monically coupled and hydrogen bonded, and that this coupling has a similar strength in the ground state (populated at lower temperatures) as the first excited state (increasingly populated with increasing temperature). In such a situation, the occupation of accepting modes, and hence T_1, does not depend on temperature. Also, the density of ice does not change appreciably over the studied temperature range and that of pure water only increases by about 5% from 273 to 363 K. Thus, changes in bulk density or water-water interactions are considered to contribute negligibly to the observed temperature dependence of T_1.

In contrast, liquid water shows a strong dependence of hydrogen-bond strength on temperature. Earlier spectroscopic studies showed that with increasing liquid temperature, the hydrogen-bond strength decreases. In this situation, "if the hydrogen bond forms one of the accepting modes of the vibrational energy, this will lead to an increase of the vibrational lifetime" (*3*). Similar arguments were used to explain early measurements of T_1 in hydrogen-bonded systems with hydroxyl groups on silica and in micas (*8*), mixed acid-base complexes (*9*), and in zeolites (*10*).

Woutersen *et al.* incorporated the relative change in hydrogen-bond strength with temperature into an analytical hydrogen-bonding model (*11*) to estimate T_1 lifetimes for the water OH-stretch. The fit of the model results to the experimental data is excellent (see figure), suggesting that the T_1 temperature dependence may indeed be dominated by changes in average hydrogen-bond strength within the medium. Molecular dynamics simulations of liquid water as a function of density and temperature, using the best available potentials, have also obtained hydrogen-bond lifetimes of 10 ps or less (*12*), similar to measured lifetimes (*3–5, 13*).

Can we conclude then that their interpretation is correct? The answer is not simple. Other transient IR investigations of HOD:D_2O at room temperature (*5*) and up to 343 K (*13*) with longer IR pulses have also yielded T_1 lifetimes of about 1 ps. However, using sophisticated two-color IR pump-probe spectroscopy with 1- to 2-ps pulses, one finds that the high-frequency OH-stretch absorption (3200 to 3600 cm^{-1}) is actually composed of three sub-bands (about 45-cm^{-1} wide) from ice-like, dimeric, or extended species, which exhibit T_1 vibrational lifetimes of 0.8 to 1.5 ps and reorientation times in the 3- to 15-ps range. The extremely short, spectrally broad IR pulses used by Woutersen *et al.* (200 fs, 100 cm^{-1} full-width at half maximum) may mask the dynamics of such multiple substructures existing at short time scales because these studies trade spectral resolution for high time resolution.

We are beginning to elucidate the properties of bulk water and ice through measuring its ultrafast molecular dynamics with sophisticated laser techniques. However, further investigations will be required before we will fully understand the intriguing properties of this ubiquitous and important but complex system.

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PERSPECTIVES: BIOMEDICINE

Putting Stem Cells to Work

Davor Solter and John Gearhart

Embryonic stem (ES) cells—pluripotential stem cells that give rise to all adult cell types—can be derived from the blastocyst, a preimplantation stage embryo (*1*), or from primordial germ cells, cells of the early embryo that eventually differentiate into sperm and oocytes (*2*). The derivation of human ES cells has opened up exciting new possibilities for therapy as well as a Pandora's box of legal and ethical controversies.

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Assuming that the currently available human ES cells (or those derived in the future) are similar to their mouse counterparts (an assumption by no means certain), it is likely that they will eventually be used in cell and tissue replacement therapy (*3*, *4*). Mouse ES cells are pluripotent—that is, they can differentiate into many cell types—but whether they are totipotent (capable of developing into all cell types) is unknown. The same is true for human ES cell differentiation. Although it is likely that human ES cells form various cell types and simple tissues, their capacity to build complex organs in culture is entirely unexplored. The judicious exploitation of cell and tissue interactions and the use of extracellular matrices should eventually enable the production of complex organs such as the kidney or lung. One can even imagine using existing organs (human or otherwise) as scaffolding, replacing the original cells with those derived from ES cells.

Using ES cells therapeutically carries inherent dangers. Mouse ES cells are tumorigenic, growing into teratomas or teratocarcinomas when injected anywhere in the adult mouse. There is no reason to believe that human ES cells will not be tumorigenic in humans. Whatever means we use to separate the undifferentiated ES cells from the desired, differentiated progeny to be injected, we will have to be absolutely sure that the separation is complete. As yet, we do not know the minimal number of ES cells necessary to form a tumor or the length of time necessary for tumor development. The answers to these questions will not come from experiments with mice because mice are too short-lived to provide an adequate test. It is entirely possible that we will have to provide some genetically designed fail-safe mechanism, a "suicide" gene, which will enable us to destroy transplanted cells if they become tumorigenic.

Many questions related to the possible therapeutic use of human ES cells have not been addressed in mouse ES cells simply because of the lack of interest. Fortunately, our understanding of the molecular pathways of differentiation and the molecules that mark specific cell types is extensive. This knowledge should help us to answer the following questions: Can human ES cells be forced to differentiate along a desired pathway? Can we make *all* ES cells

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in a culture simultaneously develop along that pathway? What exactly are the intermediary cell types and how can they be defined? Which markers and which methods can be used to sort out the desired cell type? Human ES cell lines will provide many of the answers to these questions.

At the outset it was realized that the full therapeutic potential of ES cells will depend on using ES cell lines derived from the patient's own cells for tissue replacement (*5*, *6*). There may be ways to circumvent this necessity, such as by generating large panels of different ES cell lines so that everybody will find a match or by eliminating or altering the histocompatibility antigens, thus creating "universal" donor lines.

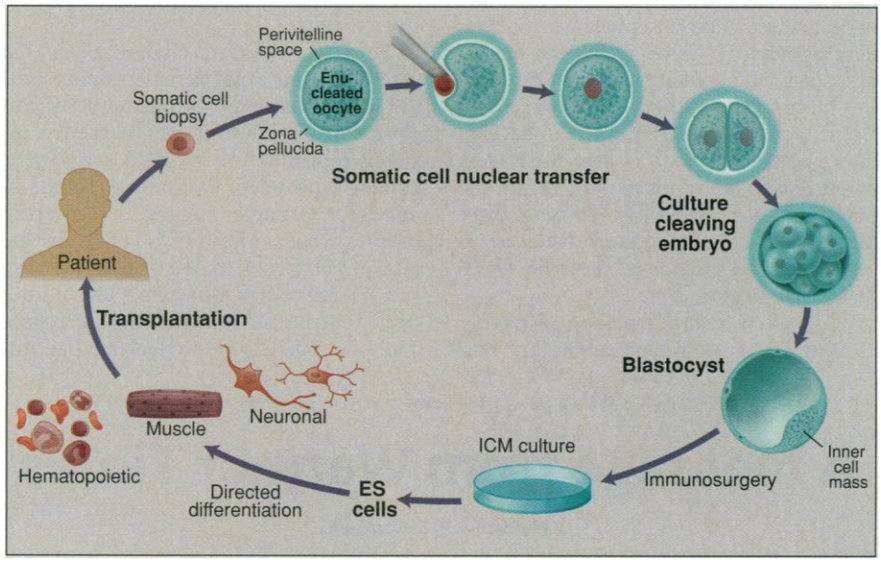
How can we derive ES cell lines that have the genome of a specific individual? In essence, we are asking how to reprogram one of the cells from an adult to become an ES cell. There are three principal approaches (although there may be more). The first is currently called "therapeutic cloning" and involves replacing the genetic material of the human oocyte with the nucleus of the adult cell, as if cloning of the individual were intended (see the figure). The difference is that, instead of placing the resulting embryo in the uterus, it would be cultivated in vitro until the blastocyst stage and then ES cells would be derived from the inner cell mass of the blastocyst (*1*). This is the most straightforward approach, but it involves taking a human blastocyst whose capacity for developing into a human being is uncertain but does exist, and subverting its development to yield cells that no longer have this capacity.

The second possibility is to use any mammalian oocyte as the nuclear recipient. Such an approach has been tried—the nucleus from an adult human cell was transferred into an enucleated cow oocyte—but it proved impossible to assess the success of the procedure (*7*). The limited studies in mice testing the possibility of nuclear transfer across species have not yielded encouraging results (*8*). If this approach does work, the exact nature of the resulting blastocysts would certainly pose a significant scientific, ethical, and legal quandary. For example, would the embryo developing from a human adult cell nucleus transferred to an enucleated cow oocyte be considered human? Could this embryo develop to term?

The third possible approach is to reprogram the nucleus of adult cells using ES cell cytoplasm. Such "cybrids" would be prepared by fusion of ES cell cytoplasts with somatic cell karyoplasts. Fibroblast nuclei transplanted into hepatoma cell cytoplasts expressed one liver-specific gene, but the extent of nuclear reprogramming was not determined (*9*).

A debate is now brewing about whether ES cells should be considered embryos (*10*). Studies of mouse ES cells may help to resolve this debate. An ES cell is not a fertilized egg and it does not have the capacity to develop into a conceptus, that is, an embryo and its extra-embryonic membranes (those forming placental tissues). ES cells placed into the uterus will never develop into a mouse. When ES cells are transferred to the interior of a blastocyst, derivatives of the transplanted cells can be found in all embryonic cell lineages—that is, they form chimeras in which the transferred cells and their derivatives comingle with cells of the host. It has also been demonstrated with tetraploid host blastocysts that mice can be obtained that are solely derived from the ES cells transferred into the blastocyst (*11*). The embryonic cells of the tetraploid host perish, and the ES cells and their derivatives survive, producing a mouse derived entirely from the grafted ES cells. So, ES cells cannot develop into an embryo on their own; they must be placed into an artificial environment, one in which the host cells provide the placental tissues of the conceptus. Human ES cells are predicted to behave in the same way. But replicating the mouse experiment with human embryos would be unethical, as one would have to intentionally produce a defective human blastocyst in order to produce offspring derived from ES cells.

The production of chimeras has demonstrated that mouse ES cells can form all cell types of the organism, but it is not clear whether they can form all extra-embryonic tissues of the conceptus. Thus, it is not clear whether ES cells are totipotent or pluripotent, and clarifying this critical issue will depend on our ability to mimic the events of embryogenesis in a culture dish. The term "totipotent" has also been used in a different context to mean a cell that is capable of making an entirely new embryo, fetus, and adult organism. Clearly, under this definition, ES cells are not totipotent.



Tissues for transplantation. A cell biopsy is taken from the patient, and the nucleus of the somatic cell is transferred into an enucleated donor oocyte with the nuclear transfer techniques pioneered in mice and sheep. The resulting embryo is allowed to develop until the blastocyst stage. The inner cell mass (ICM) of the blastocyst is then recovered by immunosurgery and cultured, and the embryonic stem (ES) cells are harvested from it. The ES cells are then directed to differentiate into the particular cell type required (for example, dopaminergic neurons to replace those lost in Parkinson's disease, pancreatic islet cells for patients with diabetes, hepatocytes to treat liver cirrhosis) and are transplanted into the patient.

To derive ES cells that carry the genome of a patient, it will be necessary to transfer genetic material from cells of the patient into an enucleated human oocyte, allow the embryo to develop to the blastocyst stage, and then derive ES cells from the blastocyst. The vast majority of embryos created in this way would not be able to develop further (as far as current experimental data suggest), but the procedure itself prevents us from definitely excluding such a possibility. Society must decide whether the therapeutic benefits justify denying full development to the constructed embryos. A recent ruling decreed that federal funds can be used for research on ES cells that have already been

derived from studies funded privately, but cannot be used to derive new cell lines from blastocysts. Obviously, if the procedure is legally prevented, the embryos will not be created (at least not with federal funds) and the question of their full development will remain moot.

The nuclear transfer procedure is required for both ES cell therapy and for cloning. This methodological identity has led to confusion of the two in people's minds. To avoid this problem, the terms therapeutic cloning and reproductive cloning have been introduced, but these terms must be precisely defined and the public constantly reminded of the definitions. Therapeutic cloning would involve transfer of the nucleus with the desired genetic material into an enucleated oocyte, development of the oocyte to the blastocyst stage, and derivation of ES cells from the blastocyst for therapeutic purposes. But if the blastocyst is allowed to develop into a newborn, which is then used as an organ donor, the result is also therapeutic but clearly not permissible. To avoid these difficulties, it would be much better to reserve the term cloning to describe reproductive cloning.

Current attempts at reproductive cloning in livestock and laboratory animals indicate that it is not a very safe procedure. The majority of clones fail sometime during development, and some fail after birth. If the recent "success" of cloning cows in which four out of eight calves died soon after birth (*12*) is anything to go by, it would be irresponsible and unethical to attempt the cloning of humans because this would almost certainly condemn a large fraction of the infants to death or malformation. We know very little about the events involved in reprogramming the nucleus—activation of previously silent genes, initiation of DNA synthesis, alteration in chromatin structure—and even less about the molecular pathways set in motion once the adult nucleus is placed into an enucleated oocyte.

For most, the term cloning means the creation of an exact genetic duplicate of the nuclear donor. The technique of nuclear transfer, however, can be used in assisted reproduction in such a way that cloning is avoided. Preliminary results in mice, for example, suggest that infertile couples whose infertility is caused by the lack of germ cells in one or both partners can be helped in this way. The nuclei from somatic cells of the infertile couple could be transferred to enucleated germinal-stage oocytes and, after meiosis, two haploid genomes (one from each parent) could be combined in a single oocyte. If this approach worked, the resulting child would be a random genetic combination of the parental genomes, the same as every other human.

It is our view that these and other benefits of nuclear transfer and cloning far outweigh the possible harm, but they can only be achieved through determined experimental effort. It will ultimately be up to society to decide which way to go, but one must hope that the decision will be an informed one and not based on irrational fear, ignorance, and prejudices.

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PERSPECTIVES: MARTIAN CLIMATE

A Message from Warmer Times

Matthew P. Golombek

When the Sojourner rover crawled over the martian surface last year, the images returned by the mission indicated that the site had changed little from when it was created by catastrophic floods some 1.8 to 3.5 billion years ago (Ga) (*1, 2*). This observation provides quantitative constraints on the rate of change at the landing site since that time. The Pathfinder data, taken together with those from the recent Global Surveyor missions and the 20 year old data from the two Viking landers, suggest an early warmer and wetter environment with vastly different erosion rates and a major climatic change on Mars between then and now.

Pathfinder observed a rocky surface composed of ridges and troughs, perched, imbricated, and partially rounded tabular rocks, and streamlined hills that is analogous to catastrophically deposited fans on Earth, such as the Ephrata Fan of the Channeled Scabland in Washington State (*1–4*). This similarity argues for the site being little altered since it formed (*4*) roughly 1.8 to 3.5 Ga (*5*).

Erosional features such as an exposed former soil horizon, sculpted wind tails, coarse pebble-rich surfaces (see figure), and ventifacts (rocks abraded by windborne particles) are abundant at the Pathfinder landing site, suggesting that the site has undergone net deflation or loss of material (*2, 6*). The 5- to 7-cm-thick redder band along the base of several rocks, interpreted as a deflated soil horizon, and the sculpted erosional wind tails behind rocks that are less than 3 cm high (*2, 6*) suggest extremely low deflation rates of around 0.01 to 0.04 nanometers (1 nm = 10^{-9} m) per year. Coarse pebble-rich surfaces and at least some of the dunes, such as Mermaid Dune, appear to be composed of poorly sorted material beneath an armoring veneer of dark gray granules, as could be seen in the trenches created by the rover. These have been interpreted as lag deposits (*7*) left behind after loss of finer windborne material, and thus also indicative of net erosion or deflation of the landing site. The presence of fluted and grooved rocks also argues for erosion crystalline sand-size particles carried by the wind (*8*). In contrast, wind deposits at the Pathfinder site are limited to a few dunes, including a crescent-shaped feature imaged by the rover. These features were most likely formed from sand-size grains entrained in the wind (*6*). The immaturity of the ventifacts and their different orientation from the dunes and wind trails has led to the suggestion that the dunes may have formed earlier when the supply of sand-size particles was greater (*8*).

The rim heights of small craters at the site are similar to those expected for fresh martian craters. This places similar (<1 nm/year), albeit less precise, constraints on erosion rates at the Pathfinder (*9*) and the Viking 1 landing sites (*10*) and suggests that a cold and dry environment, similar to today's, has prevailed since 3.1 to 3.7 Ga.

A variety of observations by Pathfinder indicate that the earlier martian climate was warmer and wetter than today's desiccating environment. Rounded pebbles and cobbles (*7*), evidence for abundant sand-size particles (*6*), and possible conglomerates (*7*) at the Pathfinder landing site sug-

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