Tracking Genes in Developing Mice

Developmental research and new methods of gene manipulation are converging—much to researchers' delight

"The type of experiment I like to do is open-ended," Beatrice Mintz says. "You have a specific question to pose and you can expect to get an answer. But the experimental situation allows for the likelihood that you can get an answer for questions that you didn't know enough to pose." The example she cites is a series of experiments on mammalian development that she began shortly after arriving at the Fox Chase Institute in 1960.

The experiments that Mintz set out to do required the creation of mice containing two genetically distinguishable populations of cells obtained from two different embryos. Because the genetic differences provided built-in markers for the cells, she would be able to trace their fates during development.

> Developmental biologists are on the verge of achieving a long-sought goal, the creation of new strains of mice bearing foreign or systematically altered genes. Such strains may finally lead to an understanding of development at the molecular level, of how genes are turned on or off in specific tissues during the orderly course of differentiation.
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> For the past 20 years, Beatrice Mintz of the Fox Chase Institute for Cancer Research has been at the forefront of attempts to bridge the gap between the molecular biology of the gene and development. In a recent interview with *Science* she discussed her work.

The prevailing view at that time suggested that the animals would not develop normally, however. Many investigators believed that the developmental fates of the embryonic cells of mammals became fixed at a very early stage, perhaps even in the fertilized egg itself. The cytoplasm of the egg was known to be inhomogeneous, leading to the hypothesis that, if the plane of the first cell division was random, two unequal cells would be produced and would then follow different developmental pathways. The experiments Mintz was planning would disturb the normal arrangements of cells in the embryo, and their subsequent development might be disrupted as well.

But she explains, "I didn't believe that. Any cell you look at, young or old, has inhomogeneities in the cytoplasm. These need not be causally related to developmental events."

She took mouse embryos at roughly the eight-cell stage and treated them with an enzyme to remove their gelatinous coverings. When treated embryos of different strains were brought together in culture fluid at 37°C, the cells readily aggregated to form giant embryos. These embryos were surgically implanted in the uteri of foster mothers, which had been hormonally prepared for pregnancy by being mated with vasectomized males.

The resulting animals were mosaics of cells derived from the two original embryos. Mintz called them allophenic mice because they had the characteristics (phenotypes) of two different genetic types. The proportions of the two types of cells could vary from animal to animal and even from tissue to tissue within the same animal. But the experiments showed that the cells of embryos containing as many as 32 cells were still totipotent. Even when shown in labeling experiments to be rearranged, the cells could differentiate to form all kinds of tissue.

According to Mintz, "Most people expected that I would get monsters. But we never saw a monster although we made more than 1000 animals this way. . . . These and other experiments contradicted the conclusion that developmental paths in the mammalian embryo are determined at a very early stage. No matter what we did we got normal mice."

The experiments with allophenic mice proved to be open-ended because they yielded many surprises, as when Mintz traced the lineages of the cells that formed a particular tissue. "If you bring together the embryo cells of a pure black mouse with those of a pure albino mouse, you can predict that you would get a black-and-white mouse. But you couldn't predict how the pigment cells of the respective colors would be situated."

By observing the patterns produced by the genetically different cells, Mintz was able to draw inferences about the number of cells that underwent the initial commitment to a specific developmental pathway and the approximate time at which the commitment occurred. The coat color patterns of the allophenic mice consisted of transverse stripes that were sharpest on their backs and became more blurred on the ventral surfaces. Often the stripes were arranged asymmetrically on the left and right sides. "If you looked at enough mice," Mintz continues, "you could see an archetypal pattern of which everything else was a modification. . . . It was like getting a new pair of eyeglasses."

She concluded that the archetypal pattern for the coat pigment cells (melanocytes) consisted of 17 stripes on each side of the body and that each stripe was

Beatrice Mintz

formed of a clone of pigment cells that was produced by a single cell. In other words, all the melanocytes of a mouse coat came from only 34 cells that made the initial commitment to become pigment-producing cells. Asymmetry resulted because the 17 cells lined up on the left dorsal surface of the embryo cannot migrate to the right side, nor can those on the right migrate to the left. In addition, there may be modifications of the archetypal pattern. For example, adjacent clones may be of the same color.

The time at which the commitment occurs could also be estimated from the

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clonal patterns. In the case of the melanocytes this had to be after day 4 of embryonic life. Otherwise there would not be enough cells to make the melanocytes and the rest of the mouse. The latest time for commitment would be day 7. At this time, the paired neural folds, which are separated by a valley along the dorsal surface of the embryo, begin to come into contact and join together. Cells would then be able to migrate back and forth and asymmetry could not be maintained.

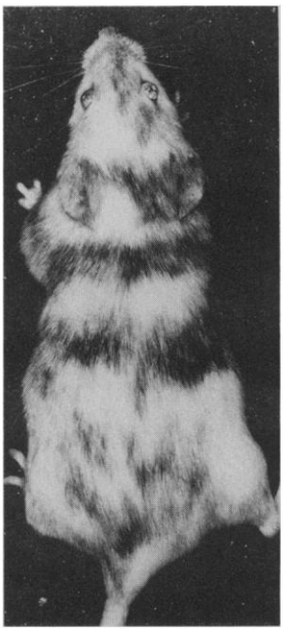
When Mintz looked at the patterns formed by other cell types, either in the skin or in other organs, she found that "every kind of cell I looked at had a unique story, not only in the number of clones, but the patterns were also different, which was not surprising."

This tracing of cell lineages in the allophenic mice provided direct support for the hypothesis that commitment to differentiate along a particular pathway occurs fairly early in embryonic life, although not as early as had once been thought, and that relatively few cells make the initial commitment. Mintz says, "It is much more sensible to have those major decisions made early, as they are, in a small number of cells and then perpetuated, although other decisions may be made later."

The big question, of course, is still unanswered. That is, what events cause an undifferentiated embryonic cell to become committed to a particular developmental pathway? Most investigators consider the genome to be the target of the change, and Mintz is no exception. Because specific clonal commitments are often maintained for a long time, she suggests that the critical change is at the transcriptional level, causing a gene to be copied into messenger RNA (mRNA).

Transcription of a gene into mRNA does not mean that the message will be immediately translated to produce the corresponding protein, however. Mintz points out that skin pigment cells are committed well before they start making the dark pigment melanin. Additional steps, such as those needed for messenger splicing, may also help to control when the final product is made.

In the 1960's, when Mintz was doing the work on allophenic mice, the need for messenger splicing was unsuspected. Only within the past few years have investigators discovered that the genes of higher organisms contain stretches of DNA that do not code for protein. These intervening sequences, which are sometimes called introns, are transcribed into mRNA but must be spliced out before the message is decoded into protein.



Allophenic mouse

The mouse developed from an embryo formed by aggregating two embryos of different strains. The dark bands are pigment cell clones of genetically black cells; the light bands are clones of genetically albino cells. [Source: B. Mintz, Proc. Natl. Acad. Sci. U.S.A. *58, 344 (1967)]*

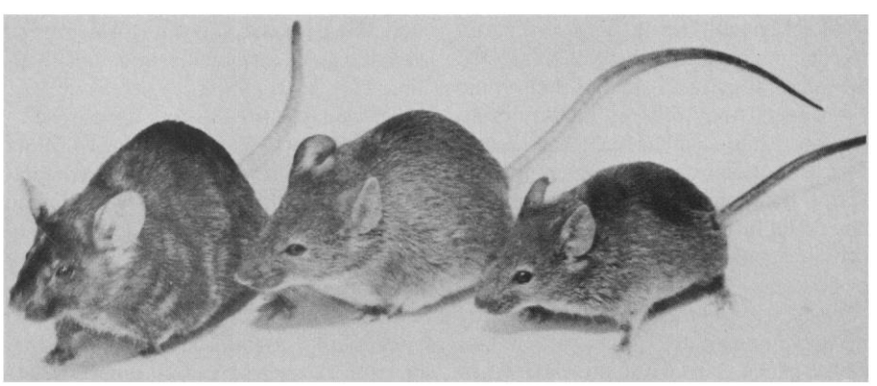
As early as 1969, Mintz's analyses of clonal patterns led her to propose that the genes of mice and other higher organisms are not the simple linear stretches of DNA that they appeared to be in bacteria. She had concluded that the coat color patterns in ordinary multicolored mice resembled those in the allophenic animals and were produced in similar fashion, from a small number of initially committed cells that expanded to form clones.

The most intriguing nonallophenic animals were homozygous for coat color—they had two copies of the same autosomal gene—but nonetheless had patterned coats of two or more colors. This situation did not have a simple explanation. From the information available in the late 1960's it was not clear how a gene could specify one color in some pigment cell clones and a different color in others.

Mintz says, "I believed that there must be something novel about the structure of genes in mammals to account for this. I therefore proposed a dozen years ago that many, or even most, genes are complex in structure and consist of a series of units tandemly arranged. Transcription could thus involve what I then called variable reading length." For example, a gene might consist of five units, with the first four being transcribed to give one color and all five to give the other. "At that time no one knew about intervening sequences," Mintz says. "I didn't imagine them either."

Now there is evidence that introns separate gene segments that code for functional subregions of protein molecules. Walter Gilbert of Harvard University suggested that this kind of gene arrangement may facilitate evolution by allowing the coding regions to be easily reassembled to form new products. Mintz speculates that introns, by providing boundaries between gene subregions, might allow differential assembly of the subregions to form variants of a given product during normal development. Her work led her to the view that even normal animals are mosaics in which the cells of a tissue might consist of clones characterized by such variants.

With methods being developed in several laboratories, including that of Mintz,



Tumor cell descendants

The female on the left is a mosaic produced by injecting cultured teratocarcinoma cells of a chromosomally normal line into an embryo. The coat shows stripes of agouti color, characteristic of the tumor strain, and black, the embryo's color. Mating of this female with a black male produced a daughter with an all-agouti coat (middle). This mouse was mated with one of her brothers, who also had an agouti coat, and produced a second generation of tumor-derived animals, represented by the mouse on the right. [Source: T. A. Stewart and B. Mintz, Proc. Natl. Acad. Sci. U.S.A. **78**, 6314 (1981)]

developmental biologists may soon have the ability to explore the relationship between genes and specific developmental events. This has long been a goal of Mintz, who maintains, "Studying molecular biology alone can tell you a great deal about cell biology or evolution, but not about development. For that it will be necessary to relate molecular information to the appearance of new cell lineages. As far as possible I want to unravel these things in the organism."

In the early 1970's she set out to develop a method for introducing specific mutations into living mice, where their developmental consequences could be assessed. Mutations directly induced in mice may be transmitted in the germ line to their progeny, but there is no way to produce specific changes. Moreover, the mutations usually cause gross abnormalities in the animals, and the underlying molecular bases of the alterations have rarely been characterized. Mutations induced in cultured cells may be more readily analyzed, but do not yield much information about development. Mintz wanted to fuse the two approaches, to take advantage of the strengths of both in vivo and in vitro methods.

Her idea was to induce characterizable mutations in cultured cells and then put the cells into a living animal, in a manner analogous to that used for making allophenic mice. A source of totipotent cells was needed for these experiments. Because embryos were unsuitable for the kind of in vitro manipulations Mintz was contemplating, she turned instead to cells from teratocarcinomas, tumors arising from undifferentiated embryonic cells.

In the first series of experiments, done with Karl Illmensee, who is now at the University of Geneva, teratocarcinoma cells were injected into early mouse embryos. Although these cells ordinarily produce malignant tumors when injected into mice after birth, in the embryonic environment they differentiated normally. The resulting mice were mosaics of cells of teratocarcinoma and embryonic origins. The tumor cells were totipotent; they could form all kinds of mouse cells, including germ cells, as shown by mating experiments.

Mintz had originally intended to study the developmental effects of mutant mouse genes, but about the time when she was doing the teratocarcinoma work the advent of recombinant DNA technology made it possible to clone genes, to make large quantities of virtually any gene from any species. In addition, genes could be altered in known ways, by deleting DNA segments thought to be involved in the turning on or off of gene expression, for example. These advances greatly enlarged the opportunities for studying gene expression in development. At the same time several investigators were developing reliable methods for introducing genes into cultured cells.

A problem with teratocarcinoma cells still had to be solved before they could be used as vehicles for introducing new genes into mice, however. When maintained in culture, they developed chromosomal abnormalities that were incompatible with the production by mosaic mice of viable offspring derived from tumor cells. The teratocarcinoma cells that Mintz and Illmensee had used for the earlier experiments were chromosomally normal but had to be maintained as transplantable tumors in living animals, which precluded the introduction of foreign genes or the selection of cells bearing specific mutations.

> " . . . You can produce mice essentially engineered in a dish. You only need one germ line animal with the desired change to perpetuate a new strain."

Within the past year or so, several investigators have produced lines of cultured teratocarcinoma cells that appear to have normal chromosome complements (karyotypes). Mintz and Claire Cronmiller, who has now left Fox Chase for graduate school, have produced one such line. But Mintz notes, "The presence of a normal karyotype could mask all sorts of genetic defects. The important thing is, will the line form normal somatic and germ cells in a host embryo?"

The answer for the line developed at Fox Chase is "yes," according to Mintz and Timothy Stewart. When they injected the cells, which were derived from an agouti-colored strain of mice, into embryos of black mice, Mintz says that "we got extremely gratifying results. There were a good number of animals with two coat colors." Of a total of 312 mice that developed from injected embryos, 41 had two-color coats. This was a higher proportion than seen in previous experiments, including Mintz's own. And she thinks that, if anything, the percentage is lower than it might have been. "In the middle of the experiment," she explains, "our animals started dropping dead with a Sendai virus infection." The strain of mice from which the tumor cells were derived is especially vulnerable to the infection, and Mintz thinks they may have lost a disproportionate number of mosaic animals.

That the cultured tumor cells are totipotent was indicated by analysis of the internal tissues of the mosaic mice. In one animal with a mostly agouti-colored coat, all the internal organs had an enzyme marker showing that they contained at least some cells from the tumor strain.

In addition, mating experiments showed that the tumor cells were capable of giving rise to reproductively functional egg cells. Nine female animals with part-agouti coats were mated to black males. (Mosaic males were not mated because the tumor cells were female, or chromosomally X/X, and could not produce functional sperm.) One female produced two females and one male with coats of the dominant agouti color out of a total of 48 progeny in five litters.

Matings of the two females with their brother produced a second generation of progeny descended from the cultured tumor cells. Both the first and second generation of mice carried other markers, in addition to their agouti coat colors, characteristic of the tumor cell strain.

Stewart and Mintz have not yet introduced a foreign or mutant gene into the teratocarcinoma cells, which have been given the designation METT-1 (for first line of mouse euploid totipotent teratocarcinoma cells), before injecting them into the embryos. But a gene transfer experiment is one of the next logical steps. Mintz, Erwin Wagner, and their colleagues at Fox Chase, in collaboration with Richard Axel's group at Columbia University's College of Physicians and Surgeons, have already shown that another, but chromosomally abnormal, line of teratocarcinoma cells takes up the genes for a viral enzyme (thymidine kinase) and for human β-globin. The cells also make the viral enzyme.

Referring to the two generations of mice descended from the METT-1 cells,

Mintz says, "These individuals are the perfect model of a new strain. You can produce mice essentially engineered in a dish. You only need one germ line animal with the desired change to perpetuate a new strain."

Mintz thinks that the capabilities of experiments with the cultured teratocarcinoma cells will partly overlap those of methods in which cloned genes are injected into fertilized eggs, which have also been yielding very encouraging results. This recent work was foreshadowed by experiments done in 1974, before cloned recombinant DNA was available, by Mintz and Rudolf Jaenisch, who is currently at the Heinrich-Pette Institut in Hamburg. They showed that DNA from a virus (SV40) could be injected into embryos and retained throughout development.

A few months ago, Mintz, Stewart, and Wagner reported that embryos that developed from fertilized eggs which had been injected with the linked genes for thymidine kinase and human β-globin carried intact copies of both genes. One of the embryos made large quantities of thymidine kinase.

Soon after this report came four more showing that foreign genes, when injected into fertilized mouse eggs, could enter the germ lines of the resulting animals and be transmitted to their progeny. In two cases the investigators injected rabbit globin genes. One of these groups included Thomas Wagner of Ohio University and Peter Hoppe of the Jackson Laboratory; the other consisted of Franklin Costantini and Elizabeth Lacy of the University of Oxford, England.

In the remaining two cases the investigators injected the viral gene for thymidine kinase. These experiments were performed by researchers from the laboratories of Ralph Brinster at the School of Veterinary Medicine of the University of Pennsylvania and Richard Palmiter of the University of Washington and by Jon Gordon and Frank Ruddle of Yale University. The Wagner-Hoppe and Brinster-Palmiter groups reported that the injected genes produced their protein products in some of the animals that developed. Mintz, Stewart, and Erwin Wagner have now also found transmission of injected gene sequences to progeny.

Comparing the egg injection method with the use of teratocarcinoma cells, Mintz says, "In both cases you can introduce an intact or manipulated gene. The egg injection route has the advantage that with luck you can get the DNA into all the cells at once. But the teratocarcinoma route has the advantage that you can preselect the positive cells and characterize them with regard to the state of the gene you introduce."

In addition to their use to study the molecular biology of development, Mintz thinks that the methods may make possible the production of laboratory animal models that can be used to investigate human genetic diseases for which such models are now lacking. Mice having Lesch-Nyhan syndrome, a severe neurological condition caused by a single defective gene, or the thalassemias, which are anemias caused by defective globin genes, are two examples.

Within the past 2 to 3 years researchers have made rapid progress toward the ability to produce mice with hand-tailored genes. As Mintz sums up the situation, "I think it is exciting, what is happening in this area. It's great being alive in science today. I would hate to have missed this."—JEAN L. MARX

Additional Reading

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3. T. A. Stewart and B. Mintz, "Successive generations of mice produced from an established culture line of euploid teratocarcinoma cells," *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6314 (1981).
4. Egg injection experiments: E. F. Wagner, T. A. Stewart, B. Mintz, *ibid.*, p. 5016; T. E. Wagner, P. C. Hoppe, J. D. Jollick, D. R. Scholl, R. L. Hodinka, J. B. Gault, *ibid.*, p. 6376; F. Costantini and E. Lacy, *Nature (London)* **294**, 92 (1981); R. L. Brinster, H. Y. Chen, M. Trumbauer, A. W. Senear, R. Warren, R. D. Palmiter, *Cell* **27**, 233 (1981); J. W. Gordon and F. H. Ruddle, *Science* **214**, 1244 (1981).

Alzheimer's Research Poses Dilemma

Legal and ethical issues in obtaining informed consent are particularly thorny because patients are senile

Alzheimer's disease afflicts 1.5 to 2 million Americans, causing a progressive and irreversible senility, starting with memory loss for recent events and continuing to a point where most patients cannot feed themselves, no longer recognize their families, and do not even know their own names. These patients also have life expectancies that are one-half to one-third those of healthy persons of the same age, according to Robert Katzman of the Albert Einstein College of Medicine. "We're dealing with a disease that is in many ways as malignant as cancer," Katzman says.

In recent years, medical scientists have found clues to what might cause the symptoms of Alzheimer's disease and what treatments might help. In particular the neurotransmitter acetylcholine is not as abundant as it should be in the brains of these patients (*Science*, 6 March 1981, p. 1032). But now researchers are confronted with a difficult legal and ethical problem. How can they obtain informed consent to do research on these patients? All too often, the patients themselves cannot give consent. As Hilda Pridgeon, the wife of an Alzheimer's disease patient and a founder of the Chicago-headquartered Alzheimer's Disease and Related Disorders Association, explains, "By the time you know a patient has Alzheimer's disease, the patient can't deal with intangibles. They're down to dealing with how to brush their teeth or take a shower."

The National Institute on Aging held a meeting* to discuss the ethical and legal questions relating to research on Alzheimer's disease patients. The central issue, as Andrew Jameton of the University of California in San Francisco says, is that "We're in a bind. We're interested in doing research on exactly the group that, according to our notions of voluntariness and informed consent, we shouldn't do research on."

The sorts of research that are causing the most concern are invasive procedures offering no therapeutic benefits to patients. To take the most emotionally charged example, there was discussion at the meeting of doing brain biopsies on patients before enrolling them in clinical studies in order to be sure they really have Alzheimer's disease. As many as 30 percent of patients thought to have Alzheimer's on the basis of their clinical

*The conference on "Senile Dementia of the Alzheimer's Type and Related Diseases: Ethical and Legal Issues Related to Informed Consent" was held on 23 and 24 November.

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