ter is only cold. Because the main problem with $\Omega = 1$ cosmologies containing only cold dark matter is that the amplitude of the galaxy-scale inhomogeneities is too large compared with those on larger scales, the presence of a little hot dark matter could be just what is needed.

If the new data (*21*) from high-redshift supernovas suggesting that $\Omega \approx 0.4$ are confirmed, then the amount of neutrino mass allowed is decreased, because there will be less cold dark matter (*18*). But the success (*1*) of the cold + hot dark matter model in fitting the cosmic microwave background and galaxy distribution data certainly suggests that low-Ω cosmologies with mostly cold and a little hot dark matter should be investigated in more detail (*22*).

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DEVELOPMENTAL BIOLOGY

Cloning for Profit

Gary B. Anderson and George E. Seidel

The genetic "parent" of Dolly—the cloned sheep that captured the imagination of the scientific community and the general public last year—was the nucleus from a single adult mammary gland cell (*1*). This nucleus was substituted for the chromosomes normally provided by the sperm and egg at fertilization. The resulting embryo, transferred to the oviduct of a surrogate mother, grew to be Dolly. While the media concentrated on the potential benefits and pitfalls of cloning in humans, after Dolly, embryologists have been using cloning procedures to efficiently generate transgenic farm animals with defined—and commercially useful—genotypes (*2*). A report in last week's issue highlighted this progress in the biology of cloning. Cibelli *et al.* (*3*) reported the birth of normal, genetically identical calves whose genetic "parents" were a line of transgenic fibroblasts derived from a 55-day bovine fetus.

To many people, cloning was invented with the birth of Dolly. In fact, cloning has been practiced for millennia in plants and for decades in mammals, and Dolly's birth followed an orderly progression of experiments that started with cloning mammalian embryos. Broadly defined, cloning is asexual reproduction that results in a genetically identical organism. For example, when a plant cutting is rooted, a new clone is produced, completely identical to the parent. Cloning of mammalian embryos first became a useful laboratory technique in the 1970s when procedures were developed for the culture of individual blastomeres. This allowed commercialization of procedures in cattle in which single embryos were split into several pieces (usually two), each giving rise to a complete embryo. Such efforts have produced many thousands of cloned calves that are routinely used for cattle breeding. Because such split embryos (as well as embryos in which the nucleus has been replaced) can be cryopreserved, researchers with sufficient foresight could freeze some members of genetically identical sets. Thus, it already was possible, even before Dolly, to copy an adult from a cryopreserved, genetically identical embryo (*4*).

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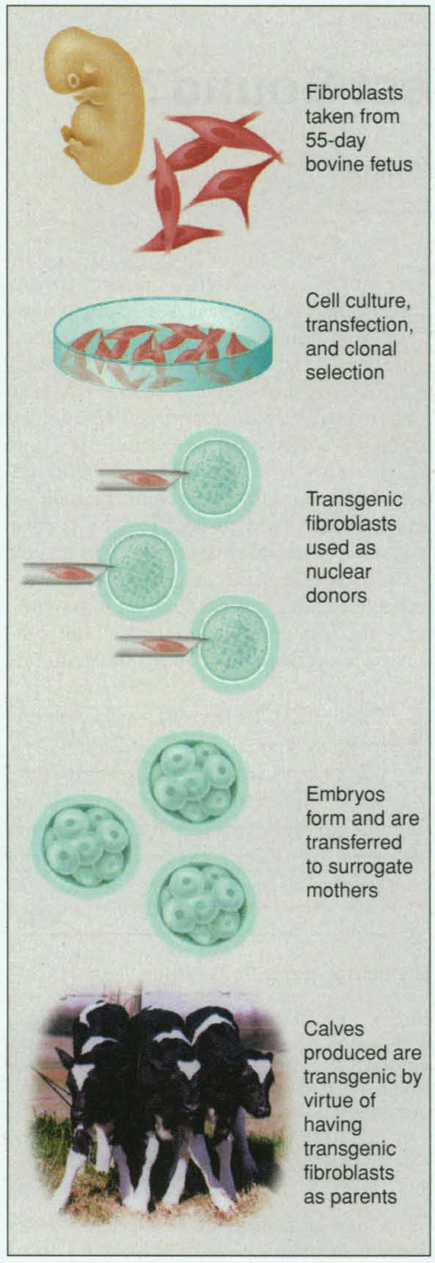
The first successful mammalian cloning by nuclear transfer, in which cells from cleavage-stage sheep embryos were fused with unfertilized sheep eggs, was reported in 1986 (*5*). The reconstituted embryo contained not only the nucleus of the donor (parent) cell, but its cytoplasm as well. This procedure, therefore, results in two sources of mitochondria, producing a mitochondrial mosaic if the donor and recipient cells are from different maternal lines. Subsequent experiments defined the conditions that were needed for survival and development of nuclear-transfer embryos, and also extended nuclear donation to cells from older and older embryos (*6*). Successful cloning from older embryos (and ultimately from an adult cell, in the case of Dolly) challenged conclusions from work on amphibia and mammals that indicated that, as cells become more differentiated, they become unable to support normal development. Re-

cently, lambs were successfully generated from eggs with nuclei from embryonic cell lines that had been established and maintained in long-term culture (*7*, *8*) and appeared to have differentiated. Fetal fibroblasts also have been used as a source of donor nuclei to produce live lambs (*1*, *2*).

Practical application of cloning technology is the norm in the plant field; entire forests are composed of genetically desirable, cloned trees. When the appropriate technology becomes sufficiently reliable, cloning could be used to multiply unique animal genotypes for agricultural production, but the first application will be to produce transgenic animals for commercial use. Initial examples are sheep that were engineered to produce the human blood-clotting protein factor IX in their milk (*2*); the efficient generation of transgenic calves expressing a marker gene (*3*) illustrates the same principle.

The most common procedure for producing transgenic mammals uses microinjection of DNA into one-cell zygotes, but the efficiency of this technique in large domestic species is disappointingly low and the cost discouragingly high. An alternative approach is to manipulate the genome of cells in vitro and use these genetically engineered cells to produce transgenic animals by nuclear transfer. Schnieke *et al.* (*2*) produced transgenic lambs by introducing a foreign gene into fibroblasts subsequently used for nuclear transfer. The Cibelli *et al.* (*3*) report of deriving transgenic calves from transgenic fibroblasts cements the validity of this approach, which requires fewer embryos than direct DNA microinjection into zygotes. Cloning from transfected cells has other advantages for making transgenic farm animals (see the figure): This method allows verification of proper transgene integration in vitro, before the transgenic animals are produced. The sex of the nuclei can also be determined beforehand, and the problem of mosaicism in the transgenic founder, which can result in failure of transgene transmission in the gametes, is eliminated. In the future, manipulation of somatic cells could allow transgenic animals to be made with a change at a targeted site in the genome.

Despite the high-profile successes with nuclear transfer in mammals, the basic cell biology of the cloning process is not yet well understood. Originally it was believed that differentiated cells would be inappropriate as nuclear donors because of changes in the DNA during differentiation—for example, epigenetic methylation of cytosines. Individual cells of an early embryo are totipotent, but as embryonic cells differentiate, their lineages become defined and totipotency is lost, or at least becomes difficult to reestablish. Now it is clear that some differentiated cells can regain totipotency, but to do so their nuclei must be able to repeat the developmental events initiated in the recently fertilized egg. Little is known about the changes in the DNA resulting from a cell's taking on a specialized function and about how these changes are reversed when a nucleus is transferred to the recipient egg's cytoplasm; apparently some cell types are more amenable to undergoing these changes than others.



Cattle on demand. Production of transgenic animals by somatic cloning.

Nuclear transfer with embryonic and fetal cells more reliably results in live offspring than does nuclear transfer with adult cells—for unknown reasons. Perhaps this difference is due to decreased and variable telomere length in adult cells. Random selection of nuclei may yield those with sufficiently long telomeres only rarely, which may explain why few adult cells [1 of 247 in the paper by Wilmut *et al.* (*1*)] are successful donors. Species differences also exist. For example, most nuclear transfer procedures in mice have been unsuccessful except with nuclei from embryos at the earliest stages of development.

The list of somatic cell types that can support development of animals to term after nuclear transfer is short—including only mammary cells, fetal fibroblasts, and fetal muscle tissue. But if the cells are taken from the embryo, fetus, or neonate, additional cell types [for example, fetal or neonatal skin (*9*, *10*)] can establish pregnancy from embryos reconstituted by nuclear transfer. Differences among cell types might be expected in their response to nuclear transfer, but prediction of which cell types are most likely to be successfully reprogrammed for normal development to term is not yet possible. An intuitive conclusion is that adult cells that no longer divide, such as neurons, would be poor candidates, and conversely, cells that continuously divide and differentiate, such as epidermal cells, would be good candidates; however, no definitive conclusions can be drawn without additional research.

An important requirement in nuclear transfer is that the stage of the cell cycle for the nuclear donor and the host cytoplasm be compatible. Compatibility can be managed in several ways. Some investigators have argued that forcing the donor cell into a quiescent G_0 stage of the cell cycle by reducing the serum content of the donor cell's culture medium enhances nuclear reprogramming, perhaps by switching off genes in the differentiated cells. But Cibelli *et al.* have shown that calves can be generated after nuclear transfer with actively growing fibroblasts, probably from those that chance to be in G_1 of the cell cycle. Thus, serum starvation is unnecessary for term development. The rapid pace of mammalian cloning experiments promises new knowledge in developmental biology and numerous opportunities to apply cloning and related biotechnologies.

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