Gene Transfer into the *Drosophila* Germ Line

A transposable element can efficiently transfer genes into the germ line of fruit flies, thereby correcting a genetic defect in their progeny

To trace the fate of individual genes during the development of complex organisms has long been a goal of biologists. The ability to isolate pure genes, to prepare them in large quantities, and to modify their structures in systematic ways opened the way to achieving that goal, but was not sufficient of itself. To study how genes are turned on or off in specific tissues as a many-celled organism develops from a single cell requires that the pure gene be introduced into the germ lines of experimental animals or plants so that its developmental fate can be examined in their progeny.

In the past few years investigators have made a great deal of progress toward introducing foreign genes into the germ lines of mice. They have had much less success with the fruit fly (*Drosophila melanogaster*) until recently.

In this issue of *Science*, Gerald M. Rubin and Allan C. Spradling of the Carnegie Institution of Washington (Baltimore), describe a new method for introducing foreign genes into the fruit fly germ line. "We can put the genes into the germ line," Rubin says, "and get them passed on to future generations."

The new method differs from other gene transfer techniques by using a transposable element, a segment of DNA that can move from place to place in the genome, to carry the foreign gene and facilitate its entry into the recipient DNA. According to Rubin and Spradling, the transfer is accomplished efficiently and without rearrangement of the transferred gene. Moveover, the gene persists in a stable form and may retain the ability to perform its normal function. In one series of experiments, the transferred gene corrected a genetic defect in the progeny of the original recipients. Igor Dawid of the National Institute of Child Health and Human Development says of the transposon method, "It gives a fantastic advantage. For *Drosophila*, it is the difference between not working and working."

The most immediate use of the method will be to study how genes are regulated during the development of the fruit fly, a particularly useful species for these studies because so much is already known about its physiology and genetics. As Spradling explains, "The genes are functional in a relatively normal way. Now we can mutate a gene and find out how particular sequences are working in development." But, Rubin and Spradling suggest, it may eventually be possible to use the method to induce beneficial changes in other plants and animals.

The *Drosophila* genome harbors several types of transposable elements. The one chosen by Rubin and Spradling for their gene transfer vehicle is the "P element," which moves at high frequency but only under certain well-defined, and controllable, genetic conditions.

Rubin, with Margaret Kidwell of Brown University and Paul Bingham of the State University of New York in Stony Brook, recently obtained direct evidence that P elements cause the complex of genetic abnormalities seen in the hybrid offspring produced by crossing certain fruit fly strains.* These abnormalities, which include sterility, frequent and unstable mutations, and chromosome rearrangements, occur in the progeny of matings between males of the P (for paternally contributing) strain and females of the M (maternally contributing) strain. The reciprocal cross, M males with P females, does not produce hybrid dysgenesis, as the complex of genetic abnormalities is called.

A few years ago, William Engels of the University of Wisconsin at Madison made the suggestion that hybrid dysgenesis is caused by a family of transposable elements, the P factors. According to this hypothesis, multiple copies of the P factor are dispersed through the P strain genome but absent from the M strain genome. The hypothesis further requires that the movement of the factors be repressed in the P cytotype (environment), but not in the M cytotype, and that the maternal strain determines the nature of the cytotype. If that were so, the factors could move in hybrids produced by mating P males with M females, thus producing the hybrid dysgenesis, but not in hybrids produced by mating M females with P males, which do not display this dysgenic behavior.

Rubin, Kidwell, and Bingham analyzed a series of seven independently derived mutations of the same genetic locus in dysgenic hybrids. Two of the mutations were caused by insertion of members of the *copia* family of transposable elements, many copies of which are found in the *Drosophila* genome. The other five were caused by members of a different family of insertion elements, which ranged in length from 0.5 to 1.4 kilobases (kb) and which Rubin, Kidwell, and Bingham called P elements. The evidence suggests that these are, in fact, the P factors predicted by Engels and the cause of the dysgenic behavior. For example, reversion of the mutations to the wild type was accompanied by precise loss of the P elements. Moreover, the excisions occurred much more frequently when the cytotype was M than when it was P, as would be expected if the movements of the P factors are repressed by the P cytotype.

Rubin says, "A number of lines of evidence indicate an association between the elements and hybrid dysgenesis, but there was a puzzle about how these elements could cause hybrid dysgenesis." The transposable element that causes hybrid dysgenesis needs to code for at least two functions, he explains. These are a "transposase" to catalyze the movement of the elements and a regulator of some kind to determine when the transposase is made. Production of the transposase has to be turned on in the M cytotype but off in the P cytotype. The P elements identified by Rubin, Kidwell, and Bingham were not large enough to carry that much information.

The simplest explanation seemed to be that these elements were incomplete versions of a large P element that had not yet been discovered. If this were so, then as long as the complete element is present in the genome to provide both transposing and regulatory functions, the incomplete elements can move or not, depending on the cytotype. Rubin points out that both the maize and bacterial systems of transposable elements provide precedents for such a relation between elements. "There has been genetic evidence consistent with this for 30 years."

With Kevin O'Hare, also at Carnegie, Rubin set out to find the larger element. "We just looked for other sequences homologous to the smaller elements. And we found them." The larger element contains about 3 kb. "It has the coding capacity to make the proteins you might need." About one-third of the 30 to 50 P elements found in P strain cells are large.

*G. M. Rubin, M. G. Kidwell, P. M. Bingham, *Cell* **29**, 987 (1982); P. M. Bingham, M. G. Kidwell, G. M. Rubin, *ibid.*, p. 995.

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Structural analyses show that the small elements are formed by the loss of internal segments of varying lengths from the large element. All of them, large and small, have the same 31–base pair inverted repeats at the ends. This may be the site of action of the P element transposase, although the presence of *copia* elements in two dysgenic mutants suggests that the P element might also activate the movement of other families of transposable elements.

"The identification of the P elements suggested a fairly obvious idea," Rubin says. "They might be ideal vectors for gene transfer. We knew that they would transpose at high rates in cells that didn't have them." The method chosen by Spradling and Rubin was very similar to the transfer of DNA by P strain sperm into an M strain egg. They injected the 3-kb P element, still contained in the cloning plasmid, into very young M strain embryos at about the time when the germ line precursor cells were forming.

To screen the progeny of the resulting fruit flies for the presence of the P elements, Spradling and Rubin wanted to use some visible change rather than the large number of biochemical assays that would otherwise be required. The embryos to be injected were from M strains that also carried an unusual mutation called *singed-weak* (sn^w), which affects the bristles and hairs on the fruit fly cuticle. This mutation, which was identified by Engels, is stable when it occurs in M strain fruit flies but in the presence of P factors is very unstable, either becoming more extreme in appearance or reverting to the wild type. The sn^w mutation is apparently caused by the insertion in the *singed* locus of one or two small P elements that cannot move on their own but can move if transposase activity is provided by a larger P element.

Spradling and Rubin found that up to 50 percent of the progeny of fruit flies that developed from embryos injected with the 3-kb P element displayed high mutability of the sn^w mutation. The mutation was stable in the progeny of uninjected embryos.

When the investigators analyzed the DNA from the parent sn^w strain and from stable and unstable strains derived from injected embryos, they found that DNA from the unstable strains contained one to five copies of the large P element in addition to the two P element sequences seen in the parent fruit flies. The DNA from stable strains did not have these additional copies. Although the P element with which Spradling and Rubin injected the embryos was contained in the plasmid used for cloning, only P element sequences, and not those of the cloning vehicle, could be detected in the DNA of transformed fruit flies. (Transformed here means that the fruit flies have acquired a foreign DNA.)

Having demonstrated that P elements would transpose from the plasmid into the DNA of fruit flies, Rubin and Spradling went on to the next step—use of the P element to introduce into the fruit fly germ line a foreign gene, the *rosy* gene, which was supplied to them by Welcome Bender of Harvard University and Arthur Chovnik of the University of Connecticut at Storrs. The *rosy* gene codes for xanthine dehydrogenase, an enzyme needed for purine metabolism. Fruit flies that cannot make this enzyme have a darker than normal eye color. The wild-type brick-red color can be restored by the production of as little as 5 percent of the normal enzyme activity, which does not need to be present in the eye itself.

Rubin and Spradling spliced the *rosy* gene into a 1.2-kb P element, which was cloned in a bacterial plasmid. Because the 1.2-kb element does not encode its own transposase, they had to supply the activity from another source. They did this either by injecting the recombinant plasmid into dysgenic embryos produced in the standard fashion by mating P strain males with M strain females or by injecting it together with the plasmid carrying the complete 3-kb element. The injected embryos were *rosy* mutants that have the abnormal eye color.

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Both methods work, according to Rubin and Spradling. From 20 to 50 percent of the injected embryos carried the *rosy* gene in their germ lines, as indicated by the ability of the resulting fruit flies to produce progeny with the normal eye color. These strains carried intact copies of the 1.2-kb P element with the *rosy* gene, which was stably inherited along with the normal eye color.

The use of transposable elements as gene carriers has two important advantages over other gene transfer systems now in use, say Rubin and Spradling. Rubin notes, "Our system is much more well defined and reproducible, and it works in the germ line of the whole organism." Gene transfer into cultured cells has proved valuable for studying some aspects of how genes are controlled, but cannot answer questions about how genes are turned on and off during the normal development of multicellular organisms.

Despite the recent progress toward introducing functional new genes into the germ lines of higher animals, primarily mice (*Science*, 28 August 1981, p. 996, and 1 January 1982, p. 44), these efforts are often hindered by problems associated with the gene transfer methods used. The problems include low efficiency of transfer and major rearrangements of the DNA that is transferred. Transfer by the transposable elements is more efficient, according to Rubin, and the boundaries of the DNA inserted into the recipient genome are defined by the ends of the P element. The transferred DNA does not appear to rearrange.

Rubin and Spradling suggest that it may be possible to use transposable elements to introduce new genes into the germ lines of plants and higher animals. If P elements themselves do not work, then elements indigenous to the species may. Most investigators think that all species will prove to have their own transposable elements, although with a few exceptions, including those of *Drosophila* and yeast, they have not yet been isolated from higher organisms.

Although the ability to introduce genes into the germ lines of higher organisms would have obvious implications for genetic engineering, the requirements that had to be satisfied to correct the fruit fly defect were not very stringent. Only 5 percent of the normal enzyme activity had to be restored and this did not need to be present in the target organ, the eye. Moreover, the correction took place even though the *rosy* gene integrated at a variety of chromosomal locations. The 50 integration sites identified thus far are scattered throughout the *Drosophila* genome, although there is a small cluster near the normal *rosy* gene site. However, Spradling notes that these insertions are "not close on a molecular scale" and the cluster might not be indicative of any specificity of integration.

The availability at last of a reliable method for introducing foreign genes into the *Drosophila* germ line will no doubt spark a surge of research on gene control during the development of this species, and eventually perhaps of others. Rubin notes that they have already sent P element clones to some 70 laboratories. Beatrice Mintz of the Institute for Cancer Research in the Philadelphia suburb of Fox Chase says, "The *Drosophila* workers will certainly reach their stated goal."—JEAN L. MARX