Biology

Julie Ann Miller reports from the National Institute of Child Health and Human Development (NICHD) in Bethesda, Md., at the conference "Molecular Genetics of Development in Flies, Frogs and Mice"

Gene transfer cures mouse defect

A hereditary defect in synthesis of a crucial blood protein in mice, which constitutes a disease similar to beta thalassemia in humans, has been corrected by the transfer of the corresponding human gene into mouse reproductive cells. Kiran Chada and Frank Constantini of Columbia University in New York City report successful transplantation of the human adult beta-globin gene. They report that in many of the recipient mice and their descendants, the gene is active in producing human beta-globin protein only in the appropriate location, red blood cells. The gene was also expressed at the appropriate time in development—in fetal and adult red blood cells but not in embryonic red blood cells. These results indicate that the regulatory instructions for the expression of the beta-globin gene sit near the gene itself, because they are contained within the DNA segment the scientists have snipped out of the human chromosome.

The finding that a "good" gene can compensate for a "bad" one that is still present is encouraging for potential clinical uses of gene transfer, comments Heiner Westphal of NICHD. While gene transplantation into human reproductive cells is only a distant prospect, gene transfers into bone marrow cells are considered imminent. "Now one worry can be set aside," Westphal says, "that transferred genes will not be expressed in appropriate amounts."

The timing of a transplanted gene's expression, however, cannot always be predicted from its behavior in the animal contributing the genes. In other experiments, Chada and Constantini transferred a gene that is expressed in different developmental stages in mouse and human. Once transplanted into a mouse, the human gamma-globin gene shifted its activity to the stage appropriate for the mouse gene, behaving as an embryonic rather than as a fetal gene.

Embryonic start-up: Mom's influence

In the earliest stages of its developmental journey, an embryo depends entirely on substances originally packed into the egg cell by the mother. But even as the embryonic genes assume control, their activity pattern is directed by prepackaged maternal factors. In the frog *Xenopus,* embryonic genes first become active when the embryo is a blastula (a hollow sphere with a single layer of cells) about eight hours after fertilization. At this time, different genes are activated in specific regions of the embryo.

One hypothesis for the differential activation of embryonic genes is that information-carrying molecules, such as maternal messenger RNA molecules, are differentially distributed in the embryo. Douglas Melton of Harvard University now reports experimental support for this idea. He finds that a few types of maternal messenger RNA, making up less than 0.1 percent of the total, are localized to specific regions of an unfertilized egg and are subsequently distributed to different regions of the early embryo. These molecules may be responsible for the characteristic pattern of early gene activation—the first step in the sequence of events by which genetically identical cells develop into all the different tissues of the body.

Down the road to skin . . .

Among the first genes to "turn on" in development, the most active are several that encode proteins making up the filaments that give structure to skin, report Thomas Sargent, Igor Dawid and colleagues at NICHD. "These keratin genes are the earliest tissue-specific gene expressed in *Xenopus,*" says Sargent. "And skin is the first organ to differentiate."

Because these early keratin genes are expressed even in dissociated embryonic cells, they are not triggered by contact with other cells. Instead, the major means of differentiation seems to be suppression of gene activity. These keratin genes are turned off in the areas that develop into neural tissue, Sargent reports. In addition, they are inactivated by experimentally introduced contact with certain other embryonic cells. But even in the cells that become skin, by the time a tadpole develops into a frog, other genes take over their role. "No gene first turned on in the blastula . . . is also expressed in the adult," Sargent says.

. . . and to muscle

A gene for a protein found in muscle of older embryos is activated in *Xenopus* development slightly later than the genes for keratin. John B. Gurdon of Cambridge (England) University reports this gene is turned on only in the cells that eventually become muscle. The specification of this gene activity, like that of genes studied by Douglas Melton (see above), appears to be spatially determined in the original egg. Gurdon and his colleagues have cut the fertilized egg into various zones. They find that the part of the egg necessary for muscle-gene activation lies just below the egg's equator.

Studies of the embryo at the 32-cell stage demonstrate that cells in an equatorial band, which make up two out of four tiers of cells, normally can develop into muscle. But Gurdon reports that if the embryo is cut on the equator between the two muscle-making tiers (called tiers 2 and 3), only the lower of the two tiers can form muscle. The higher tier now forms skin and nerve. He concludes from additional experiments that a muscle-inducing substance is located in tier 3, and it diffuses into tier 2 when the cells are in contact.

Gene action and cancer in eye lens

The lens of the eye is an unusual tissue, constructed for transparency and lacking a blood vessel system. Ophthalmologists have observed that the lens has a special immunity to cancer, even when the nearby retina or iris has a tumor. This resistance is not an inherent property of the lens cells, but rather reflects an inaccessibility to tumor-causing agents, says Heiner Westphal of NICHD. He and his colleagues have demonstrated that a cancer-associated gene experimentally targeted to be expressed in the lens can produce malignant transformation there.

To direct gene expression in the lens, the scientists used a control region of DNA from the gene for alpha-A crystallin, a natural protein of the lens. When this control region was attached to a bacterial gene, and that DNA was injected into single-cell mouse embryos, the gene was expressed only in the lens and only at the time during development when the crystallin gene is normally active. Next the scientists attached the crystallin control region to a viral gene known to cause tumors. This DNA was injected into 50 mouse embryos, resulting in eye tumors for all 50 mice, Westphal reports.

Chemical basis of a biological clock

The ticking of a fruit fly's biological clock involves a chemical called a proteoglycan, a long chain of sugar subunits attached to a protein. This result, reported by Michael Rosbash of Brandeis University in Waltham, Mass., comes from studies of a fly gene known as *period* (abbreviated as *per),* which is required for the insect's biological rhythms. By moving pieces of DNA into fly reproductive cells, Rosbach located the *per* gene on a segment of DNA that is active in the embryonic nervous system and again late in the pupal stage and in adulthood, especially in the fly head. The nucleotide sequence of the *per* gene was determined, and its predicted product resembles a mammalian proteoglycan. In further experiments, Rosbach showed "the *per* gene does indeed code for a proteoglycan."