Ann. Rev. Genet. 1984. 18:271–93

THE EVOLUTIONARY IMPLICATIONS OF MOBILE GENETIC ELEMENTS

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INTRODUCTION

The genetic rearrangements induced by certain mobile elements provide direct selective value to the organism or its immediate offspring, thus giving a simple and satisfying answer to the question of the biological function of these

0066-4197/84/1215-0271$02.00

elements. The somatic cell genome rearrangements that accompany formation of active antibody-producing cells and the yeast-mating type switch are clear examples of such mobile genetic elements. As suggested by Campbell (16), in this review I include in this category those cases where the mobile element is an integral part of a cyclical change through which a species passes, such as phase variation in pathogenic bacteria and trypanosomes and most of the traits associated with bacterial plasmids. The drug-resistance transposons and the attachment-site function of IS2, IS3, and gamma delta in F plasmid integration are such plasmid traits. Those traits of the mobile elements that clearly fall into this class will not be considered here.

Many elements, however, produce no clear phenotypic manifestation other than their ability to induce mutations. Many of the transposable elements fall into this category. Because of this lack of phenotypic manifestation, it has been suggested that the biological function of this class of elements is the generation of a genetic diversity that helps drive evolution. Alternatively, it has been suggested that the transposable elements are merely genomic parasites. In this review I describe those results indicating a role for movable elements in evolutionary change. For a general review of the possible role of plasmids, temperate phages, and insertion sequences in bacterial evolution, see (17). In addition, a number of reviews have described the general properties of transposons; this subject will be treated only briefly here (11, 14, 18, 33, 72, 86, 90, 94, 97). Recently, however, considerable information has been presented on how transposons regulate their own movement. Because these regulatory mechanisms directly influence transposons' potential mutational activities, this subject will be considered in some detail. I will then cover some traits of transposable elements that seem relevant to their evolutionary role. Finally, the discussion will broaden to consider a possible role for animal viruses in the evolution of their hosts. I will describe transposable elements from bacteria, yeast, and metazoans. Similar phenomena for many of the examples were first documented in maize and, although many of the questions to be discussed were anticipated by McClintock (68), maize genetics will not be included in this review.

The Evolutionary Hypotheses

The debate on the evolutionary role of mobile elements has been framed in terms of two competing hypotheses. The first hypothesis is that mobile elements are sustained by direct selection acting on genetic variability (24, 72). If evolution is a race among evolving species, those species that give rise to successful variants at the highest rate will be favored (96). This "selectionist" hypothesis maintains that transposable elements operate as a complex molecular apparatus driving the speciation process, which in turn ensures the survival of the elements themselves. There are many known mutational mechanisms

besides those provided by transposable elements; these include such changes as point mutations as well as the same kinds of genetic rearrangements that are induced by transposable elements. We can therefore ask whether the changes induced by transposons are different enough to be selected for in this way. I believe that transposons have the potential to introduce highly complex changes in a single event, changes that are not easily induced by other mechanisms.

The second hypothesis, which in some of its initial formulations could be called the null hypothesis, is that the transposable elements need not provide selective advantage to the hosts that bear them in order to account for their presence (44, 75, 87). This is popularly called the "selfish-gene" theory; the mobile element is essentially treated as a chromosomal parasite that has been selected by its own ability to maintain itself independent of selection acting on the organism.

General Considerations

The parasitic gene hypothesis has gained some support because many transposons appear to duplicate themselves upon transposition, conserving the maternal copy and inserting a daughter copy into a new location. The simplest explanation for the presence of transposons does not account for the element's survival in terms of selective value to the host; rather, it describes their duplicative process as adequate insurance for the survival of the element. Mere self-perpetuating ability is consistent with the selfish-gene hypothesis, but it is also consistent with the selectionist hypothesis. If a transposon has no selective value for individuals that bear it during periods of stasis [to use the terminology from the theory of punctuated equilibria (39, 66)], but is only selected for during periods of change, then this self-perpetuating property may be required to ensure element survival during the stasis periods. The proponents of the selfish-gene theory have criticized the selectionists for resorting to group-selection arguments. I argue that it is not necessary to rely on a group selection mechanism, as Doolittle defines it (28, 65), since selection for the element at the level of the individual is possible. Assume for a moment that a population has a transposable element whose only trait is genetic variability. This confers no selective advantage to any individuals during periods of stasis. However, during periods of change, when only a few individuals are producing mutations that will become fixed in the entire future population, then by definition these mutations are strongly selected. What is unique about many of the mutations induced by transposable elements is that the elements themselves become part of the new traits. Thus, the trait of genetic variability is carried along by selection for the new mutation (17). This means that the trait of genetic variability associated with transposable elements alternates between being highly selected and being neutral or even slightly detrimental at the organismal level.

Two lines of evidence have motivated speculation that transposons may play a major evolutionary role. One is that gross genetic rearrangement appears to be an important mutational mechanism in evolutionary change. Wilson and coworkers have compared the rates of change in structural genes and in genome organization for both rapidly evolving species and slowly evolving species (12, 106). They found that the rate of change in the sequences of structural genes is basically the same for both groups of species. However, changes in genome organization were found to be more frequent in the rapidly evolving species. This correlation between changes in genome organization and changes in gross morphology has led to the inference that genome reorganization may be a major mutational mechanism for gross morphological change. (An alternative view is that gross chromosomal rearrangement is a major isolating mechanism, insulating the genotype of a daughter species from the parental gene flow.) In either case, the ability of transposons to induce gross genetic rearrangements would position them conveniently to play a role in this special type of mutation.

A second line of evidence invokes studies isolating bacterial mutants that have acquired new metabolic traits. These studies are considered an experimental model for progressive evolution. Mutations in the most common class involve changes in the regulatory patterns of preexisting enzymes, not in the creation of new enzymes (40, 58, 62, 105, 107). These regulatory changes involve mutations in regulatory proteins, activation of promoters, and genome rearrangements such as tandem duplications and transpositions. As we will see in the following sections, transposons are particularly well adapted to induce regulatory mutations.

BACTERIAL INSERTION SEQUENCES

Studies of the properties of the insertion sequences (IS) in *E. coli* form a general introduction to the properties of transposable elements. A bacterial insertion sequence, depending on its class, is a defined DNA sequence with two specific ends that has the ability to insert linearly into genomic loci. Sequences vary in size from about 800 to a few thousand base pairs, enough space for one or a few genes. Their presence was first detected because their insertion can cause a gene-inactivating mutation. Later IS's were identified as components of composite transposons (a composite transposon consists of two insertion sequences flanking a gene, often an antibiotic-resistance gene). In addition to their capacity for simple transposition, IS's are implicated in a rather complex series of chromosomal rearrangements. Since this topic has been reviewed elsewhere (17, 54), I will mention just some of these events.

Figure 1 illustrates two reactions that these elements promote. First is simple transposition, showing conservation of the maternal copy. The second is the cointegration reaction that many elements are able to induce. These two

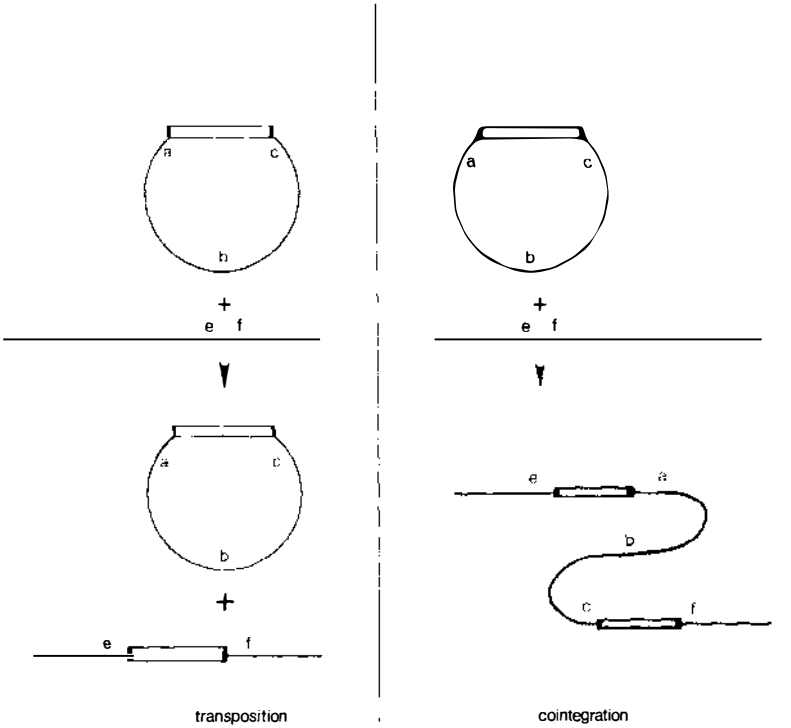

Figure 1 *Transposition:* An insertion sequence located between sequences *a* and *c* on the circle transposes into another chromosome between sequences *e* and *f*. Though the mechanism is not known, the result is preservation of the maternal copy in the descendent clone. Hence, insertion into a new site dupicates the element.

Cointegration: Some of the insertion sequences and the Tn3 class of transposons have the capacity to fuse two replicons together, giving a single chromosome with two copies of the element determining the novel junction (i.e. sequences e-element-a and c-element-f). In this reaction the transposable element probably duplicates itself by a replicative mechanism.

reactions divide the bacterial transposons into two classes. One is the Tn3 class, in which the transposition pathway includes the cointegration reaction as an intermediate step (89). The other is the IS class, in which it appears that transposition and cointegration are alternative, but closely related, pathways (35). In this latter class, the cointegration reaction has only been shown for a few elements, such as IS1 (74), IS50 (47) [but see (4) for an alternative interpretation], and IS21 (81).

The simplest rearrangement is precise excision, or the exact removal of the element. Recall that transposition of many transposons is a duplicative process in which the maternal copy is preserved; mechanistically precise excision is unrelated to movement. An insertion sequence can also stimulate the deletion

of sequences adjacent to its site of residence in reactions that may or may not delete the element itself. Inversion of the DNA sequences adjacent to the element can also occur. Both the deletion and inversion reactions are probably the result of the intramolecular transposition or cointegration reactions.

Two copies of the same element located in different regions of the chromosome can cooperate and promote transposition of sequences between them. In this way these elements can induce chromosomal translocations, which may be of great significance in higher organisms with multiple chromosomes. Most of these rearrangements can also occur without the mediation of insertion sequences, but the rate of these rearrangements can be greatly increased by the presence of the transposable element. For example, spontaneous deletions in the *His* operon of *Salmonella typhimurium* is probably stimulated 1,000-fold if a copy of Tn10 with its two copies of IS10 is present in that region (54).

Those elements that have been well-characterized in *Escherichia coli* include IS1, 2, 3, 5, 10, and 50 (54). Each of these elements has the ability to insert into a large number of different sites on the bacterial chromosome. The most intensively studied insertion sequences are IS10 and IS50. Because these elements are not naturally found in *E. coli* (5, 54), it has been possible to compare bacteria harboring these elements with those that do not. This comparison has not been possible for studies of IS1, 2, 3, and 5.

Self-Regulation of IS10 and IS50 Movement

Most mutations have undesirable consequences to the organisms that have experienced them. It is clear that a transposable element that is too highly mutagenic threatens the survival of its host. It is not surprising, therefore, that each transposon that has been sufficiently studied appears to have complex regulatory systems that modulate gene movement. Since the details of self-regulation have not yet been reviewed, and since an understanding of the mechanism of regulation is important to the discussion of mutational mechanism, I will illustrate these points with IS10 and IS50. However, I want to point out that transposition regulation was first documented using Tn3 (37, 43).

Biek & Roth (7) demonstrated transposition regulation with IS50. They showed that when Tn5, whose transposition is determined by its copies of IS50, first enters a host cell that does not contain a copy of Tn5, called a naive cell, the transposition frequency is quite high. Conversely, this transposition activity is inhibited in cells in which Tn5 is established. Subsequently it was shown that this phenomenon can be attributed to proteins that are expressed from IS50R, the right stem of Tn5 (48, 49, 80).

In Tn5, two nearly identical copies of IS50, referred to as IS50R and IS50L, flank a central region that carries a kanamycin-resistance gene. IS50L differs from IS50R at a single base, yet this difference provides IS50L with an outward-reading promoter (85). IS50R encodes at least two proteins on over-

lapping structural genes. One of these proteins, p1, is used to activate transposition of IS50R (or Tn5, as the case may be) and the second protein, p2, inhibits the transposition process. The single base-pair difference in IS50L renders its proteins inactive in both inhibiting and activating transposition. Apparently p2 does not regulate expression of the IS50 genes; rather, it inhibits the transposition reaction directly (48, 49).

IS10 is found in the composite transposon Tn10; two nearly identical IS10 elements flank a tetracyclin-resistance gene. One of these, IS10R, encodes both a protein that is required for its transposition and an RNA molecule that serves to regulate IS10 transposition. This RNA molecule inhibits translation of the mRNA from which the activator protein is made (92).

IS10 and IS50, though they show differences in details and regulatory mechanism, have similar phenotypic consequences. Both display an additional means by which transposition is modulated. Each activator product acts preferentially on the transposon from which it was synthesized. This was demonstrated in genetic complementation tests of activator-deficient mutants, where it was found that the wild-type activator behaved as a cis-acting protein. Therefore, when multiple copies of the transposon are present in a cell, one transposon's activator will not stimulate a second copy of the same transposon.

Extreme activator specificity and diffusible inhibitors thus combine to regulate transposition frequency and perhaps transposon copy number. As described above, transposition is a duplicative process; hence, transposon copy number tends to increase. This prompts the question of whether a regulatory mechanism exists that prevents excessive accumulation of copies in the affected host. The properties of IS50R (50) and IS10R (92) suggest that copy number control may be a consequence of the inherent properties of the cis-acting activator and the trans-acting inhibitor. For example, using these two features, it can be shown that the transposition frequency *per* transposon will necessarily decrease with increasing copy number. This follows because a transposon's inhibitor can act on another identical transposon but its activator can not. Johnson & Reznikoff (50) directly measured transposition frequency of Tn5 against varying copies of IS50. They found that the frequency of Tn5 movement decreased linearly with copy number over a 20-fold range. These results imply that the trans-acting inhibitor and the cis-acting activator combination, though clearly limiting the rate of IS50 spread, is not sufficient to limit the number of copies that may accumulate in a cell. An additional factor is required.

This model for copy number limitation depends on the activator being cis-acting. It is interesting to note that Tn3, which has a trans-acting activator, is able to regulate its own copy number through an entirely different mechanism. A Tn3 resident in a given replicon contains a cis-acting sequence that prevents the insertion of a second Tn3 in that replicon. This control, called

transposition immunity, is independent of the diffusible repressor of Tn3's activator (57, 103). Thus, the number of copies of Tn3 is determined by the number of replicons.

It seems reasonable to deduce that insertion sequences carry the genetic information that promotes and inhibits their own transposition. These transposition-controlling signals may be arranged to govern copy number. The ability of transposable elements to regulate their own movement has important implications for their possible evolutionary role. The fact that the transposition frequency can become very high through disruption of the negative regulatory factor (for example, by introduction into a naive cell or by mutation) provides the transposon with the potential to be highly mutagenic. It is tempting to speculate that such activated transposons could have possible benefits during periods of intense selection for new species.

The Effect of IS Insertion on Gene Expression

Insertion of an IS element into an operon usually causes highly polar mutations resulting in the inactivation of all genes promoter-distal to the element. This polarity is often the result of transcriptional terminators that reside in the elements. These terminators possibly are required for the normal maintenance of the insertion sequences themselves. For IS1 (63), IS10 (41, 91), and IS50 (88), it appears that the act of transcription from external promoters across the ends of the respective elements affects the ability of these elements to transpose. An exception to polar insertions was discovered when certain insertions of IS2 were found to activate expression of adjacent genes (97). Subsequently, IS10 and IS50 have also been shown to have a similar effect (6, 23). Expression of these adjacent genes is due to new promoters introduced by the element and not to transcriptional readthrough. Apparently, IS2 does not carry a complete outward-reading promoter but, rather, has the capacity to create one (38). Hinton & Musso observed a promoter created from the novel DNA sequences found at the insertion site (45). Insertions of IS3 also have the ability to activate adjacent genes transcriptionally, but the mechanism of this activation is not understood (38). Since the insertion sequences native to *E. coli* probably exist in multiple, non-identical copies, generalizations based on a single example may not be valid. Different copies of a given IS may produce different phenotypes upon insertion at the same site.

With IS10 there is a good case for the presence of a promoter that can transcribe adjacent genes. This promoter is capable of expressing genes in the *His* operon of *S. typhimurium* (23). The RNA molecule transcribed from this outward-reading promoter is apparently the same one that regulates IS10 transposition (91); hence, it can be viewed as contributing to the maintenence of IS10. With IS50L, where the outward-reading promoter transcribes the kanamycin-resistance gene in Tn5, this promoter does not obviously function

to maintain IS50 in general. On the contrary, the presence of this promoter renders IS50L incapable of promoting its own transposition and makes it dependent upon IS50R for its transposition functions.

IS1 and IS5 can have a profound influence on the regulatory patterns of adjacent genes. Insertion of IS1 or IS5 into the silent *bgl* genes renders these genes inducible by their normal substrate, ß-glucoside sugars. The molecular mechanism of IS1 or IS5 activation of the *bgl* genes is not understood, but apparently insertion of these elements near the *bgl* promoter increases its activity. This example is particularly interesting because the activated gene is still regulated, not constitutively expressed (79). Similarly, Barany et al (2) have isolated an erythromycin-resistance R-plasmid from Gram(+) bacteria that is able to replicate in and confer a degree of erythromycin resistance to *E. coli*. Spontaneous mutations to higher levels of resistance in *E. coli* were found to correlate with insertions of IS1, IS2, and IS5. It seems possible, though the results are still preliminary, that the insertion sequences cause an increase in transcription from the normal promoter.

In summary, transcription terminators in most of the bacterial insertion sequences, in addition to the outward promoter on IS10, are probably important in the regulation of transposition and are probably maintained for the benefit of the element itself. The incipient promoter in IS2, and maybe IS3, the outward-reading promoter in IS50L, and the promoter-enhancer activity associated with IS1 and IS5 are not obviously required for maintaining or regulating the respective insertion sequences. The capacity to introduce a complex regulatory function in a single mutational step is a feature that clearly would increase the evolutionary potential of any cell harboring these factors.

Growth Competition Experiments

I have argued that transposable elements, by their ability to induce gross chromosomal rearrangements and highly complex regulatory changes, seem to be uniquely positioned to influence evolution. Now I will describe recent data that show that at least one insertion sequence actually does provide a selective advantage to *E. coli* at the level of genetic variability. The possible benefits of both IS10 and IS50 to the populations of *E. coli* that bear them have been directly assessed by growth competition experiments in chemostats.

The effect of IS10 was studied by Chao et al (22). A strain containing Tn10, the IS10 source, was grown along with an isogenic strain lacking Tn10 in tetracyclin-free glucose-limited chemostats. The result was that the Tn10-containing strain clearly increased its proportion in the chemostat by about 200-fold. This advantage to the Tn10-bearing strain appears to be the result of the mutagenic affect associated with Tn10. It has been known for some time that populations of mutator strains have a selective advantage over wild type in long-term competition in chemostats [(27, 36); reviewed in (26)]. For example,

when *mutT* (21) is initially placed in a chemostat with wild-type cells, no differences in growth are observed for the first 50–150 generations, after which the *mutT*-containing strain increases its relative proportion by about 200-fold over the next 50 generations. The increase is caused by the appearance of a faster growing mutant in the *mutT* population. This outcome is seen only when the ratio of *mutT* to wild-type cells in the initial inoculum is 10^{-3} or greater. The long lag in growth advantage and the dependence on the frequency of the initial inoculum are also seen when Tn10-carrying strains are grown in pairwise competition against wild types. In addition, when the victorious Tn10-containing clones are analyzed, an additional copy of IS10 is found at a new location. Tn10-carrying clones derived from chemostats where the wild type dominated did not show additional copies of IS10. Interestingly, in four separate chemostat experiments in which the Tn10 strain won, the new IS10 was inserted in a PvuII restriction fragment of identical size, suggesting that the same mutational event is selected each time.

In chemostat competition experiments with IS50R reported by Hartl and coworkers (8, 42), an entirely different pattern was observed: the IS50R-containing strains grew more rapidly for about 50 generations. The competitive advantage was immediate upon inoculation and showed no frequency dependence, indicating a direct growth advantage for IS50R. The advantage was not observed with IS50R mutants unable to synthesize p1 and p2, and was seen only when fresh cultures were first placed in the chemostat. After 25 generations the growth advantage IS50R provided was lost; furthermore, if the two strains were pregrown for 75 generations in monoclonal chemostats before mixing, no growth differences were seen. Finally, when victorious IS50R clones were analyzed, there was no indication of IS50R-mediated rearrangements or transpositions. The most reasonable explanation for these events is that IS50R confers a direct growth advantage to its host, an advantage that is seen only immediately after transfer into the glucose-limiting chemostat.

Results similar to those obtained with IS50R have been obtained in growth competition experiments between lysogens of phage λ and non-lysogens. Edlin and coworkers (61) have observed that λ lysogens outperform non-lysogens immediately after inoculation into a glucose-limited chemostat. In addition, as with IS50R, the advantage appears to be frequency-independent and to persist only for a limited number of generations, since the lysogens never completely overtake the entire population. Lysogens of phages mu, P1, and P2 also behave in a similar manner when compared to their respective non-lysogens (30). The molecular mechanism conferring this growth advantage is not understood, but preliminary indications hint that cellular respiration is directly or indirectly involved (61).

To summarize the results of the growth competition experiments: IS10-containing strains are selected over wild type because the IS10 induces a

selectively advantageous mutation showing directly that IS10 increases its proportion in the chemostat because of its phenotype of genetic variability. IS50R-containing strains are selected over wild type because of some physiological trait. The λ, P1, P2, and mu prophages behave like IS50R does. None of these experimental results conforms to the predictions of the selfish-gene hypothesis.

TRANSPOSONS IN YEAST

We have discussed the properties of the bacterial insertion sequences and how they may influence gene activity. A heterogeneous class of transposons in yeast, called Ty, appear to carry an even larger variety of gene-regulatory signals. The Ty elements seem to be highly coadapted with the rest of the genome in that they preferentially insert into noncoding regions of structural genes, thus avoiding unnecessarily destructive mutations (31). For example, from a collection of 8 Ty induced auxotrophs (5 *lys*$^-$ (31), 2 *his*$^-$ (83), and 1 *ura*$^-$ (84)), 6 are located at the 5' end of the respective gene, while only 2 are structural gene insertions. Reversion studies on one of the *his* auxotrophs, induced by a Ty insertion 5' to the normal *his*4 promoter (Ty917), has revealed a large spectrum of possible Ty phenotypes. Roeder & Fink (82) selected for a gene conversion event between the Ty sequence in Ty917 (*his*$^-$) and other Ty sequences in the cell. The resulting strains were found to have a variety of His phenotypes: some remained *his*$^-$, while others were leaky or cold-sensitive *his* auxotrophs. In one class, *his* gene expression was placed under the control of the mating-type configuration. This result demonstrates the heterogeneity among Ty elements, even though each one must have had sufficient homology to recombine with the sequences in Ty917.

The ability of Ty insertions to alter the regulatory pattern of genes was initially observed in a screen of cis-dominant constitutive mutants at a variety of loci (34, 104). One of these constitutive mutants in alcohol dehydrogenase is controlled by a combination of the mating-type configuration and glucose levels—these signals represent a qualitatively different pattern of gene expression as compared to the wild-type (100). In these various cases, it is unclear if a promoter in Ty is responsible for the different regulatory responses or if Ty activates an adjacent promoter. Because of this regulatory versatility, Errede et al (34) have suggested that Ty sequences normally play a role in coordinate gene expression and differentiation, although direct evidence for this is not yet in. Clearly the ability of Ty elements to selectively insert into the 5' controlling regions of genes and to introduce such a variety of regulatory responses provides obvious selective possibilities to populations of yeast (31). It is difficult to imagine how a selfish Ty element could have evolved so many and

such complex regulatory responses without these having been selected at the organismal level.

In addition to Ty's ability to transpose, they have been implicated in a variety of genomic rearrangements, including deletions, tandem duplications, inversions, and chromosome translocations, as has been reviewed elsewhere (83). These various rearrangements appear to be the result of general recombination between different homologous Ty elements and not the result of the transpositional activities associated with Ty.

TRANSPOSONS IN DROSOPHILA

A variety of transposable elements have been discovered in flies (86, 94). In some respects, their evolutionary role is better understood than that of other elements because systematic studies on the distribution of these elements in natural populations have been conducted. The properties of these elements have been reviewed elsewhere, but a few points relevant to the present topic will be mentioned. Transposons in Drosophila, like those in bacteria and yeast, promote gross genetic rearrangements. Systematic studies of rearrangements induced by *P* elements at a variety of loci (33) and by *foldback* at the *white-crimson* allele (25) have been described.

Copy Number Regulation

It appears quite likely that Drosophila's transposons experience active copy number control. A copy number control scheme affecting the *copia*-like elements seems probable. Young (108, 109) examined the distribution of *copia* in about a dozen different geographical isolates and found considerable insertion-site polymorphism from one population to another. From among all the isolates, nearly 200 different insertion sites could be inferred. However, no single isolate contained more than 30 to 50 *copia* copies. Thus, it appears that the upper limit of 30–50 copies per haploid genome is not maintained by a lack of available sites, but more likely by some factor that restricts further transposition after a certain point. The finding that the genomes from fly cells grown in tissue culture for many generation increase their copy number two- to three-fold demonstrated that the *copia* elements had not lost the ability to transpose (76). Preservation of copy number in whole flies implies that either natural selection acts directly to maintain copy number or that *copia* is self-regulated. An alternative explanation, that the distribution is simply the result of random gain (by transposition) and loss (by excision) of copies, predicts a much broader distribution of copy number than the one observed.

A stronger case for self-regulation of copy number is seen with the *P* transposons (33). There are strains of Drosophila lacking *P* transposons into which the *P* transposons can be introduced. This event has occurred repeatedly

in nature as well as in the laboratory. At low copy number, the transposition frequency is seen to be quite high and copy number invariably increases until 30–50 elements have accumulated. At this point, observable transposition events decrease and further accumulation is not seen.

Regulatory Mutations Induced by Transposons

Many different insertion mutations have been characterized in Drosophila and some of these have phenotypes suggesting altered regulatory patterns. However, so little is known about gene expression in flies that it is difficult to distinguish whether the insertion mutations disrupt a normal regulatory response or introduce new regulatory features. Nevertheless, there are three cases that may indicate an ability among some of Drosophila's transposons to influence the expression of genes adjacent to the site of the respective insertions.

Rubin and coworkers (59) have presented evidence showing that a *foldback* element insertion restores a lost gene function. This was seen in revertants of a specific white-eyed mutant called *white-ivory*. This mutation is a tandem duplication in or near a gene that controls eye pigment. There is a class of partial revertants, called *white-crimson,* that are induced by a *foldback* insertion into the tandem duplication. Too little is known about the organization of the *white* structural gene to suggest any mechanism except to say that the *foldback* element, in at least this case, can partially activate a silent gene.

McGinnis et al (69) have identified an insertion of *hobo* in the *sgs4* gene that causes a 50-fold reduction in the larval salivary gland glue protein. *Hobo* is inserted just 5' to the TATAA box and presumably disrupts normal promoter activity. The leaky activity in the mutant is now provided by four different transcripts, as compared to one in wild type, and two of these originate within *hobo*. The interesting result is that these fusion transcripts are properly regulated; they appear only in salivary glands at the proper time of development. In addition, no other *hobo* transcripts can be detected in these cells or in the ancestral wild type, indicating that the other *hobo* copies in the genome, including the parental, are not transcribed. Thus, at least one *hobo* copy carries a silent promoter activity that can be activated and developmentally regulated when it inserts into the proper locus.

Gypsy, one of the *copia*-like elements, is another transposon in Drosophila. Mutations induced by *gypsy* insertion have been characterized at a variety of loci and many of these have been found to be suppressed by an unlinked recessive mutation known as *su(Hw)* (71). This suppressor is specific to mutations that are *gypsy* insertions. The simplest interpretation is that *su(Hw)*$^+$ is a stable chromosomal gene that makes a product influencing *gypsy*'s ability to affect the gene expression of adjacent sequences. This implies that gene expression sites in *gypsy* are controlled by a normal Drosophila gene—a

situation that would be expected if *gypsy*-induced mutations were of selective advantage during Drosophila's evolution. Alternatively, *su(Hw)*$^+$ may have evolved to protect against the destructive features of certain mobile elements.

These three transposons appear to be integrated with the fly's regulatory signals. The results can be interpreted as being due to fortuitous expressions of self-preservation functions within the transposons, but it is probably easier to explain these features as highly evolved mutator activities that were selected at the organismal level.

Hybrid Dysgenesis

The behavior of the *I* and *P* transposons provides the most direct evidence for an evolutionary role for mobile genetic elements. These two elements are responsible for hybrid dysgenesis, a phenomenon that has been the subject of two recent reviews (11, 33). The two systems differ in some details, so I will focus this discussion on the *P* element, the element for which there is more information (9). The phenomenology of hybrid dysgenesis is observed when males that carry *P* transposons are crossed with P^- females. This cross results in a variety of unusual genetic phenomena in the progeny; one of the most striking is an extremely high frequency of *P* element transposition. Indeed, nearly all of the chromosomes in the dysgenic cross will have experienced a transposition event. In addition, the chromosomes that carry the *P* elements will be highly susceptible to rearrangements such as duplications, inversions, deletions, and translocations. The *P* elements also undergo high rates of precise excision. Progeny from the dysgenic cross therefore carry an extremely large number of visible and lethal mutations. In addition, the hybrid progeny will be sterile if they are raised at high temperature (33, 52). Thus, *P* elements are responsible for a partial reproductive barrier between flies that have, and those that do not have, the element.

Certain strains of Drosophila lack *P* elements and into these strains *P*-element DNA can be introduced. This fact has furthered the progress of *P* element studies considerably. Spradling & Rubin (95), for example, were able to show that mutated *P* elements were unable to transpose but could be complemented to transpose by the wild-type *P* element. This shows that the *P* element encodes a factor that promotes its own transposition.

The dysgenic cross is not observed when P^- males mate with P^+ females, and nonhybrid crosses show none of these traits. This asymmetry has led Engels (32) to propose that the *P* transposon encodes a second factor that inhibits expression of the *P* element transposition functions. According to this hypothesis, *P*-element DNA from the P^+ male enters the cytoplasm of the egg from the P^- female, which is missing the negative factor. This causes the induction of the transposition functions, which leads to extreme mutability. In the cytoplasm of the egg from the P^+ cells, sufficient negative cytoplasmic

factor is present to repress the transposition functions. The actual mechanism of *P*-element regulation will probably turn out to be something like this, but it is necessarily more complicated. For example, the fertile females that result from the dysgenic cross carry *P* transposons but have the P^- regulatory phenotype (also called the M cytotype) (32, 33). The P^- regulatory phenotype persists until about 30 to 50 *P* transposons accumulate. Thus, full expression of the cytoplasmic inhibitor seems to be dependent on copy number. Making the picture even more complicated is the fact that the female progeny resulting from the non-dysgenic hybrid cross have the P^+ regulatory phenotype. These females presumably have the same number of *P* elements as do the females that result from the dysgenic cross. It is therefore necessary to propose some temporal regulation on P^+ regulatory expression. O'Hare & Rubin (73) provide the outlines of a *P*-element self-regulation model where the regulatory protein is required to activate itself in order to inhibit transposition. This kind of mechanism would provide a temporal delay in inhibitor synthesis, much as the synthesis of the bacteriophage λ repressor is delayed by a similar mechanism.

Distribution of P *Factors*

P^- flies are found in nature infrequently today, but they are common in laboratory strains taken from nature thirty years ago. In fact, *P* elements are not seen in flies isolated before 1945, making it appear that the *P* element has recently been introduced into wild-fly populations, as the "recent-invasion" hypothesis suggests (51). The *P* element could have originated either from an unrelated species and been introduced into Drosophila by means of viral transmission (70) or, less likely, could have arisen in an isolated Drosophila sub-population.

Alternatively, the "stochastic-loss" hypothesis proposes that all wild flies originally carried *P* elements but that the laboratory strains lost them (33). The major difficulty with this explanation is that the early flies are not just P^- in their phenotype; they have no DNA sequences that cross hybridize to *P* element sequences. It is hard to imagine how the multiple copies could have become so completely deleted in such a short period of time. If this is true, we will also have to explain why *P* DNA is so much less stable than the other middle repetitive sequences in laboratory stocks. In any case, this hypothesis is testable by monitoring the long-term stability of *P* sequences in current laboratory stocks.

If we grant that the recent-invasion hypothesis is true, it means we have witnessed a major genetic change in fly populations that represents a substantial step toward a speciation event. The importance of hybrid dysgenesis to theories of speciation is considerable and replete with consequences (51, 109). First, this phenomenon provides a mechanism for the partial reproductive isolation of a subgroup within a larger population, without a need for geographic isolation.

In addition, given their ability to spread so quickly, the *P* elements possibly provide a mechanism for the speciation of a very large population; i.e. sympatric speciation. One of the major arguments against sympatric speciation is the difficulty in introducing new genotypes into large populations. The flow of normal Mendelian traits within large populations resists the introduction of new traits. This concern has been a major impetus toward the general notion that speciation events occur in smaller founder populations in which new genotypes can first become integrated (67). The founder population is then imagined to displace the ancestral population at some later time. The *P* transposons may show how a new trait can sweep through a very large population. Not only is the spread of the trait rapid, but one of its consequences is the production of sterile offspring in the dysgenic crosses. Such a result has been traditionally used to classify different individuals into subspecies.

The *I* transposons also have a hybrid dysgenic phenotype, but it is manifested in different ways; in general, it appears to be less severe (11). The I^+ phenotype made a significant appearance in flies in the 1930s.

Laven first pointed out the potential of this type of asymmetric sterility for providing a mechanism for sympatric speciation (53, 55). In this case, he documented a phenomenon phenotypically similar to hybrid dysgenesis, but which occurred between different native populations of the mosquito *Culex pipiens*. The molecular mechanism of asymmetric sterility in these mosquitoes has not been determined and may not involve transposable elements. However, the result is sufficiently close to hybrid dysgenesis that the evolutionary implications are the same.

It is difficult to argue that the *I* and *P* elements have no evolutionary implications for Drosophila. The fact that some flies lack *P* factors argues against their need for individual survival. The question becomes: was the introduction of *P* and *I* in today's wild populations selected for or was the spread simply the result of infection? We have no evidence indicating that P^+ and I^+ strains are more highly adapted than the earlier P^- and I^- strains, although it is possible that the *P* and *I* phenotypes were directly selected by the associated mutator activities. It has been pointed out that the presumed *I*-element invasion (or *I*-element activation as the case may be) occurred during the widespread introduction of DDT and that the *P*-element invasion occurred after the introduction of the organophosphate insecticides. Perhaps other changes in human activity produced a selective advantage for mutator activities associated with these elements. The idea here is that the progeny of dysgenic crosses experienced very high mutation frequencies and that some of the resulting mutants were selected, thus fixing the respective transposable elements. If the selfish-gene explanation for the rapid spread of the *P* and *I* transposons is assumed and if two elements become randomly fixed each century, the number of these elements in evolutionary time would be unreason-

ably large (51). Perhaps it can be argued that some recent stress made Drosophila vulnerable to *I* factor and *P* factor spread, without beneficial results to the fly.

MOBILE ELEMENTS IN MAMMALIAN CELLS

A variety of DNA sequences have been encountered in higher metazoans that indicate the widespread occurrence of transposable elements. However, most of the sequences have not been seen to move; their movement has been inferred from their properties. For example, the presence of a repetitive sequence strongly suggests a single precursor that amplified itself (20). Many classes of the highly repeated sequences show greater similarity of sequence within species than between species. This suggests the occurrence of gene conversion events between the homologous sequences within the species, an event that Dover and co-workers have suggested may have profound evolutionary implications (29).

The absence of large collections of spontaneous mutations in either animals or cultured cell lines has delayed the types of studies that we have summarized with bacteria, yeast, and Drosophila. We have two different reports of insertion mutations in mammalian cells (15, 77). In these studies an SV40 shuttle vector carrying a bacterial gene was passaged through a cultured cell line that expressed the appropriate replication proteins for the SV40 origin. These plasmids were then placed back into bacteria, and those that had null mutations in the cloned bacterial gene were isolated. Among a collection of hundreds of mutant plasmids, a few percent were found to have insertions of foreign DNA that originated from the mammalian cells. In addition, foreign DNA, when transformed into cells, has been observed to directly integrate into the genome at remarkably high frequencies. This has been seen for linear and circular DNA molecules that need not have any detectable homology with the host genome. The simplest way to explain this observation is that the linear molecules are circularized by the end-joining activity present in these cells. The circles are then inserted into the chromosome (10, 19), possibly through the action of specialized sites and proteins. As yet, we do not have compelling evidence that the foreign DNA inserts into specific sites, although Stringer (98) has obtained indications that an insertion of SV40 may be adjacent to a site that may stimulate recombination.

Transforming viruses, especially the retroviruses, are frequently discussed along with the mobile genetic elements because of their structural similarity to many of the transposable elements. These viruses are obviously parasitic, but their evolutionary role could be quite profound, as we will see in the next section.

CROSS-SPECIES GENE TRANSFER

To bacteriologists, the ready transfer of mobile DNA's between distantly related species is widely accepted as important to the survival of bacterial populations in specific environments and, furthermore, as potentially important for the evolution of novel traits. Reference to the possibility that cross-species gene transfer is related to the evolution of higher organisms is not as frequently made, though there is increasing reason to believe that genes do occasionally cross species barriers. Norman Anderson (1) was the first to present arguments in favor of cross-species gene transfer as an important evolutionary force. Since then, a number of comparisons of either homologous gene or protein sequences between different species have been encountered that could be interpreted as resulting from a gene transfer between the lineages under comparison (3, 13, 46, 60, 64, 93, 99). Such examples should probably be expected if we consider that the various components of the mobile genetic elements provide a mechanism for transferring genes from one species to another. This mechanism involves the ability of broad host-range viruses to package nucleic acid that derives from one of the permissive hosts and the subsequent uncoating of this nucleic acid in the nucleus of a cell of a different species. Once this nucleic acid has entered the cell, it need only recombine into that cell's chromosome. If the in-coming nucleic acid is double-stranded DNA, it can be directly integrated into the host chromosome by the integration activities summarized above. If the nucleic acid is a single-stranded RNA that has been brought in by a retrovirus, a DNA copy can be synthesized by reverse transcriptase before the information can be integrated. This last step is not based on pure speculation; there are a number of examples of germ line sequences that are most easily interpreted as having derived from RNA transcripts. Two prominent cases are the structure of the mammalian α-globin pseudo-gene (56, 102) and a small nuclear RNA pseudo-gene (101). Thus, all of the components needed to transfer genes from one mammalian species to another are available. To firmly oppose cross-species gene transfer under these circumstances requires the additional postulate that known mechanisms are not used.

In view of the above discussion, it seems to me that the most interesting question is not whether genes do transfer across species boundaries but whether the frequency is high enough to actually influence evolutionary trends. Speculation on this topic has been opposed as a gratuitous exercise, since many evolutionists are currently satisfied with macro-evolutionary theory and see no need for major modification. A theory of evolution that incorporates a high rate of cross-species gene transfer can provide a unique explanation for a variety of unrelated phenomena. In particular, horizontal gene flow can account for biological unities. These include the uniform genetic code among all living organisms, similar spectra of hormone/receptor-protein combinations within phyla, related gene expression signals and the highly conserved embryological

development programs within classes and phyla. Traditionally, the commonality of these biological processes was considered merely a reflection of common ancestry, with functional constraint preserving these processes. However, identifying this functional constraint poses a difficult question. With an evolutionary theory incorporating cross-species gene transfer, we can provide a single explanation: the biological unities are maintained by selection at the level of evolutionary rate. A lineage whose systems diverge from the unified rules will lose the ability to incorporate certain foreign traits that may offer selective advantage; hence, its evolutionary rate would slow. This explanation presupposes that the rate of evolutionary change is significantly affected by cross-species gene transfer.

CONCLUSION

Transposons are an important class of mobile genetic elements that both promote and regulate their own movement. The regulatory schemes are probably necessary adaptations for the various elements in order to modulate their destructive potential to the genomes in which they reside. A general feature of these regulatory schemes is to limit the number of elements that accumulate in a given genome.

In addition to promoting and controlling their own movement, many of the elements carry signals that permit them to affect the regulation of adjacent genes. I have presented the various adjacent gene-regulating activities as evidence supporting the hypothesis that these features of the transposable elements evolved by selection at the oranismal level. Direct support for selection of transposons at the level of their ability to influence genetic variability was found for IS 10 during the chemostat competition experiments. Because the transposable element becomes tightly linked to any mutation it induces, its fixation in a population is guaranteed whenever it induces a highly selected mutant. If the gene-regulating activities of transposons have in fact been selected at the level of the organism, then a necessary prediction is that these elements, or the relevant sequences derived from them, will be found to be essential parts of functioning, regulated genes.

Evolutionists have not paid much attention to mutational mechanisms; they tend to be more interested in the result of mutations, that is, in the variants and how the variants can be fixed in large populations. As I have attempted to show, the properties of transposable elements and the types of mutations they induce have a direct bearing on these larger questions. First, transposable elements can induce mutations that result in complex and intricately regulated changes in a single step. This provides a mechanism for what has been termed *macromutation,* an event that has been considered by many to be unimportant in evolutionary change. If classes of mobile elements have been selected because they induce just such events, it may be that macromutation is an important

evolutionary feature. A highly evolved macromutational mechanism lends direct support to saltationist views of evolution. Second, mobile genetic elements have the potential to move extremely rapidly through large populations, which should unsettle the general notion that large populations are resistant to genetic change. The induction of hybrid dysgenesis by the *P* transposon in Drosophila, which can be considered a sub-speciation event, provides a direct mechanism for sympatric speciation. Finally, the recent discoveries of genes that seem to have moved across species boundaries imply that viral transmission of genetic information may play an important evolutionary role. If cross-species gene transfer is found to occur frequently, then this event could have a profound influence on macroevolutionary trends (78).

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