soul. This is why the epigenetic state of a cell is an indicator of its ontological identity. It reflects the organization of the cell and is therefore one of the manifestations of its formal principle. To put it another way, isolated cells of different cell types cultured in Petri dishes can be distinguished by their epigenetic states because these states reflect their organization and their behavior and thus are manifestations of their different souls.

The Proposal of ANT-Cdx2 and ANT-OAR: A Scientific Analysis

Altered nuclear transfer is a proposal that seeks to take advantage of nuclear transfer technology to develop a morally acceptable alternative to the destructive embryo research currently required to obtain pluripotent stem cells. Although ANT is a broad concept, two specific versions of the ANT proposal have been described in recent months. ANT-Cdx2 involves the functional deletion of the gene *Cdx2* in a somatic cell nucleus and the recipient enucleated egg prior to nuclear transfer.[14] A subsequent proposal, termed ANT-OAR, involves the overexpression of key reprogramming genes in the somatic cell nucleus prior to transfer.[15] Here, I will begin with the scientific analysis of ANT-Cdx2 and then continue with ANT-OAR.

To summarize the ANT-Cdx2 proposal: Like the laboratory technician attempting somatic cell nuclear transfer, the scientist performing ANT-Cdx2 would begin with the nucleus of an adult human cell. However, in contrast with the cloner, he would first alter the epigenetic state of the nucleus by deleting the *Cdx2* gene. He would then extract the nucleus and insert it in its altered epigenetic state into a cytoplasmic sac taken from a human egg that has also been altered to prevent expression of *Cdx2*, hoping that what is constituted is not an embryo, but a human cell that has the characteristics and limited developmental disposition of a pluripotent stem cell.

To understand properly the ANT-Cdx2 proposal, it is important to review the function of *Cdx2* and its role both in the self-organization of the early mammalian embryo and in the establishment of the pluripotent state associated with embryonic stem cells.

First, studies in mice have established that *Cdx2* plays an essential role in the self-organization and development of an embryo. Recall that an embryo is an organism. It manifests a species-specific self-organization involving the ordered and sequential ap-

[14] W. B. Hurlbut, "Altered Nuclear Transfer as a Morally Acceptable Means for the Procurement of Human Embryonic Stem Cells," *National Catholic Bioethics Center* 5.1 (Spring 2005): 145–151.

[15] For a statement of the ANT-OAR proposal, see "Production of Pluripotent Stem Cells by Oocyte-Assisted Reprogramming," *National Catholic Bioethics Quarterly* 5.3 (Autumn 2005): 579–583.

pearance of specialized cells and tissues, which, in mammals, can already be detected at the two-cell stage. At this stage, the two cells—called blastomeres—are already specialized cells that have different characteristics and different fates. To put it another way, at the two-cell stage, the two cells can properly be called "parts" with distinct developmental futures becoming different tissues of the developing organism, which together constitute the embryonic "whole." Recent experiments have shown that *Cdx2* is an essential determinant for this embryonic self-organization.[16] At the two-cell stage, the blastomeres are distinguishable from each other by the presence or the absence of the expression of *Cdx2*. The cell that does not contain active Cdx2 divides first and goes on to become the inner cell mass that will generate the cell lineage that, within the full environment of the natural embryo, will eventually become the body proper of the fetus. In contrast, the cell that does contain active *Cdx2* divides after its partner and goes on to become the trophectoderm that will develop into the placenta and the other extra-embryonic tissues of the fetus. Strikingly, silencing *Cdx2* in the later-dividing blastomere precludes the self-organization of mouse embryos. In the absence of *Cdx2*, cell growth either arrests at the morula stage or gives rise to visibly evident abnormal structures. These experiments demonstrate that *Cdx2* is essential for the organization of the mammalian embryo. Moreover, recent studies in mice have shown that from the very beginning—the asymmetry of *Cdx2* is already found in the egg—the mammalian embryo needs *Cdx2* to organize itself. Thus, with ANT-Cdx2, silencing *Cdx2* in the somatic cell nucleus and the egg cytoplasm prior to the nuclear transfer prevents an embryo from ever coming into being, since the newly constituted cell, from the very beginning, lacks the power for self-organization, the hallmark of an organism.

Some may still argue that the product of ANT-Cdx2 is somehow a deficient or disabled embryo that simply cannot self-organize. In response, I argue that ANT-Cdx2 not only prevents the appearance of an embryo but also initiates the transformation of the somatic cell directly into a pluripotent stem cell. In support of this claim, recent experiments have demonstrated that *Cdx2* does more than play a positive role as a master regulator gene in promoting the specific epigenetic state that characterizes the trophectoderm lineage. *Cdx2* also acts as a repressor. Its presence is responsible for turning off the transcription factor Oct3/4, which is essential in the formation of the cell lineage of the inner cell mass.[17] This is why, at the first asymmetric cell division, the cell that receives no active *Cdx2* goes on to form the inner cell mass. In fact, recent studies in mice show that deleting *Cdx2* in mouse embryos leads to the expression of genes characteristic of pluripotent stem cells, such as *Oct3/4* and *Nanog*, even in cell lineages that would not normally express these genes.[18] These two genes, *Oct3/4* and *Nanog*, control a cascade of molecular pathways that maintain the pluripotency and

[16] K. Deb et al., "*Cdx2* Gene Expression and Trophectoderm Lineage Specification in Mouse Embryos," *Science* 311.5763 (February 17, 2006): 992–997.

[17] H. Niwa et al., "Interaction between Oct3/4 and Cdx2 Determines Trophectoderm Differentiation," *Cell* 123.5 (December 2, 2005): 917–929.

[18] D. Stumpf et al., "Cdx2 Is Required for Correct Cell Fate Specification and Differentiation of Trophectoderm in the Mouse Blastocyst," *Development* 132.9 (May 2005): 2093–2102.

identity of embryonic stem cells.[19] A recent paper has shown that the overexpression of *Nanog* alone leads to the reprogramming of neural or skin cells into cells that show embryonic stem cell characteristics.[20] Thus, with ANT-Cdx2, deleting *Cdx2* in a somatic cell nucleus prior to fusion should not only prevent the appearance of an embryo, but also initiate the transformation of the somatic cell into a pluripotent stem cell.

Given the incomplete state of our current knowledge, ANT-Cdx2 has to be tested with nonhuman cells before it can be used with human cells. Two key and related questions need to be addressed: Can the functional deletion of *Cdx2* before the act of nuclear transfer prevent the generation of an embryo? Does the deletion of *Cdx2* generate a pluripotent stem cell? Clearly, the first is the more important question. Based on the philosophical analysis outlined above, I propose the following three criteria for successful ANT-Cdx2: (1) From the very beginning, the newly constituted cell of ANT-Cdx2 must not manifest the epigenetic state, that is, the gene expression profile, associated with embryos. Rather, it would manifest the epigenetic constitution associated with a pluripotent stem cell. As I argued above, this would be evidence that the product of ANT-Cdx2 is not an embryo. This would be most evident if the functional deletion of *Cdx2* in the somatic cell nucleus resulted in the reprogramming of the epigenetic state of the nucleus *prior to transfer into the enucleated egg*. Such a preemptive epigenetic alteration would preclude the embryonic state in the reconstituted cell after nuclear transfer. Therefore, one question remains to be investigated: Can one already detect the appearance of Oct-3/4, Nanog, and other pluripotent stem cell factors not found in the embryo in the somatic nucleus prior to nuclear transfer? (2) When inserted into a receptive female, the reconstituted cell of ANT-Cdx2 must not develop along the trajectory toward a mature organism.[21] As I argue above, this would be another indicator that the product of ANT-Cdx2 is not an embryo. (3) The product of ANT-Cdx2 would be able to generate a pluripotent stem cell line. This would be the goal for ANT-Cdx2. Initial experiments with mice have already shown that ANT-Cdx2 can indeed be used to produce pluripotent stem cells.[22] However, the additional experiments noted above should now be done.

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[19] H. Niwa, J. Miyazaki, and A. G. Smith, "Quantitative Expression of Oct3/4 Defines Differentiation, Dedifferentiation or Self-Renewal of ES Cells," *Nature Genetics* 24.4 (April 2000): 372–376; Y. Loh et al., "The Oct4 and Nanog Transcription Network Regulates Pluripotency in Mouse Embryonic Stem Cells," *Nature Genetics* 38.4 (April 2006): 431–440.

[20] J. Silva et al., "Nanog Promotes Transfer of Pluripotency after Cell Fusion," *Nature* 411.7096 (June 22, 2006): 997–1001.

[21] As I have argued elsewhere, it would be ideal if the product of ANT-Cdx2 developed into a teratoma. Along with the absence of the epigenetic state associated with embryos, this would be definitive evidence that the ANT-Cdx2 cell is not an embryo. For details, see the essay "Are Teratomas Embryos or Non-Embryos?" *National Catholic Bioethics Quarterly* 5.4 (Winter 2005): 697–706.; Also, see the appendix to this essay, "Knowing Embryos, Princes, and Toads: A Further Response to *Communio*."

[22] A. Meissner and R. Jaenisch, "Generation of Nuclear Transfer-Derived Pluripotent ES Cells from Cloned Cdx2-Deficient Blastocysts," *Nature* 439.7073 (January 12, 2006): 212–215.

To discuss ANT-OAR, I will start by summarizing the proposal: Like the cloner, the scientist performing ANT-OAR would begin with the nucleus of an adult human cell. Like the investigator performing ANT-Cdx2, however, and unlike the cloner, he would first alter its epigenetic state, this time by overexpressing the pluripotent stem-cell-specific transcription factor, Nanog, alone or in combination with other factors.[23] He would then extract the nucleus and insert it in its altered epigenetic state into a cytoplasmic sac taken from a human egg, with the intention of constituting, not an embryo, but a human cell that is indistinguishable in its basic biological characteristics from a pluripotent stem cell.

To understand properly the ANT-OAR proposal, it is important to review the scientific precedents for using genetic transformations, particularly the forced overexpression of genes for transcription factors, to manipulate the epigenetic states and therefore the identity of differentiated cells. First, overexpressing the gene for the muscle transcription factor, MyoD, can change the epigenetic state of a skin cell into the epigenetic state associated with muscle cells.[24] In other words, it turns on muscle-cell-specific genes and turns off skin-cell-specific genes. When this happens, the skin cell is transformed into a muscle cell.[25] As with the alterations involved in ANT-Cdx2 and ANT-OAR, this transformation is at such a fundamental level that literally thousands of molecular interactions are reordered. In another example, overexpressing the gene for the eye-specific transcription factor, *eyeless*, can change the epigenetic state of cells that normally become antenna by turning on the eye specification genes that are important for eye development.[26] When this happens, the antennae of the fly are transformed into extra eyes.[27] Here again, as with the

[23] K. Mitsui et al., "The Homeoprotein Nanog Is Required for Maintenance of Pluripotency in Mouse Epiblast and ES Cells." *Cell* 113.5 (May 30, 2003): 631–642; and S. Hatano et al., "Pluripotential Competence of Cells Associated with Nanog Activity." *Mechanisms of Development* 122.1 (January 2005): 67–79.

[24] H. Weintraub et al., "Activation of Muscle-Specific Genes in Pigment, Nerve, Fat, Liver, and Fibroblast Cell Lines by Forced Expression of MyoD," *Proceedings of the National Academy of Sciences* 86.14 (July 1989):5434–5438.; and H. Weintraub et al., "Muscle-Specific Transcriptional Activation by MyoD," *Genes and Development* 5.8 (August 5, 1991):1377–1386.

[25] S. J. Tapscott et al., "MyoD1: A Nuclear Phosphoprotein Requiring a Myc Homology Region to Convert Fibroblasts to Myoblasts," *Science* 242.4877 (October 21, 1988): 405–411; J. Choi et al, "MyoD Converts Primary Dermal Fibroblasts, Chondroblasts, Smooth Muscle, and Retinal Pigmented Epithelial Cells into Striated Mononucleated Myoblasts and Multinucleated Myotubes," *Proceedings of the National Academy of Sciences* 87.20 (October 1990): 7988–7992. For a recent review, see C. A. Berkes and S. J. Tapscott, "MyoD and the Transcriptional Control of Myogenesis," *Seminars in Cell and Developmental Biology* 16.4–16.5 (August–October 2005): 585–595.

[26] G. Halder et al., "Eyeless Initiates the Expression of Both *Sine Oculis* and *Eyes Absent* during *Drosophila* Compound Eye Development," *Development* 125.12 (June 1998): 2181–2191.

[27] G. Halder et al., "Induction of Ectopic Eyes by Targeted Expression of the *Eyeless* Gene in Drosophila," *Science* 267.5205 (March 24, 1995): 1788–1792.

products of ANT, there is a fundamental reordering of molecular interactions that in turn changes the nature and direction of development. These two classic examples are proofs-of-concept that overexpressing transcription factors can change the epigenetic states of cells and thereby transform their biological identity.

With the ANT-OAR proposal, the hope is that overexpressing the gene for the pluripotent-stem-cell-specific transcription factor, Nanog, alone or in combination with other factors, will reprogram the epigenetic state of the nucleus of the adult cell and force it to become a pluripotent stem cell directly when the nucleus is inserted into the egg-derived cytoplasmic sac. Moreover, since Nanog is specifically found in undifferentiated pluripotent stem cells and is not found in single-celled embryos, the expectation is that the change in the epigenetic state of the constituted cell will preclude the ordered molecular interactions that characterize the development of an embryo. To put it another way, proponents of ANT-OAR hope that the overexpression of *Nanog* will simultaneously turn on those genes required for the pluripotent stem cell state and turn off those genes required for the embryonic state in the transferred nucleus even before the new cell is constituted. As mentioned above, a recent study has shown that the overexpression of *Nanog* leads to the reprogramming of neural or skin cells into cells that show embryonic stem cell characteristics.[28]

The careful reader will note that ANT-Cdx2 and ANT-OAR are, in fact, not necessarily distinct approaches, but mutually complementary alterations. Both the functional silencing of *Cdx2* and the overexpression of both *Oct 3/4* and *Nanog* promote the specific patterns of gene expression that characterize pluripotent stem cells. This is not surprising since *Cdx2* and *Oct3/4* regulate each other: the functional deletion of *Cdx2* promotes the expression of *Oct 3/4* and *Nanog*, and the overexpression of *Oct 3/4* represses the expression of *Cdx2*. In natural embryogenesis, this mechanism is used to assure the distinct development of both the inner cell mass and the trophectoderm, the two earliest and distinct cell lineages of the organism. In ANT, the proposed alterations to this fundamental mechanism will assure the cellular product of ANT is a cell, that from the very beginning, will only develop into a cell line with characteristics of the cells in the inner cell mass, i.e., a pluripotent stem cell line.

Like ANT-Cdx2, ANT-OAR has to be tested with nonhuman cells before it can be used with human cells. The questions that arise are similar to those raised by ANT-Cdx2: Can *Nanog* overexpression alone or in combination with other factors prevent the generation of an embryo? Does *Nanog* overexpression alone or in combination with other factors generate a pluripotent stem cell? Again, as I noted above, I propose criteria for successful ANT-OAR: (1) From the very beginning, the reconstituted cell of ANT-OAR must not manifest the epigenetic state associated with embryos. Rather, it would manifest the epigenetic state of a pluripotent stem cell. (2) When inserted into a receptive female, the reconstituted cell of ANT-OAR must not develop along the trajectory that leads to a mature organism. (3) The product of ANT-OAR would be able to generate a pluripotent stem cell line. This would be the goal for ANT-OAR.

[28] Silva et al., "Nanog Promotes Transfer of Pluripotency," 997–1001.

ANT-Cdx2 and ANT-OAR: A Philosophical Analysis

Once again, the scientific analysis described above needs to be placed within a philosophical framework. Philosophically, how does one explain how changing the epigenetic state of a cell can alter its identity? How does one explain the transformation of a skin cell into a muscle cell with the forced overexpression of a transcription factor like MyoD, or the transformation of a skin cell into a pluripotent stem cell with the deletion of *Cdx2,* or the overexpression of *Nanog*?

In response, I suggest that overexpressing the gene for a transcription factor like MyoD or *eyeless* alters the disposition of the cell's material principle so that it is disposed to receive a new form specified by the transcription factor. Thus, overexpressing *MyoD* in a skin cell changes its epigenetic state and its identity by changing the disposition of its material principle, so it is now disposed to be informed by the formal principle associated with a muscle cell. This change in form is manifested in the change in the epigenetic state of and in the altered organization and behavior of the cell. In the same way, proponents of ANT propose that overexpressing *Nanog* or deleting *Cdx2* can so change the material disposition of the transferred human nucleus that it is now apt to receive, not the rational soul of a human embryo, but the form of a human pluripotent stem cell. This change in form would be manifested in changes in both the cell's epigenetic state—it would manifest the epigenetic state of a pluripotent stem cell—and its developmental trajectory —it would become a pluripotent stem cell line and would never develop along the trajectory of the human organism if it were ever transferred into the uterus of a woman.

Finally, to anticipate a possible objection, some may suggest that a few molecules cannot change the disposition of matter to a particular form. I beg to differ. A few molecules of cyanide can kill a man. Philosophically, I would have to say that these molecules of cyanide killed him by changing the disposition of his material principle so that it could not be informed by the human soul. We call this change in the disposition of the man's material principle and the ensuing substantial change that follows, death. In a similar way, I propose that the presence of a few molecules of *Nanog* or the absence of a few molecules of *Cdx2* are capable of changing the disposition of matter, such that the newly constituted cell can never be informed by the human soul. Instead, the cell would now be disposed to be informed by the form associated with a pluripotent stem cell.

ANT-Cdx2 and ANT-OAR: A Prudential Analysis

In this essay, I have argued that there are scientific and philosophical reasons to think that ANT would be a technically feasible and morally acceptable alternative to the embryo-destructive research currently needed to harvest pluripotent stem cells, since it would not involve the creation of embryos. This approach should now be more extensively tested in nonhuman systems. Here, I respond to three prudential questions that have been raised by critics of ANT.

First, some have suggested that ANT is not necessary, since many are persuaded that it is unlikely that research with pluripotent stem cells, like embryonic

stem cells, will lead to any therapies that cannot be developed with adult stem cells. Furthermore, they argue that, overall, research with adult stem cells is more likely to produce actual therapies than research with pluripotent stem cells.

In response, it is important to emphasize that we are in the preliminary stages of a new era in the study of developmental biology: it is simply too early to predict what scientific and therapeutic potential directions of inquiry may hold. Clearly, even if it does not lead to direct cell therapies, human pluripotent stem cell research could lead to fundamental discoveries in the basic science of human development that would *indirectly* lead to therapies. Adult stem cell work cannot be an adequate replacement for this dimension of stem cell research. As an analogy, if we want to discover the mechanisms behind language acquisition in human beings, it would be better to study infants learning a language for the first time rather than focus on adults learning a second language. In the same way, if we want to discover the molecular mechanisms behind human development, especially embryonic human development, it would be better to study pluripotent stem cells that have the inherent ability to become the many types of tissues of the human organism rather than focus on adult stem cells that remain relatively specialized. Finally, despite the assertion of many critics of embryonic stem cell research, the possibility remains that research with pluripotent stem cells could produce therapies that cannot be produced by adult stem cells. ANT, by allowing research with pluripotent stem cells, would facilitate creative invention and discovery without the destruction of human embryos.

Next, some have opposed ANT because they suggest that it would lead to the exploitation of women, especially poor women. They argue that ANT would require the use of human oocytes obtained from ovarian superovulation of donors, a procedure that involves health risks to the women without direct benefit to the donors themselves.

As an advocate of ANT, I share the medical and moral concerns associated with ovarian superovulation. Nonetheless, it is important to point out that a woman's donation of her eggs for therapeutic reasons is not intrinsically disordered. If ANT becomes a morally acceptable alternative to protocols involving the destruction of human embryos, one could imagine a scenario where a woman undergoes ovarian superovulation in order to donate eggs that will be used to generate pluripotent stem cells for the medical treatment of her dying child. Akin to organ donation, egg donation in conjunction with morally acceptable forms of regenerative medicine can be understood as an act of self-donation, a self-giving of the human person involving heroic charity. In this case, the donor's action would be morally laudable. Nevertheless, like organ donation, egg donation can be abused and must be regulated. This, however, is a separate moral concern that in itself does not undermine the liceity of ANT.

More fundamentally, there are reasons to believe that scientific advances will, in the not so distant future, allow an abundant supply of human eggs without subjecting women to the medical peril of superovulation for research purposes. These sources may include the procurement of eggs either from ovaries removed for surgical reasons or from cadavers, and the possibly direct production of human eggs from pluripotent stem cells. Of course, given the central human significance of our reproduc-

tive functions, such projects must be conducted with great sensitivity and reserved for serious medical purposes.

Finally, some have raised concerns that research with ANT involving animal cells could never lead to the moral certitude that would justify trying ANT with human cells and eggs. With data obtained from animal research alone, can we risk the lives of embryonic human beings?

In response, I point out that novel medical procedures involving human subjects—even procedures that place human life at risk—are often developed and tested in animal models before they are used with human patients. This approach to biomedical research is justified by the striking parallels between animal and human biology. There are numerous examples in the scientific literature where human genes have animal counterparts that function in the same way. For example, at least eight human genes have been discovered that resemble mouse core clock genes that are responsible for the daily sleep cycles of the mouse. Strikingly, in humans, mutations in two of these genes, *hClock* and *hPer2*, have been implicated in human sleep disorders.[29] These parallels hold in developmental biology as well; we have discovered that embryonic development in different mammalian species is often governed and regulated by the same genes and molecular factors. For example, mutations in genes in the hedgehog pathway, which is known to regulate development in flies and mice, also leads to developmental defects in human infants that parallel the effects in the animal models.[30] These discoveries—and there are many more—justify the use of animal model systems to study and to treat human disease. In the same way, experiments with animal cells, particularly mouse and nonhuman primate cells, should help us to test ANT. If these experiments show that we can meet the criteria for successful application of the ANT approaches described above, then we should have the moral certitude to believe that we could meet the same criteria in the human system.

Conclusion

In summary, philosophically speaking, ANT is a proposal to alter the material disposition of a somatic cell nucleus toward the form of a pluripotent stem cell, by using genetic transformations involving the functional deletion and overexpression of pluripotent-stem-cell-specific transcription factors. To gain further certainty in the technical validity of this approach, it should now be tested in additional model animal systems. Grounded in the philosophical and scientific principles described above, ANT is a reasonable and practical approach that could lead to a morally acceptable alternative to the destructive research currently required to obtain human embryonic stem cells.

[29] For a review that discusses the similarities between human and mouse clock genes, see Hugh D. Piggins, "Human Clock Genes," *Annals of Medicine* 34.5 (August 1, 2002): 394–400.

[30] For a review, see Allen E. Bale, "Hedgehog Signaling and Human Disease," *Annual Review of Genomics and Human Genetics* 3 (2002): 47–65.

APPENDIX

Knowing Embryos, Princes, and Toads: A Further Response to *Communio*

In recent months, several authors writing in the journal *Communio* have raised objections to the ANT proposal in both of its forms.[1] Here, I will respond to their most significant criticisms. As I interpret these objections, they deal with a fundamental question in the philosophy of nature: Can we know the substantial identity of an organism through empirical observation alone?

In several of my earlier writings, I have appealed to the Thomistic axiom *agere sequitur esse* to justify my claim that we can discover the substantial identity of an embryo by observing its organization and its behavior. I have suggested that an organism's acts reveal its nature, since its acts follow from its nature. In a recent essay, David Schindler has argued that my use of this axiom is flawed. He proposes that I commit a fatal philosophical error when I define being "not by what it is, but by what is its *first* (ontological) *effect*."[2] More specifically, he argues that I have conflated the cognitional claim that we know being in its appearance with the ontological claim that being is properly defined in terms of appearance. Thus, Schindler concludes that my argument implies a question-begging definition of being *by its consequences*. It amounts to a "species of ontological *consequentialism*."[3]

Schindler argues instead that being cannot be defined solely by its consequences, that is, its acts. More specifically, he suggests that the substantial identity of a cell or of an organism cannot be revealed by empirical observation alone:

> What it means—and what the "Response to the Joint Statement" does assert—is that *this indispensable role of empirical observation is not, and cannot be, the sole or indeed most basic criterion for ascertaining the ontological identity* [of the cell or of the organism]. On the contrary, the ascertainment requires, coincident with observation of the behavior of an entity, a philosophical judgment that presupposes but does not reduce to a merely empirical criterion.[4]

Therefore, Schindler proposes that the possibility remains open, that "in principle, an organism (embryo) might behave in a disorganized fashion (like a tumor) not be-

[1] In this response, I will focus on the arguments made in the following essays: Adrian J. Walker, "The Primacy of the Organism: A Response to Nicanor Austriaco," *Communio* 32.1 (Spring 2005): 177–187; Adrian J. Walker, "A Way around the Cloning Objection against ANT? A Brief Response to the Joint Statement on the Production of Pluripotent Stem Cells by Oocyte Assisted Reprogramming," *Communio* 32.1 (Spring 2005): 188–194; David Schindler, "A Response to the Joint Statement, 'Production of Pluripotent Stem Cells by Oocyte Assisted Reprogramming," *Communio* 32.2 (Summer 2005): 369–380; Jose Granados, "ANT-OAR: Is Its Underlying Philosophy of Biology Sound?" *Communio* 32.4 (Winter 2005): 724–743; and David L. Schindler, "*Agere sequitur esse:* What Does It Mean? A Reply to Father Austriaco," *Communio* 32.4 (Winter 2005): 795–824.

[2] Schindler, "*Agere sequitur esse,*" 795 (original emphasis).

[3] Ibid., 804 (original emphasis).

[4] Ibid., 797 (original emphasis).

cause it is a non-organism, but because on the contrary it is, or was in its original constitution, a radically defective organism."[5] He goes on to say,

> If, in other words, a non-embryo and a radically defective embryo both unfold in a radically disorganized way, and indeed (possibly) begin to do so from the first moment of their original constitution, it follows that organization and behavior do not suffice, of themselves and without further qualification, to account for the nature of the entity in question.[6]

Schindler summarizes his critique by suggesting that I and the other proponents of ANT have failed to answer adequately what remains the decisive question: "Does this intrinsic relation between the substantial identity of an organism and its epigenetic state entail that substantial identity can be known simply on the basis of and is *nothing more than* the epigenetic state?"[7] In other words, Schindler asks, Can we *really* know the substantial identity of an organism—its essence—from observing its organization and behavior alone? He concludes: "On the basis of what criteria do we render a reasonable response to this question?"[8]

In response, I would like to reply to David Schindler by moving this discussion to a more familiar context with the following questions: Can we know that an animal is a toad? Can we know its substantial identity with some certitude? All reasonable individuals would probably agree that we can. However, how can we know this? In response to Schindler, I propose that we can know an animal's substantial identity by observing it. We look at its organization and its behavior: Does it have short legs, a stout body, and a thick skin with noticeable warts? Does it have four toes on each front leg, and five toes connected by webbing on each hind leg? Are its pupils oval and black with a circle of gold around them? Does it croak with long trill sounds that each last between four and twenty seconds? Does it hop around and live in ponds and streams? Does it eat insects by extending its tongue to capture them? If an animal had all these characteristics that we associate with toads—characteristics that manifest its organization and behavior—then all reasonable individuals would know that it is toad with reasonable certitude.

With this in mind, let me return to and paraphrase Schindler's decisive question: Does this intrinsic relation between the substantial identity of a toad and its organization and its behavior, that is, having short stubby legs with four front toes and five rear ones, croaking with long trill sounds, and capturing insects with a long tongue, entail that substantial identity can be known simply on the basis of, and is nothing more than, the organization and the behavior of the toad? Or, more succinctly, is the substantial identity of a toad nothing more than its organization and behavior in themselves? Certainly not. The hopping, croaking, and capturing of insects do not make a toad. Therefore, Schindler is correct when he writes that the animal's organization and behavior do not constitute its identity. However, I also

[5] Ibid., 800–801 (original emphasis).

[6] Ibid., 801 (original emphasis).

[7] Ibid., 799 (original emphasis).

[8] Ibid., 799–800 (original emphasis).

have to affirm that we can only know an animal's substantial identity through its organization and behavior. How else could we know its identity?

Consider the following thought experiment: What would happen if, suddenly, our animal's behavior and organization changed? Let's say that it grew large, began to walk upright on its two rear legs, and began to speak the Queen's English? Could we still say that it was a toad? Again, I do not think so. We would have to conclude that the animal had been transformed from a toad into a prince. But Schindler asks, *In principle*, could the prince be a radically defective toad? Could it be that the organization and the behavior of the toad has changed—it looks like a prince, speaks like a prince, and kisses like a prince—in such a way that its underlying nature has not changed—it remains a toad, albeit a radically defective one? I admit that in principle, we could say that the prince is a radically defective toad. However, I do not think that it would be reasonable to conclude this. Instead, I suggest that all reasonable individuals would affirm that the differences in the organization and the behavior of the toad and the prince are so radically different that they reveal a substantial change in the identity of the organism has taken place.

But why do we think that the differences in the organization and the behavior of the toad and the prince are radical enough that they reveal a difference in the underlying nature of the two organisms? In contrast, we do not think that the differences in the organization and the behavior of a caterpillar and of a butterfly amount to a difference in their underlying nature. Both are simply stages of the development of the same organism, in the same way that the toddler and the postpubescent teenager are at stages in the development of the same human being.

To respond to Schindler, I propose that we distinguish radical differences in organization and behavior—differences that reveal differences in the underlying natures of the two organisms—by making philosophical judgments about our empirical observations based on our common experience of the stable and perfective natures of the organisms around us. From our experience, we know that toads do not routinely become princes. In contrast, we also know that caterpillars do routinely become butterflies. Indeed, this transformation is perfective and ordered toward the good of the caterpillar, because it is essential for the survival of the organism. Thus, we conclude—reasonably, I think—that the differences in the organization and the behavior of the toad and the prince are radical enough that they reveal a difference in the underlying nature of the two organisms, while the differences in the organization and the behavior of the caterpillar and the butterfly are not radical enough to reveal a similar ontological difference.

In the same way, I suggest that the differences in the organization and the behavior of a cell that becomes a baby and a cell that becomes a tumor—changes that are mirrored in the differences in their epigenetic states—are so radical that they too reveal that an essential difference exists between the two cells. This may not be as obvious as the differences between a toad and a prince, but I would suggest that this is simply because most reasonable people have not really observed embryos and tumors in the same way that they have observed toads and princes. To further my point, consider this second thought experiment: What would we say if the 180-pound prince was suddenly transformed into a 180-pound tumor mass, a transformation

that would be accompanied by radical changes in the epigenetic state of the prince? To make the case even clearer, let us say that the tumor is made up of the same atoms as the original prince. Despite this material identity, could we still say that the tumor mass is simply a radically defective prince who continues to live as this pulsating mass of cellular tissue? In principle, we could do so, but again, I do not think this is reasonable. Princes (and paupers) do not routinely become tumors. It is not in their nature to do that. We would say that the prince had died. He ceased to be and was replaced by something else with its own substantial identity, what we call a tumor. This tumor has its own nature directed toward its own good, its growth and increase in size, which is incompatible with the perfective good of the prince.

In support of this claim, I point out that we do not routinely treat teratomas as defective embryos—we do not remove ovarian tumors, baptize them—even conditionally—and bury them. We simply discard them as defective tissue because we judge that they do not have the substantial identity of a human being, even a radically defective one. Thus, I believe that it is reasonable to conclude that the differences in the organization and the behavior of a cell that, from the very beginning, becomes a prince and a cell that, from the very beginning, becomes a tumor—and the underlying differences in their epigenetic states—reveal that there is a difference in the underlying nature of the two cells. Therefore, these differences can serve as reliable benchmarks for evaluating ANT.

In my writings I have proposed that an organism's acts—its organization and its behavior—reveal its substantial identity since its acts follow from its nature: *agere sequitur esse*. In agreement with Schindler, I affirm that these acts do not determine or constitute the nature of the organism. However, they serve as a window—the only window—into the organism's being through which we can know and judge its nature, a judgment that is always made within the ambit of our common experience of the stable and perfective natures of the organisms that surround us.

Next, in a similar vein, Adrian Walker charges that the other proponents of ANT and I have not shown how changes in the epigenetic state of a cell cause changes in its ontological status. In particular, Walker proposes that we have not shown that "epigenetics is the primary determinant of cellular identity, not only for ordinary somatic cells, but *for totipotent single-celled embryos as well.*"[9]

In response, as I discussed in my accompanying essay, I propose that properly understood, the epigenetic state of a living system reflects its organization and its behavior and thus is a manifestation of its soul.[10] Thus, when it undergoes change, it manifests an underlying change in the ontological nature of the cell. More specifically, I have suggested that genetic manipulations that alter a cell's epigenetic state and its identity, like the overexpression of *MyoD, eyeless, Nanog,* or *Cdx2,* do so by changing the disposition of its material principle to a new form. Again, this is an argument grounded in the Aristotelian-Thomistic axiom *agere sequitur esse*, act follows from

[9] Walker, "Primacy of the Organism," 178 (original emphasis).

[10] For more discussion on the nature of the soul, see the preceding essay, "The Moral Case for ANT-Derived Pluripotent Stem Cell Lines."

being: We know that an isolated skin cell transformed into a muscle cell by *MyoD* in culture has been transformed ontologically because its organization and its behavior are different. Therefore, properly speaking, the epigenetic state of a cell does not determine its ontological status but manifests it. It is a manifestation of the soul.

But what about Walker's more specific objection—how do we know that epigenetics is related to *an embryo's* ontological status? The evidence comes from developmental studies of nonhuman embryos. There are published papers that report that α-amanitin and cycloheximide, two inhibitors of gene expression at the transcriptional and translational levels respectively, can halt embryogenesis and kill different animal embryos.[11] Biologically, these inhibitors kill by changing the epigenetic state of the embryo. Philosophically, however, we would explain their mode of action by saying that they kill embryos by changing the disposition of their material principle for its form such that they undergo a substantial change. In the end, these reports constitute empirical data that demonstrate changing the epigenetic state of an embryo can lead to changes in its essence and thus its substantial identity. I have suggested that we can explain this philosophically by proposing that changing the epigenetic state of the cell leads to changes in its material predisposition to form. Thus, proponents of ANT argue that transforming the epigenetic state of a human somatic nucleus prior to transfer can be equated to changing the predisposition of its material principle, such that it is not apt to receive the rational soul that makes the reconstituted cell an embryo.

Finally, given the similarities between ANT and somatic cell nuclear transfer (SCNT), a similarity highlighted by Adrian Walker and Jose Granados in their essays in *Communio*, we need to ask one question: Is there a point when the product of ANT and the product of SCNT are identical? For instance, Jose Granados has written, "the product of ANT–OAR and the product of SCNT are indistinguishable in their morpho-functional unity during the first stages of reprogramming."[12] Therefore, he concludes, "it must be maintained (at least by those who have reservations about [ANT]) that the product of ANT-OAR, during these first moments of its development, has the same status as the product of SCNT: that is, it is a human being."[13]

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[11] For details and references to the primary literature, see the following review articles: N. A. Telford, A. J. Watson, and G.A. Schultz, "Transition from Maternal to Embryonic Control of Development: A Comparison of Several Species," *Molecular Reproduction and Development* 26.1 (May 1990): 90–100; and E. Memili and N. L. First, "Zygotic and Embryonic Gene Expression in Cow: A Review of Timing and Mechanisms of Early Gene Expression as Compared with Other Species," *Zygote* 8.1 (February 2000): 87–96. For obvious moral reasons, these experiments should not be repeated with human embryos. However, there is no reason to believe that human embryos would behave differently to α-amanitin and cycloheximide, especially since α-amanitin is known to kill adult human beings when it is consumed in poison mushrooms.

[12] Jose Granados, "ANT-OAR," 743. This essay raises other interesting objections to the systems perspective described in my writings. Unfortunately, because of space limitations, I am not able to respond to them in this paper.

[13] Ibid., 742.

	Somatic Cell Nucleus		Genetically Engineered Somatic Cell Nucleus		Oocyte-Reprogrammed Cell Nucleus		Reconstituted Cell Nucleus
ALTERED NUCLEAR TRANSFER:	Epigenetic State A	→	Epigenetic State B	→	Epigenetic State C	→	Epigenetic State D
	Adult Skin Cell		*Cell expressing Nanog, Oct 3/4, etc.*		*Cell expressing Nanog, Oct 3/4, and other, still-unknown factors*		*Pluripotent Stem Cell*
SOMATIC CELL NUCLEAR TRANSFER:	Epigenetic State A		→		Epigenetic State X	→	Epigenetic State Y
	Adult Skin Cell		[Not done in SCNT]		*Cell without Nanog, Oct 3/4, etc., expressing instead the genes required for embryonic function*		*Cloned Single-celled Embryo*

TABLE 1. Comparison of Altered Nuclear Transfer (ANT) and Somatic Cell Nuclear Transfer (SCNT)

In response, Granados is mistaken because he misunderstands the science of epigenetic reprogramming. Reprogramming the somatic cell nucleus to become an embryo is not the same as reprogramming the somatic cell nucleus to become a pluripotent stem cell. Take the analogy of translating an English sentence into French and into Spanish. Translating the English sentence into French is not the same as translating the English sentence into Spanish. There is no time when the partially translated sentences are identical. In the same way, there is no time when the ANT and SCNT products are identical, because the reprogramming that goes on in the two cells involves two different processes, which begin with the same nucleus but follow different, non-overlapping trajectories and pass through different epigenetic states (Table 1). With ANT, the reprogramming of the somatic cell nucleus begins *prior* to its transfer into the enucleated oocyte. In contrast, with SCNT, the reprogramming of the nucleus begins *after* its transfer. Therefore, at fusion with the enucleated cytoplasm, the ANT nucleus is already distinguishable from the SCNT nucleus. They are different nuclei with different epigenetic states. Furthermore, with ANT, the reprogramming of the somatic cell nucleus involves the expression of genes important for the creation of a pluripotent stem cell, genes incompatible with the creation of an embryo. In contrast, with SCNT, the reprogramming of the nucleus involves the expression of another set of genes important for the creation of an embryo, genes that are themselves incompatible with the creation of a pluripotent stem cell. Therefore, the reprogramming trajectories are non-overlapping. Thus, the product of ANT and the product of SCNT are always distinguishable in their morpho-functional unity. Consequently, using the same criteria proposed by Granados, the product of ANT does *not* have the same status as the product of SCNT: that is, it is *not* a human being.

Can we know the substantial identity of an organism through empirical observation alone? For the authors of the *Communio* school, this is the disputed question underlying the current controversy regarding ANT. In response, I affirm that we can know embryos, frogs, and princes with reasonable certitude, and that we can know them through their acts, including their epigenetic acts—the acts that manifest their natures. *Agere sequitur esse*.