

timately had the highest medical costs: those who normally filed no medical claims and those with chronic diseases who filled monthly prescriptions for maintenance medications fewer than 10 times per year. To encourage the first group to seek primary care, managers decided to charge employees nothing for most preventive services, no more than \$20 for the most expensive ones, and \$20 for visits to primary care providers. To encourage workers with chronic diseases to take medication, the company reduced copayments on all drugs for hypertension, asthma, and diabetes to 10%. Although the company's spending on these drugs increased, its overall costs for the three diseases dropped, and it ex-

panded the policy to cover several other conditions. Today, the firm says its health costs per employee are roughly 20% below those of comparable employers.

Of course, most Americans work for much smaller employers, but Michael Critelli, chief executive officer at Pitney Bowes, believes such programs make economic sense even for those organizations, because having one or two workers with high-cost illnesses can be catastrophic for a small business. "Our philosophy was [that] people get sick for the most part because of behaviors that are preventable and changeable," Critelli said. "Taking care of your health is free. If you do it right up front, it's by far

the most cost-effective way to deliver health."

Dr. Okie is a contributing editor of the *Journal*.

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Pluripotency Redux — Advances in Stem-Cell Research

John Gearhart, Ph.D., Evanthia E. Pashos, B.Sc., and Megana K. Prasad, B.Sc.

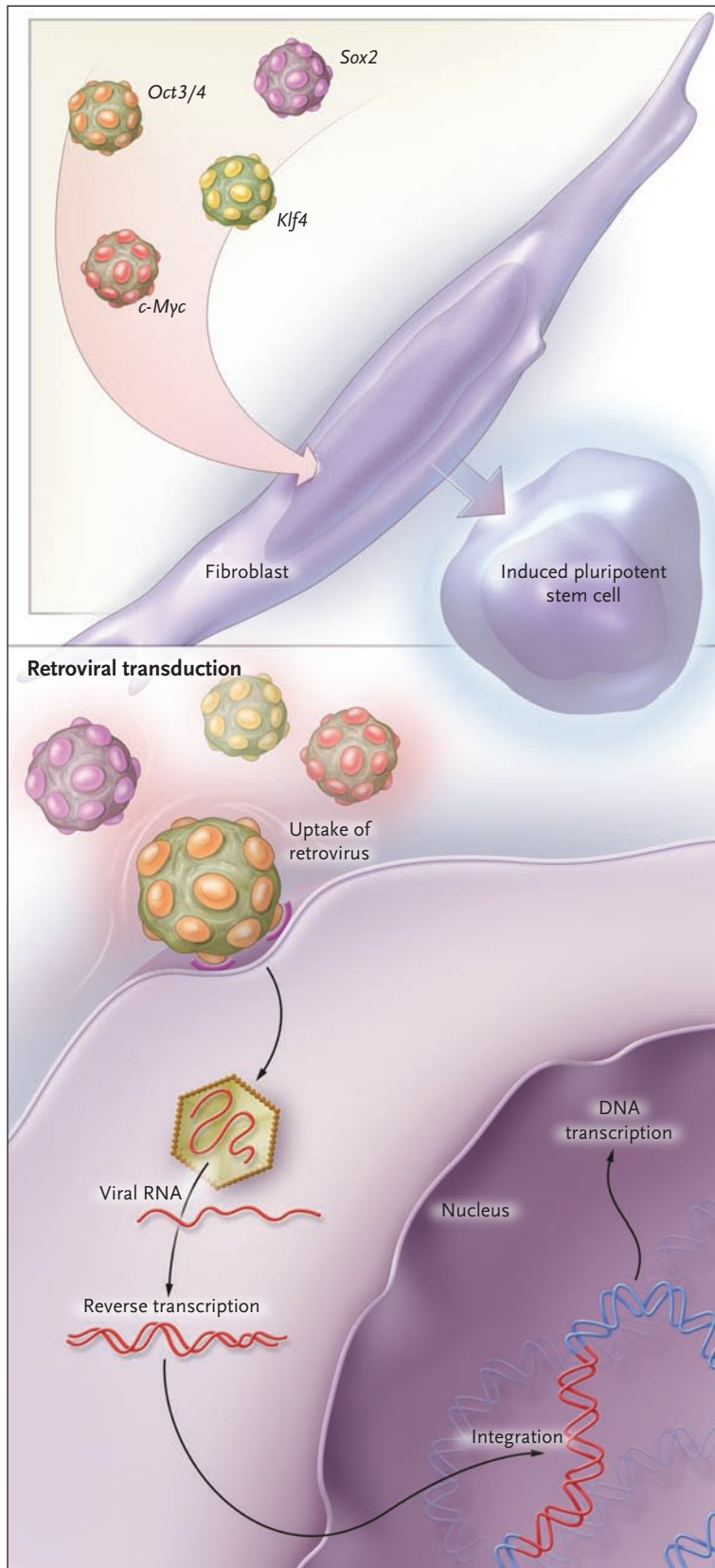
A cell's ability to give rise to all the cell types of the embryo and the adult organism is called pluripotency. Pluripotent cells are found within mammalian blastocysts and persist briefly in embryos after implantation. Embryonic stem cells, derived from the inner cell mass of blastocysts, are a renewable source of pluripotent stem cells that are proving valuable in basic science studies and may eventually become a source of cells for safe, effective cell-based therapies. Much embryonic stem-cell research has focused on determining the molecular signature of pluripotency, and a picture is emerging of a complex interaction among transcription factor net-

works, signaling pathways, and epigenetic processes involving modifications in the structure of DNA, histones, and chromatin.

Deciphering the molecular basis of pluripotency will facilitate the development of procedures for efficiently deriving patient-specific stem cells. In somatic-cell nuclear transfer, which has held the greatest promise for generating such cell lines, the nucleus of a somatic cell is introduced into an enucleated oocyte or mitotic zygote and is "reprogrammed" to an embryonic state, resulting in the formation of a blastocyst from which embryonic stem cells can be derived. Although this procedure has been demonstrated in animals, it

has yet to be accomplished with human oocytes or zygotes. An alternative approach to reprogramming a somatic cell is to fuse it with an embryonic stem cell, but the resulting hybrid pluripotent cell is tetraploid and of limited practical application.

Against this background, a study published last year by Takahashi and Yamanaka¹ surprised and excited stem-cell biologists. Using a novel strategy, the investigators showed that fibroblasts derived from tissues of adult and fetal mice could be induced to become embryonic-stem-cell-like cells with the introduction of four genes expressing transcription factors. Twenty-four genes



Induction of Pluripotent Stem Cells through Retroviral Transduction.

Retrovirally encoded transcription factor genes were introduced into mouse embryonic and adult fibroblasts. After integration and expression of the transgenes, the fibroblasts were reprogrammed to pluripotency.

were initially chosen as candidates on the basis of their preferential expression in embryonic stem cells or their known roles in the maintenance of such cells or in cell-cycle regulation. These genes were introduced into fibroblasts isolated from mouse embryos and adult tail tips in a combinatorial manner through retroviral transduction.

Fibroblasts that are induced to become pluripotent stem cells were selected through the expression of *Fbx15*, a gene known to be expressed in pluripotent cells. The investigators discovered that only four factors — encoded by *Oct3/4*, *Sox2*, *Klf4*, and *c-Myc* — were sufficient to induce pluripotency (see diagram). The induced pluripotent stem cells had some properties of embryonic stem cells: they formed teratomas when grafted into immunocompromised mice, and they formed embryoid bodies (aggregates of embryonic stem cells that can spontaneously differentiate). However, they differed substantially from embryonic stem cells in their gene-expression and epigenetic profiles, and they failed to form live-born chimeric pups when injected into blastocysts.

Recently, the generation of higher-quality induced pluripotent stem cells has been reported in three independent studies.²⁻⁴ The new lines not only resemble embryonic stem cells more closely in their transcriptional and chromatin-modification profiles but

are capable of generating adult chimeric mice with contributions to the germ line — the most rigorous test for pluripotency. The main procedural difference between the production of these lines and that of Takahashi and Yamanaka's line was the selection scheme for identifying the reprogrammed cells. The initial strategy relied on the induced expression of *Fbx15* in the transduced fibroblasts. This gene is expressed in embryonic stem cells but is not required for pluripotency. The recent studies were designed to select for expression of *Nanog* or *Oct3/4*, which are essential for pluripotency and embryonic stem-cell identity.

The molecular changes characteristic of pluripotency occurred gradually during weeks in culture. How these four factors induced reprogramming is unknown, but their known roles suggest hypotheses. *Oct3/4* and *Sox2*, along with *Nanog*, form a core regulatory network for pluripotency in embryonic stem cells. *Oct3/4*^{-/-} embryos die in utero because of defects in the inner cell mass; *Oct3/4* repression in mouse embryonic stem cells results in a loss of pluripotency and differentiation into trophectoderm, and *Oct3/4* overexpression leads to the loss of pluripotency and differentiation into primitive endoderm and mesoderm. Similarly, *Sox2*-null mice die during the peri-implantation period because of epiblast defects, and *Sox2* knockdown in embryonic stem cells leads to trophectoderm differentiation. (Pluripotency is known to be maintained by a few transcription factors, including *Oct3/4*, *Sox2*, and *Nanog*. We hypothesize that the dispensibility of *Nanog* as an introduced factor in these experiments can be ex-

plained by the induced expression of the endogenous *Nanog* gene by cooperativity between *Oct3/4* and *Sox2*.)

The proto-oncogene *c-Myc* is believed to regulate the expression of 15% of all genes, including genes involved in cell division, cell growth, and apoptosis. It exerts its effects on transcriptional targets through various mechanisms — there are positive effects from recruitment of histone-modifying enzymes, general transcriptional machinery, and chromatin-remodeling complexes and negative effects from recruitment of DNA methyltransferases. In many contexts, *c-Myc* drives cell proliferation and inhibits differentiation, as in mouse embryonic stem cells. Such cells proliferate and remain in an undifferentiated state in culture when the cytokine leukemia inhibitory factor is provided, but forced expression of *c-Myc* eliminates the requirement for this factor. Surprisingly, *c-Myc* behaves quite differently in human embryonic stem cells, where it induces apoptosis and differentiation. Given that tumors developed in 20% of mice derived from induced pluripotent stem cells owing to the reactivation of the *c-Myc* transgene,² it is clear that we must determine which role of *c-Myc* is essential to reprogramming to obviate the need for introducing the proto-oncogene into cells. The use of a series of *c-Myc*-deletion mutants may clarify its role in reprogramming.

Klf4 promotes self-renewal of mouse embryonic stem cells, probably through a leukemia-inhibitory-factor–dependent pathway. A recent report suggests that *Klf4* behaves in a context-dependent manner, mediating a cytostatic function by repressing *p53*, a re-

pressor of *Nanog*, or by promoting cell proliferation in collaboration with the H-RasV12 oncogene.

The identification and characterization of the responsive cells in the target population of primary fibroblasts may also help us understand these results. The low efficiency with which induced pluripotent stem cells were generated (<0.01%, despite a 50% transduction rate) may indicate that only a subgroup of cells can be induced to pluripotency. The skin, a complex tissue with robust regenerative capabilities, contains a variety of stem cells, including epidermal, mesenchymal, neural crest–derived, and stem cells or progenitors from the circulation. Fibroblasts derived from the skin represent a heterogeneous population of cells. Skin fibroblasts from various anatomical sites have distinct gene-expression patterns, varying in terms of the genes involved in pattern formation, cell-cell signaling, extracellular matrix synthesis, and fate determination. Furthermore, fibroblasts from a single skin region have widely varying morphologic and physiological characteristics. Perhaps the induced stem cells are derived from a rare fibroblast subpopulation that is already multipotential and more easily induced to pluripotency. Determining the identity of such a subpopulation may aid in increasing the efficiency of reprogramming. Repeating these experiments with a variety of differentiated cell types and subpopulations of fibroblasts from various tissue sources will be informative.

Whether these four factors (or others) will be capable of inducing pluripotency in human cells that will prove safe for use in cell therapies remains unknown. Differences between human and

mouse embryonic stem cells in the mechanisms of pluripotency suggest that other factors may be required to achieve similar results with human cells. Further investigation of the factors is needed to elucidate their roles in reprogramming and to ensure that we can avoid any detrimental effects they may have on cells. Transient expression of factors (using vectors that do not integrate into the genome) in fibroblasts or the identification and use of small molecules that mimic the effects of the factors would enable researchers to avoid the possibility of generating mutations in the genome through random insertions and reactivation of transgenes in the retroviral vectors.

Reprogramming of adult cells to generate patient-specific therapies represents the future for stem-cell biologists. Inducing plu-

ripotent stem cells is the first successful way of instructing somatic cells to become pluripotent by introducing defined factors. A recent report on identifying induced pluripotent stem cells on the basis of morphologic criteria alone brings us a step closer to translating this work safely into human cells. Such identification would obviate the need for transgenic reporter genes in the donor fibroblasts.⁵ Despite these encouraging results, research on human embryonic stem cells should not be impeded; such cells remain the gold standard for determining the molecular basis of human tissue development and for developing cell-based therapies for human diseases.

An interview with Dr. Douglas Melton, a scientific director of the Harvard Stem Cell Institute and a professor in the Department of Molecular and Cel-

lular Biology at Harvard University, can be heard at www.nejm.org.

Dr. Gearhart is a professor of gynecology and obstetrics, physiology, comparative medicine, and biochemistry and molecular biology and the director of the Stem Cell Biology Program at the Institute for Cell Engineering, Johns Hopkins Medical Institutions, Baltimore, where Ms. Pashos and Ms. Prasad are Ph.D. candidates in the Human Genetics and Molecular Biology Program.

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Hematopoietic-Cell Transplantation at 50

Frederick R. Appelbaum, M.D.

September 12, 2007, marked the 50th anniversary of E. Donnall (Don) Thomas's initial report of a radical new approach to cancer treatment: radiation and chemotherapy followed by the intravenous infusion of bone marrow.¹ That publication represented the beginning of a long series of laboratory and clinical investigations; more than a decade would pass before the procedure achieved its first successes. Yet Thomas's persistence in the face of criticism and clinical failure ultimately paid off in a new form of therapy that was used to treat approximately

50,000 people worldwide in 2006 (see timeline).

Thomas's interest in the possibility of hematopoietic-cell transplantation was sparked in 1949, during his residency at Peter Bent Brigham Hospital in Boston, when he learned of Leon Jacobson's experiment showing that a mouse exposed to otherwise lethal irradiation would survive if its spleen, or in later studies its marrow, was shielded.² That its survival was due to a cellular rather than humoral effect was proven several years later, when researchers showed that irradiated mice giv-

en an infusion of marrow with a chromosome marker recovered with marrow cells exclusively of donor origin. With that experiment, Thomas became convinced of the clinical potential of human marrow transplantation.

In 1955, he moved to the Mary Imogene Bassett Hospital in Cooperstown, New York, and began working with Joseph Ferrebee. Thomas and Ferrebee's 1957 article describes the first experience with allogeneic marrow transplantation in humans: six patients were treated with irradiation and chemotherapy and then intravenous