Biology

Julie Ann Miller and Robert Pollie report from Bethesda, Md., at a National Institutes of Health workshop "Expression of Cloned Genes in Development"

New genes unpredictable in mice

With their newfound ability to isolate and alter genes and make abundant copies, scientists are eager to plug genes back into living organisms. Such an accomplishment is necessary for any medical applications of genetic engineering and also for a wide range of experiments investigating how the genes act during normal development. Scientists are now reporting success in inserting genes into animals' cells. But they have yet to master the task of making the transplanted genes function appropriately.

"What has come as something of a surprise is how easy it is to incorporate foreign genes into a mammal," says Beatrice Mintz of the Institute for Cancer Research in Philadelphia. Experiments in her laboratory and others demonstrate that foreign genes injected into a fertilized egg can be retained and transmitted to all cells of the adult mouse and even passed on to the mouse's descendants. The foreign genes seem to have established themselves within chromosomes, because they are inherited according to the Mendelian rules. Frank Constantini of Columbia University, who has introduced a rabbit globin gene into mouse eggs, finds the gene can take up residence in a variety of positions, probably random, on the chromosomes. But so far he has not observed the rabbit gene at the site of the corresponding mouse gene. Although there has been a report of rabbit globin gene expression in mouse blood cells (SN: 9/12/81, p. 164), Constantini only occasionally saw evidence of gene activity, and it was in inappropriate tissues.

Evidence for natural regulation of one transplanted gene was described by Ralph L. Brinster of the University of Pennsylvania. Working with Richard Palmiter of the University of Washington, Brinster made an artificial gene combining the gene for a viral protein with the control region of a mouse gene, whose activity is regulated by metals and hormones. He finds the viral protein, produced in response to cadmium and zinc, in about half the animals successfully injected with the hybrid gene. But the gene does not respond to hormones, and in only one case did its activity show the expected pattern among the mouse tissues. Brinster suggests the variability among results may reflect the variety of positions transplanted genes assume on the chromosomes. He says the next big step will be to insert genes into selected sites on mouse chromosomes.

Development as an expanding repertoire

Development is generally pictured as sets of genes turning on and then off in sequence. But in the case of the microscopic soil worm, the nematode *Caenorhabditis elegans,* one aspect of maturation seems to involve activating more and more genes. David Hirsh of the University of Colorado examined more than 50 genes from the nematode at different stages of the life cycle—egg, last of four larval forms and adult. The genes all code for similar collagen proteins, the structural components of the nematode's outer covering. This cuticle is replaced each time the developing nematode molts.

The largest group of the 50 collagen genes is those active throughout nematode life, in the eggs, larvae and adults, Hirsh finds. A second sizable group is active in larvae and adults, and a third smaller group becomes active only as the larvae develop into adults. Just a few genes appear to be turned off during development. These are active only in the eggs, only in the larvae or only in the eggs and adults.

Hirsh speculates that the nematode's pool of active genes expands by adding on banks of genes to give the cuticle characteristic of progressive stages. He says, "It seems to add new building blocks." Mutant worms with abnormal cuticle provide further evidence for this idea: a mutation expressed at one larval stage is also expressed during later stages. Hirsh points out that the different cuticles of the first larval stage, later larval stages and adults may have arisen in parasitic, rather than free-living soil, nematodes. Parasitic nematodes experience vastly different environments during different stages of life.

Research on nematode collagen genes has produced other surprises. There are more than 100 similar collagen genes, but they do not seem to be clustered. Compared to the collagen genes of vertebrates, the nematode genes are small and have few intervening, non-coding stretches of DNA. Scientists are now working on genetic engineering techniques to introduce specific genes into nematodes.

Genes egg snails on

When the sea snail *Aplysia* lays its eggs, it's engaging in one of the most closely studied behaviors in the animal kingdom. Neurophysiologists have diagramed the behavior as a small set of actions—the snail stops feeding, begins releasing eggs, and so on—in turn coordinated by a relatively simple relay network of nerve cells, neurotransmitter substances and peptide hormones. And the analysis hasn't stopped there. The neurological apparatus may be controlled at a still higher level by *Aplysia*'s genes, through mechanisms currently being elucidated by researchers at Columbia University. The group's recent findings on the structure of hormone-encoding genes have brought scientists a step closer to understanding the genetic control of behavior in many organisms.

The work began two years ago as an attempt to locate the genes for egg-laying hormone (ELH), a short peptide released by certain of *Aplysia*'s nerve cells and known to evoke several phases of the egg-laying behavior. Surprisingly, molecular probes indicated that at least five different genes contained DNA sequences that could code for ELH. Moreover, each gene appeared to produce ELH only as part of a larger "polyprotein" strand, which could presumably be chopped up by cellular enzymes to yield ELH together with an assortment of other, possibly neuroactive, peptides.

These early findings led the researchers to formulate a novel "combinatorial hypothesis" of neuropeptide action: Each small peptide in the polyprotein complex might have a specific influence on the snail's egg-laying behavior. The exact nature of the behavior produced by ELH would therefore depend on the particular ELH gene expressed, since each gene produces a different combination of ELH and other peptides.

The hypothesis was extraordinarily appealing to scientists studying the genetic basis of behavior, since it explained how genes could orchestrate and modify a great variety of behavioral responses simply by deploying different sets of a few neurotransmitters.

But recent, still-unpublished studies have not borne out some features of the hypothesis, Columbia researchers James Jackson and Richard Axel report. Detailed examination of the proposed ELH genes indicate that there is only one true ELH gene after all, present in three copies. Two other genes (known as A and B, respectively) are both remarkably similar to the ELH gene in overall structure, but they code for shortened, possibly nonfunctional versions of ELH — along with several other peptides known to be involved in egg laying. The A and B genes may be evolutionary descendants of an original single ELH gene that have been genetically modified to further refine the egg-laying behavior. All three genes do appear to code for collections of several peptides, which may fan out in *Aplysia* to coordinate the diverse operations involved in egg laying.

The recent findings, therefore, call for a revision of the combinatorial hypothesis, but leave intact one of its central arguments, summed up by Axel: "Peptides elicit individual units of behavior, and more complex behaviors are built by the expression of different combinations of peptides."