GENETIC ENGINEERING

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A mobile genetic element gives scientists powerful new ways to tackle one of biology's oldest problems

By GARDINER MORSE

Since Aristotle founded the discipline of embryology over 23 centuries ago, the basic mechanisms of development have remained obscure. How, for instance, do genes control the transformation of a fertilized human egg into an adult whose 50 trillion cells form vastly complex, interacting organs and tissues? And what accounts for errors in this process that result in a spectrum of defects?

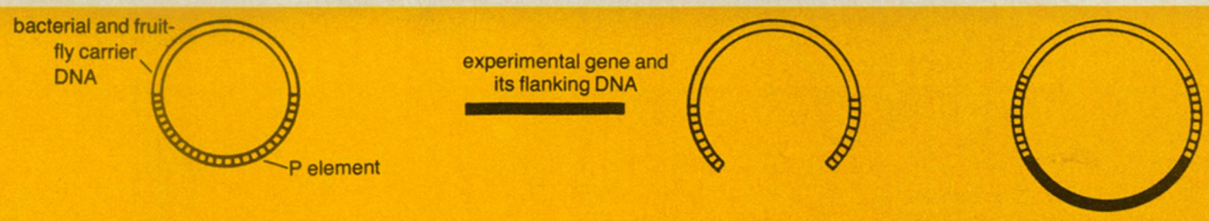
Now, a jumping gene, or transposon, harnessed and put to work in gene transfer experiments, promises to shed new light on these and related questions. Recently, scientists have discovered a way to use a transposon to carry functioning genes into fruit flies. Jim Posakony of Harvard University's department of biochemistry and molecular biology in Cambridge, Mass., says of the technique, "It's going to completely revolutionize our ability to do genetics."

In the fruit fly *(Drosophila melanogaster)*, as in corn plants where Barbara McClintock first discovered transposons more than three decades ago, these segments of DNA shuttle from site to site among a cell's chromosomes. Allan Spradling and Gerald Rubin of the Carnegie Institution in Baltimore, have succeeded in inserting a gene into an isolated fruit fly transposon, called the P element, and injecting this DNA into fly embryos. Under carefully controlled conditions the P element and its passenger gene insert into chromosomes in the embryo's germline precursor cells, which become sperm or eggs. Thus the progeny resulting from these transformed cells contain the foreign gene. The first gene they transferred this way was a gene for red eye color called "Rosy."

Since Spradling and Rubin's report a year and a half ago (SN: 10/23/82, p. 260), several laboratories have published papers describing results of gene transfers mediated by P elements. What these reports, and new data from Spradling and Rubin, make clear is that the genes transferred into *Drosophila* by P elements function reliably. Not only are transferred genes expressed at near-normal levels (although sometimes affected by where and on what chromosome they insert) but they show apparently normal developmental regulation — that is, they are expressed in the right fly tissues and at the right times as the fly develops from embryo to adult.

Jay Hirsh and colleagues at Harvard Medical School are studying a gene called Ddc. This gene codes for the dopa decarboxylase protein, an enzyme with various functions in the fruit fly, among them the hardening of the fly's cuticle, or exoskeleton. Ddc expression is developmentally regulated; the gene is most active during cuticle synthesis when the fly molts, and it shows tissue specificity, functioning predominantly in the fly cells that make the cuticle.

Mutant flies that lack the normal Ddc gene fail to make normal cuticles when raised at room temperature and appear greenish instead of a healthy brown. But when these embryos receive a normal Ddc



1 *Recombinant DNA methods are used to construct a DNA ring containing an intact or altered P element, the jumping gene vehicle for gene transfers.*

2 *The P element is cut open with enzymes and mixed in a tube with fragments of DNA carrying the experimental gene and sequences that may control its function.*

3 *The ends of the experimental gene and its flanking DNA are chemically joined to the P element vehicle.*

AND THE JUMPING GENE

These fruit flies come from a stock that received a hybrid gene by the P element gene transfer method. This gene activates at high temperature, producing a product in the fly's tissues that turns blue when chemically treated. The fly on the right in the picture was heat shocked, activating the hybrid gene.

gene by P element mediated gene transfer, progeny inheriting this gene from genetically engineered parents form healthy cuticles at appropriate times during development. Stringent biochemical tests have further established that the gene follows its usual program.

David Goldberg, Tom Maniatis and Jim Posakony at Harvard report that the alcohol dehydrogenase gene, Adh, which confers alcohol resistance to flies, is also regulated normally in the insects following transfer by the P element. These findings underscore the remarkable dependability of P element mediated gene transfer in flies.

Among the discoveries already resulting from these gene transfer experiments is how little DNA flanking these genes is needed for their proper regulation. The Ddc structural gene—which codes for the entire DDC protein—is 4,000 subunits, or bases, long. Hirsh's group included 2,500 bases at one end of the gene, where control sequences often lie, and 1,000 bases at the opposite end. Both the Rosy and Adh genes used for fly transformation similarly included short segments of flanking DNA which, it turns out, are sufficient to permit proper gene regulation.

But these successes follow years of often disappointing attempts. While methods for inserting working genes into simple organisms such as viruses and bacteria have become commonplace during the past decade, experiments to genetically transform metazoans—multicellular organisms with defined organs—have lagged behind. Until recently, scientists have lacked a vehicle to insert a gene into the host's chromosomes so that the gene works normally and most attempts to transform metazoans, notably mice, were bedeviled by rearrangements, duplications and loss of sections of the transferred genes. What's more, these genes were often expressed abnormally, if at all, and often in the wrong tissues (SN: 10/16/82, p. 252). While researchers using modified viruses to carry genes into animal cells and embryos have recently reported striking successes, none yet matches the dependability of Spradling and Rubin's gene transfer method, which overcomes these problems—at least in fruit flies.

This reliable method for transferring functioning genes into a higher organism gives the best opportunity to study the precise relationship between the structure of higher organisms' genes and how they are regulated in the living creature. Using recombinant DNA techniques, scientists can isolate a fruit fly gene, alter its structure, insert it into a P element and then put the gene into a fly. This allows them to study how structural changes affect a gene's behavior. Spradling explains that the technique "provides the kind of assay that was never available for higher organisms," a direct, living assay of what parts of a gene and adjacent DNA play what roles in controlling the gene's function during development. Steve Scholnick, one of Hirsh's colleagues at Harvard, sums

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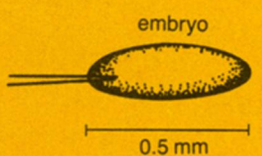

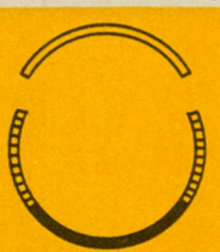

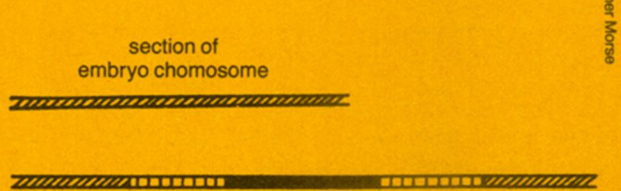

Gardiner Morse

4 Under a microscope, newly formed fruit fly embryos are injected with copies of the P element and its passenger gene as well as intact P elements.

5 In the embryo cells the P element and experimental gene separate from the carrier DNA and insert into the chromosome. The exact mechanism is not known but the process probably occurs in a single step and is driven by a transposase, an enzyme encoded by the intact P element injected at the same time.

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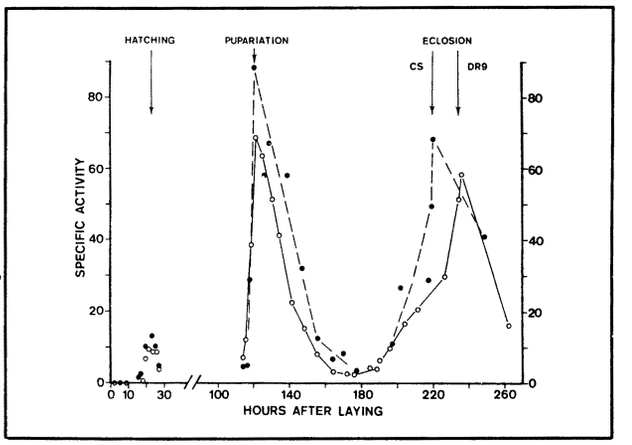
up this research strategy. "The hot thing to look for now," he says, "is elements that control the gene."

But Spradling cautions that this type of assay, even when successful, will not alone "get you the whole way to understanding gene regulation." While the simple switching on and off of individual genes figures importantly in the control of gene expression, a variety of other factors influence gene function, including the structure of the chromosome where a gene lies, the presence of activity-boosting "enhancer" sequences and the number of gene copies present. Gene expression can be controlled by DNA base sequences lying close to the gene or thousands of bases away, and by other, perhaps distant genes.

Despite these and other influences governing gene expression in higher organisms, P element mediated gene transfer experiments will answer some specific and central questions. Hirsh explains that these experiments should pinpoint sequences required for the correct expression of genes important in development.

To find these sequences, scientists are using a recombinant DNA method called in vitro mutagenesis, the introduction of precise changes into isolated genes by rearranging, deleting or inserting DNA fragments. To pinpoint control sequences in genes such as Ddc, Adh and Rosy, pieces of DNA as large as hundreds of bases, and as small as specific, single bases, can be deleted from the DNA fragment containing the gene. By removing a section of the DNA flanking, or even within a gene, and then using the P element vehicle to transfer this altered gene into a fly, scientists can quickly determine the importance of sequences contained in the missing DNA to the gene's regulation. If a transferred gene lacking a particular 200 bases fails to regulate properly in such a situation, but works normally when intact, then bases among those 200 must play some role in its regulation. More precise deletions could then be used to ferret out just those sequences essential to normal regulation. Ultimately, the role of individual bases can be shown.

John Lis and colleagues at Cornell University in Ithaca, N.Y., report in the December issue of CELL (Part 1) a promising new tactic in fly gene regulation studies. They constructed a "fusion gene" combining a fruit fly heat-shock gene, which activates at high temperatures, with a bacterial gene whose product is easily detectable. When flies transformed with this fusion gene are incubated at high temperature (37°C), the heat shock gene becomes active, causing production of the fused bacterial gene's product. This gene product turns blue in the presence of certain chemicals, and so its location in fly tissues is easily assayed. Lis explains that this system will be valuable in studying the expression of developmentally regulated fruit fly genes by allowing scientists to visualize when and where genes function.



Normal fruit flies show a distinctive pattern of DDC enzyme activity during development (black circles). Mutant flies that make abnormal DDC enzyme show nearly identical enzyme activity once they are transformed with the normal Ddc gene (white circles), demonstrating how reliably the transferred gene works.

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In fact sequences controlling the heat shock gene can be fused to any isolated fly gene. This gene fusion should then be regulated by high temperature, giving scientists a way to switch genes of interest on or off at will. These preliminary experiments suggest myriad research and practical applications for transposons. One obvious idea is to see if the P element will insert passenger genes into organisms other than the fruit fly. So far, attempts to use the P element to transfer functioning genes into mice, frogs, plants, sea urchins and nematodes (a type of worm) have failed, Spradling says, but he suggests that it may be possible to modify the P element's structure so that it will work in other organisms.

Alternately, transposons found in other complex organisms might be used to carry genes into the same organism from which the transposon was isolated, where it would be most likely to function. Scientists should have abundant opportunities to test this idea. Explains Spradling, "It's startling just how many of these elements there are. In *Drosophila,* 10 percent of the total DNA is transposable elements, and in other organisms, even a higher fraction of the DNA may be transposons."

The genetic material of some viruses is structurally similar to transposons, and Posakony speculates that "it may turn out that viruses are the closest thing you'll find in mammals to the P element." Recent work with these viruses shows their promise as a vehicle for introducing functioning genes into higher organisms.

It hasn't escaped notice that ways of transferring working genes into complex organisms have clear practical applications. Spradling and Rubin write, "In the long term, transposable elements may be of significant use in modifying plants and animals for beneficial purposes." Richard Palmiter of the University of Washington in Seattle, who has made important progress in attempts to transfer working genes into mice (SN: 12/18/82, p. 389), notes that methods allowing the introduction of functioning genes into animals may provide ways to accelerate animal growth, correct genetic diseases, and produce valuable gene products such as hormones. Harvard's Scholnick suggests that it may become possible to use transposable elements in gene transfers that could "modify plants for resistance to certain diseases or herbicides."

These optimistic forecasts are likely to prove accurate, although it is impossible to predict when such benefits will be realized. While Spradling agrees that transposons may be put to work in making "significant improvements in animals and plants used in agriculture," he says that the exact nature of the required genetic engineering is not necessarily straightforward. "Whether it would involve the germline or exactly how one might go about it is another problem," he explains.

While direct practical applications of gene transfer experiments in higher organisms may be several years in coming, basic research into the gene structures essential to normal gene regulation is moving rapidly forward. Although studies of gene regulation in fruit flies may seem only remotely related to questions about gene function in people, Spradling observes that "the laws of genetics were worked out in flies, and we now know that all those principles are directly applicable to other higher organisms." He concedes that some differences in the mechanisms of gene regulation may occur between flies and other higher organisms, but adds that regarding "these basic regulatory phenomena, there are probably far more similarities than differences."

Investigations of gene regulation in fruit flies should answer questions about related processes in other higher organisms, and may ultimately improve our understanding of gene regulation in humans. But predictions of far-reaching applications for transposon-mediated gene transfer shouldn't obscure the only certainty in this young field—that the P element does a spectacular job in *Drosophila.* This fact alone justifies widespread enthusiasm about the new technique for putting genes into fruit flies. Says Posakony, "Right now, the major obstacle to the P element's use for all the huge number of things you can think of to do with it is really the time it takes to raise the flies." □

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