The Dynamic Genome: Transposable Elements

1. Mutations in gal can be generated, and from these strains, λdagl phage isolated. Through hybridization of denatured λdagl DNA containing the mutation with wild-type λdagl DNA, some of the molecules will be heteroduplexes between one mutant and one wild-type strand. If the mutation was caused by an insertion, the heteroduplexes will show a “looped out” section of single-stranded DNA, confirming that one DNA strand contains a sequence of DNA not present in the other.

The text also illustrates a method to compare the densities of gal+-carrying λ phage with gal–-carrying phage. In this experiment, the gal–-phage are denser, indicating that they contain a larger DNA molecule.

If the gal genes are cloned, direct comparison of the restriction maps or even the DNA sequence of mutants compared with wild type will give specific information about whether any are the results of insertions.

Using primers that flank the gene, PCR can be used to determine the size of the intervening fragment. Insertions would result in a larger-sized fragment compared to wild type.

2. In replicative transposition, transposable elements move to a new location by replicating into the target DNA, leaving behind a copy of the transposable element at the original site. If, on the other hand, the transposable element excises from its original position and inserts into a new position, this is called conservative transposition.

To test either mechanism, experiments must be designed so that both the “old” and “new” positions of the transposon can be assayed. If the transposon remains in the old site at the same time that a new copy is detected elsewhere, a replicative mechanism must be in use. If the transposon no longer exists in the old site when a copy is detected elsewhere, a conservative mechanism must be in use.

Figure 13-13 in the companion text describes how replicative transposition can be observed between two plasmids. The same general protocol could be used to detect conservative transposition, but of course the results would be different. Kleckner and co-workers actually demonstrated conservative transposition by following the movement of a transposon that contained a small heteroduplex within the lacZ gene. The DNA of two derivatives of Tn10 carrying different lacZ alleles (one being wild-type and the other being mutant) were denatured and allowed to reanneal. In some cases, the DNA molecules that reformed were actually heteroduplexes; one strand contained the lacZ+ allele, and the other strand contained the lacZ– allele. Transpositions of theses heteroduplexes were then followed. Based on the mechanism of movement, two outcomes are possible. If replicative transposition occurred, the semiconservative nature of DNA replication would generate two genetically different transposons: instead of the heteroduplex lacZ+/lacZ– DNA, one would now be all lacZ+ and the other all lacZ–. If transposition was conservative, the lacZ gene would still be heteroduplex
lacZ+/lacZ− after transposition and the first cell division would resolve the heteroduplex. This is what was observed. (The “sectored” colonies are the result of the original cell still having the lacZ+/lacZ− heteroduplex after transposition. After the first division one cell was now lacZ+ and the other was lacZ−). The experiment is outlined below:

3. R plasmids are the main carriers of drug resistance. These plasmids are self-replicating and contain any number of genes for drug resistance, as well as the genes necessary for transfer by conjugation (called the RTF region). It is R plasmid’s ability to transfer rapidly to other cells, even those of related species, that allows drug resistance to spread so rapidly. R plasmids acquire drug-resistance genes through transposition. Drug-resistance genes are found flanked by IR (inverted repeat) sequences and as a unit are known as transposons. Many transposons have been identified, and as a set they encode a wide range of drug resistances (see the table in the companion text). Because transposons can “jump” between DNA molecules (e.g., from one plasmid to another or from a plasmid to the bacterial chromosome and vice versa), plasmids can continue to gain new drug-resistance genes as they mix and spread through different strains of cells. It is a classic example of evolution through natural selection. Those cells harboring R plasmids with multiple drug resistances survive to reproduce in the new environment of antibiotic use.

5. P elements are transposable elements found in Drosophila. Under certain conditions they are highly mobile and can be used to generate new mutations by random insertion and gene knockout. As such, they are a valuable tool to tag and then clone any number of genes. P elements can also be manipulated and used to insert almost any DNA (or gene) into the Drosophila genome. P element-mediated gene transfer requires inserting the DNA of interest between the inverted repeats necessary for P element transposition. This recombinant DNA, along with helper intact P element DNA (to supply the transposase), are then co-injected into very early embryos. The progeny of these embryos are then screened for those that contain the randomly inserted DNA of interest.