latter view is in line with the observations that the early mammalian embryo is a highly regulative system, and that the body axes do not become fixed until the end of cleavage or early gastrulation.

EXPERIMENTAL MANIPULATIONS OF CLEAVING EMBRYOS

Much of the knowledge about the developmental properties of early mammalian embryos is the result of more recently devised techniques for experimentally manipulating them. Typically, the use of these techniques must be combined with other techniques that have been designed for in vitro fertilization, embryo culture, and embryo transfer (see Chapter 2).

Classic strategies for investigating the developmental properties of embryos are (1) removing a part and determining the way that the remainder of the embryo compensates for the loss (such experiments are called **deletion** or **ablation experiments**) and (2) adding a part and determining the way that the embryo integrates the added material into its overall body plan (such experiments are called **addition experiments**). Although some deletion experiments have been done, the strategy of addition experiments has proved to be more fruitful in elucidating mechanisms controlling mammalian embryogenesis.

Blastomere deletion and addition experiments (Fig. 3-10) have convincingly shown the regulative nature (i.e., the strong tendency for the system to be restored to wholeness) of early mammalian embryos. Such knowledge is important in understanding why the exposure of early human embryos to unfavorable environmental influences typically results in either death or a normal embryo.

One of the most powerful experimental techniques has been the injection of genetically or artificially labeled cells into the blastocyst cavity of a host embryo (see Fig. 3-10B). This technique has been used to show that the added cells become normally integrated into the body of the host embryo, providing additional evidence for embryonic regulation. An equally powerful use of this technique has been in the study of cell lineages in the early embryo. By identifying the progeny of the injected marked cells, investigators have been able to determine the developmental potency of the donor cells.

A technique that is providing great insight into the genetic control mechanisms of mammalian development is the production of **transgenic embryos**. Transgenic embryos (commonly mice) are produced by directly injecting foreign DNA into the pronuclei of zygotes (Fig. 3-11A). The DNA, usually recombinant DNA for a specific gene, can be fused with a different regulatory element that can be controlled by the investigator.

Transgenic mice are created by injecting the rat growth hormone gene coupled with a metallothionein promoter

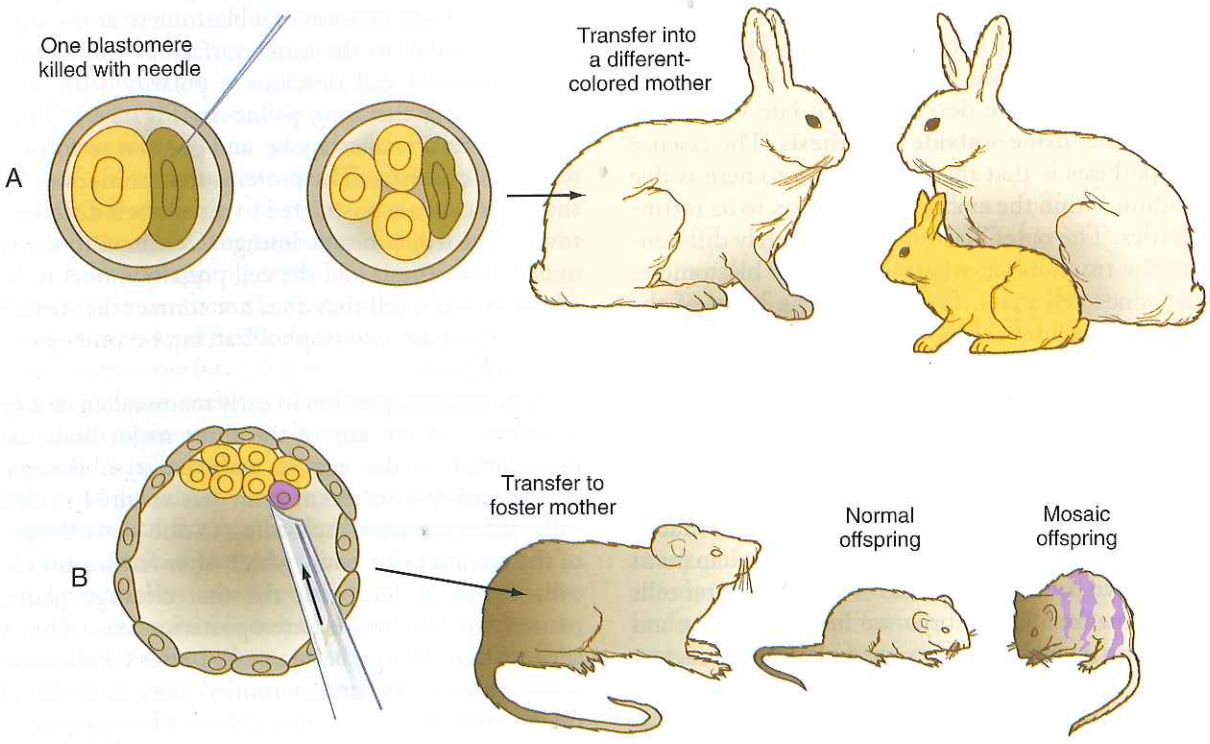

FIGURE 3-10. Blastomere addition and deletion experiments. **A,** If one blastomere is killed with a needle, and the embryo is transferred into a different-colored mother, a normal offspring of the color of the experimentally damaged embryo is produced. **B,** If a blastomere of a different strain is introduced into a blastocyst, a mosaic offspring with color markings characteristic of the strain of the introduced blastomere is produced.

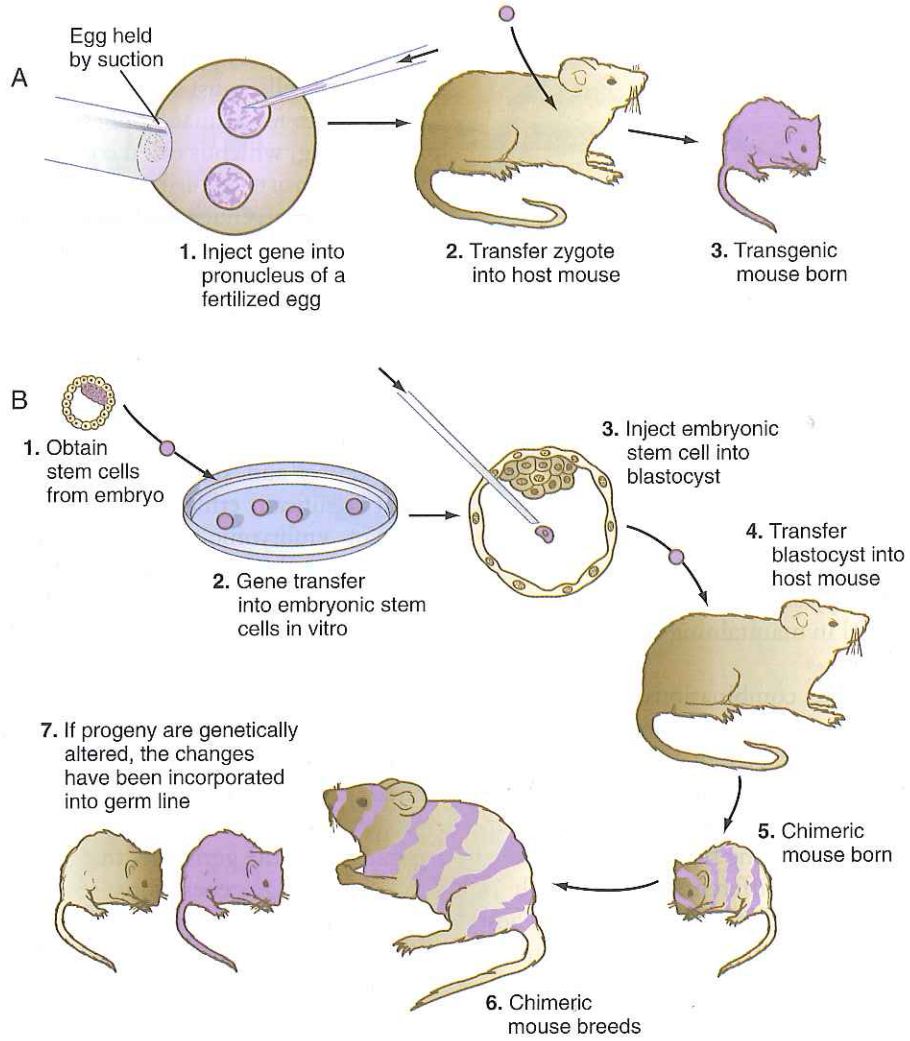

FIGURE 3-11. A, Procedure for creating transgenic mice by pronuclear injection. **B,** Procedure for inserting genes into mice by first introducing them into embryonic stem cells and then inserting the transfected stem cells into an otherwise normal blastocyst.

region (MT-I) into the pronuclei of mouse zygotes. The injected zygotes are transplanted into the uteri of foster mothers, which give birth to normal-looking transgenic mice. Later in life, when these transgenic mice are fed a diet rich in zinc, which stimulates the MT-I promoter region, the rat growth hormone gene is activated, causing the liver to produce large amounts of the polypeptide growth hormone. The function of the transplanted gene is obvious; under the influence of the rat growth hormone that they were producing, the transgenic mice grow to a much larger size than their normal littermates (Fig. 3-12). The technique of producing transgenic embryos is being increasingly used to examine factors regulating the expression of specific genes in embryos and to disrupt genes in the host embryos. In addition, the efficacy of this technique to correct known genetic defects is being increasingly explored in mice.

An important technological advance is the creation of lines of embryo-derived stem cells (**ES cells**). ES cells are originally derived from inner cell masses and can be propagated in vitro as lines of pluripotential cells that can be

FIGURE 3-12. Photograph of two 10-week-old mice. The one on the left (normal mouse) weighs 21.2 g. The one on the right (a transgenic littermate of the normal mouse) carries a rat gene coding for growth hormone. It weighs 41.2 g. (From Palmiter RD and others: Dramatic growth of mice that develop from eggs microinjected with metallothionein-growth hormone fusion genes, *Nature* 300:611-615, 1982.)

maintained in an undifferentiated condition or stimulated to undergo specific lines of differentiation.

STEM CELLS AND CLONING

A major development in biomedical research at the turn of the 21st century was the realization that certain cells (**stem cells**) in both human embryos and adults have the capacity to develop into a variety of cell and tissue types in response to specific environments. In embryos, stem cells can be derived from the inner cell mass (**embryonic stem cells**) or primordial germ cells (**embryonic germ cells**). In adults, stem cells have been isolated from tissues as diverse as bone marrow, skeletal muscle, brain tissue, and fat. Regardless of their origin, stem cells are maintained and propagated in an undifferentiated state in culture. Characteristically, stem cells express *oct-4* (see p. 47), which is involved in maintaining the undifferentiated state.

In response to specific combinations of exogenous agents, such as cocktails of growth factors, added to the culture medium, stem cells can be induced to differentiate into specific adult cell types—for example, red and white blood cells, neurons, skeletal and cardiac muscle, or cartilage. When introduced into living tissues, poorly defined local factors can direct the differentiation of adult or embryonic stem cells into specific adult cell types. These techniques have tremendous potential for the treatment of a variety of conditions, including diabetes, parkinsonism, blood diseases, and spinal cord injury, but many complicating factors, such as immune rejection of the implanted cells, must be dealt with before they are practical and safe for human application.

Cloning, which is often confused with stem cell technology, consists of fusing or introducing an adult cell or nucleus into an enucleated oocyte and allowing the hybrid cell to develop into an embryo and ultimately to mature into an adult. Although forms of cloning have been successfully accomplished for more than 50 years, the creation of the sheep Dolly has had the greatest influence on the public imagination. Cloning is not easily accomplished, and there seems to be a significant incidence of abnormal development in cloned individuals.

Cloning and stem cell technology have brought to light significant ethical and societal issues. For example, human embryonic stem cells have been introduced into mouse blastocysts in an attempt to determine the influences that control their differentiation. It will be fascinating to see how these issues, all sides of which have profound implications, are resolved.

It is possible to genetically engineer specific genes in ES cells. When such genetically manipulated cells are introduced into blastocysts, they can become incorporated into the host embryo (see Fig. 3-11B). If the progeny of a genetically engineered ES cell become incorporated into the germline, the genetic trait can be passed to succeeding generations. Some types of twinning represent a natural experiment that shows the highly regulative nature of early human embryos, as described in Clinical Correlation 3-2.

CLINICAL CORRELATION 3-2

Twinning

Some types of twinning represent a natural experiment that shows the highly regulative nature of early human embryos. In the United States, about 1 pregnancy in 90 results in twins, and 1 in 8000 results in triplets. Of the total number of twins born, approximately two thirds are **fraternal**, or **dizygotic**, twins and one third are **identical**, or **monozygotic**, twins. Dizygotic twins are the product of the fertilization of two ovulated eggs, and the mechanism of their formation involves the endocrine control of ovulation. Monozygotic twins and some triplets are the product of one fertilized egg. They arise by the subdivision and splitting of a single embryo. Although monozygotic twins could theoretically arise by the splitting of a two-cell embryo, it is commonly accepted that most arise by the subdivision of the inner cell mass in a blastocyst, or possibly even splitting of the epithelial epiblast a few days later (Fig. 3-13). Because most monozygotic twins are normal, the early human embryo can obviously be subdivided, and each component regulates to form a normal embryo. Inferences on the origin and relationships of multiple births can be made from the arrangement of the extraembryonic membranes at the time of birth (see Chapter 7).

Apparently, among many sets of twins, one member does not survive to birth. This is a reflection of the increasing recognition that perhaps most conceptuses do not survive. According to some estimates, as many as one in eight live births is a surviving member of a twin pair. Quadruplets or higher orders of multiple births occur very rarely. In previous years, these could be combinations of multiple ovulations and splitting of single embryos. In the modern era of reproductive technology, most multiple births, sometimes up to septuplets, can be attrib-