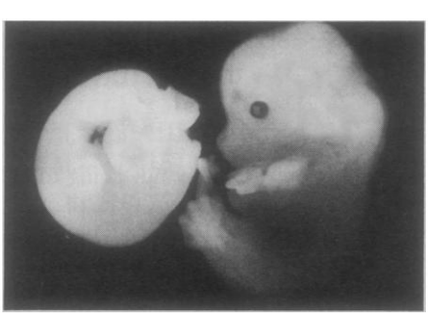
Injected Virus Probes Fetal Development

Besides opening the possibility for selective therapy, the insertion of genetic material into animal embryos can provide insight into fetal development. For instance, in one series of experiments Rudolph Jaenisch of the University of Hamburg and his collaborators specifically and dramatically halted mouse embryologic development following the insertion of a retrovirus into a collagen gene. This approach, if generalized, offers a way of dissecting embryonic development at the molecular level.*

Jaenisch and his collaborators have developed a series of 14 mouse strains, each of which has a single copy of the Moloney retrovirus in different chromosomal sites. In several of those strains, the viral genes remain intact, and the animals develop viral disease. The chromosomal position of the viral genes significantly influences the time during development when those genes will become activated—a finding that was not clear from other studies where multiple gene copies are inserted into a recipient animal's chromosomes, Jaenisch points out.

Only one of the 14 strains, which is called MOV-13 (and was created by microinjecting the virus slightly later after fertilization than in the other strains), carries an obvious mutation. But it is striking. Although innocuous when the viral genes are present on only one of a pair of chromosomes (possibly chromosome 11, although it has not yet been mapped), the mutation is fatal when those genes are present on both. The mice begin to show characteristic signs of degenerative illness toward the end of the second week of fetal development, and all are dead by about day 14.



The degenerating mouse embryo (left) will not survive past day 14; its littermate (right) is developing normally.

Circumstantial evidence and a series of good guesses led Jaenisch and his colleagues to realize that the virus inserts into a collagen gene in the MOV-13 mouse—specifically into the proximal (5′) end of the first intron of the α-1 collagen gene. At first, this finding seemed straightforward: Expression of this key gene is blocked and the mice die because of the absence of the α-1 collagen protein. However, Jaenisch's most recent findings have undermined this simple explanation, leaving him with a puzzle that challenges current ideas about the role played by this collagen in the body, particularly during development.

The genetic analysis of the MOV-13 mouse is clear-cut. The retrovirus is located precisely 15 nucleotide bases into the first intron of the α-1 collagen gene. Although introns are not part of the gene specifying the protein product, this portion of the collagen intron is probably important because its sequence is highly conserved between mouse and man, Jaenisch says. In any case, the gene containing the retrovirus sequences cannot make α-1 collagen messenger RNA, which ordinarily appears at day 12 of embryonic development.

The α-1 collagen is believed to stabilize structures, including bones, skin, and tendons. "Why do the embryos die at day 12?" Jaenisch asks. "There are no bones at this stage." Histologic examination of the dying embryos shows that several kinds of tissue, particularly mesenchymal cells surrounding peripheral muscle and nerves, are degenerating by day 12. However, degenerative changes in the liver are "most characteristic" of the developmental abnormalities that afflict these mice, he says. "The first sign of degeneration is in the erythropoietic [red blood cell–forming] cells in the liver." Such cells are undergoing a rapid burst of proliferative growth at this stage, and ordinarily would multiply about 70-fold during the next few days, he notes, but "this doesn't explain" why the mice die. Very recent findings by pathologist Jürgen Löhler indicate that the rupture of major blood vessels throughout the body—following closely after this widespread cell degeneration—is the cause of death.

One other series of experiments, conducted by Klaus Kratochwil from Salzburg while visiting Jaenisch's lab, embellishes this picture and, so far, further weakens established notions about α-1 collagen's role in the body. Explanted cell samples from a mouse embryo's organs can be grown in vitro, where under proper conditions the cells coalesce, taking on the shape and other characteristics of the organs from which they were derived. It has been thought that α-1 collagen plays a vital role in this process, by stabilizing the clefts that develop along the surface of a growing organ and protecting the basal lamina against degradation. However, explanted cells from the organs of homozygous MOV-13 mice grow "absolutely normally" in culture, even though they cannot make α-1 collagen, Jaenisch says.

These two sets of results thus challenge the established view about α-1 collagen, he says. First, α-1 collagen has not been thought to be involved in stabilizing blood vessels, a role attributed to collagen 3. However, both the histologic and pathologic studies implicate α-1. Moreover, the timing and the seeming involvement of degenerating hematopoietic cells in the liver as a separate event suggest to Jaenisch that α-1 collagen could serve more than one role during early embryonic development. Second, the successful growth and development in organ culture of tissue explants that lack α-1 collagen also suggest that its supposed role as stabilizing organ morphogenesis must be reconsidered.

This reassignment and possible dual reassignment for the α-1 collagen gene during embryonic development, though inviting, is not ironclad. The main reason for caution is that more than one mouse gene could be affected. Currently, Jaenisch is sequencing the DNA adjacent to the collagen gene to see whether a recognizable gene resides there. "We know the virus has changed the chromatin structure at the collagen gene," he says, "but we don't know if that change extends beyond the 5′ [end] to the adjacent gene, and we don't know if there is another gene adjacent."

He plans to address this problem in another way—one that gets back to the realm of gene therapy. His group will test whether the α-1 collagen gene, when added back to MOV-13 mice, rescues the embryos.—**JEFFREY L. FOX**

*Jaenisch discussed his findings at the Fourth Annual Congress for Recombinant DNA Research in San Diego, California, 19 to 22 February 1984.