Assimilating Surplus DNA

GENETIC recombination is a central process in heredity, for its construction of DNA which is a composite of that from the two parents gives rise to the fundamental rearrangements of genetic material on which evolution depends. But working out the details of recombination has proved to be an exceedingly difficult task. A principal obstacle has been the lack of a system amenable to both genetic and biochemical analysis. Bacteriophages now seem to provide an answer, however, and on page 13 of this issue of *Nature New Biology*, Cassuto and Radding report the development of a model system from phage lambda. Essentially, what they have done is to synthesize a new form of lambda DNA, which they suspect may resemble an intermediate stage in recombination, and use this as a substrate for a nuclease enzyme coded by the phage.

Recombination requires the DNA duplexes of the two parents to break and to rejoin crosswise to produce chromosomes which thus have some genetic material derived from each parent. To avoid losing pieces of DNA, the crossover must take place at exactly corresponding sites in each parental chromosome; this is probably achieved by making use of the specificity of Watson–Crick hydrogen bonding between complementary single strands of DNA. That is, the individual strands of the parental DNA duplexes must break, unwind from their complements, and unite with the appropriate strand from the other parent. Such an event would produce two duplexes of DNA, each consisting of a length of duplex DNA from one parent linked to a duplex from the other parent by a length of "hybrid DNA", a curious duplex structure which has one strand derived from each parent.

There is more than one system for recombination in phage lambda, but mutations which affect the general ability of the phage to undergo recombination have been located in two (*red*) genes, which code for an exonuclease and the so-called β-protein. Cassuto and Radding have investigated the behaviour *in vitro* of the complex of both proteins, but the exonuclease can probably work by itself, so the need for β-protein is not clear. At any rate, the enzyme(s) seem to be able to tidy up the immediate product of recombination and may also help to create the hybrid DNA.

An intermediate stage of recombination in bacteriophages is formation of a "joint molecule"; this comprises two lengths of parental DNA, joined by hybrid DNA, but with gaps at the sites of joining so that the two pieces of parental DNA are not yet covalently linked. Cassuto and Radding now take some delight in introducing a new term; they suggest that a "redundant joint molecule" may be an earlier product of recombination. They have prepared redundant joint molecules synthetically by hybridizing one of the two strands of lambda DNA to a short complementary sequence at one end; the entire complementary strand is then added, and hybridizes to the remaining single stranded region. This produces a duplex DNA with a gap at the redundant joint where the complementary strands switch over. Circularizing the DNA then forms the substrate used for the enzyme.

Lambda exonuclease converted this to a simple circular structure, as shown in Cassuto and Radding's Fig. 2*c* (page 14). Because the different strands of the circular substrate had been labelled with different radioactive isotopes, Cassuto and Radding were able to see which part of the molecule was degraded by following the release of soluble radioactive nucleotides. This showed that what the enzyme does is to start at the redundant joint and degrade one strand of the DNA duplex, thereby allowing the excluded part of the complementary strand to join in the duplex. The product is the joint molecule intermediate. The substrate remained in the circular form, which implies that the exonuclease must have stopped at the nick—otherwise it would have continued to the next gap (only twelve nucleotides further) and this would have released the lambda DNA into a linear form.

Starting from this action of the exonuclease, Cassuto and Radding propose that the enzyme may itself create hybrid DNA by forming redundant joint molecules. Once two parental DNAs are ready to be recombined (that is, they must previously have been broken at nearby sites), the exonuclease could attach to the two free 5′ ends and degrade far enough from each to free complementary single strands of the two parents (see step *a* in Fig. 1 on page 14). When these unite by hydrogen bonding, two redundant joints are produced at the sites where the enzyme molecules are located (step *b*). Further degradation then allows the free overlapping single strands to be assimilated into the duplex (step *c*) to yield a joint molecule. Recombination is completed if a ligase enzyme covalently seals the gaps.

But does this mechanism work *in vivo*? Caution is needed when extrapolating from enzyme activities *in vitro* to functions *in vivo*, as the present controversy about the involvement of DNA polymerase in replication in *Escherichia coli* well shows. The beauty of Cassuto and Radding's model is that, unlike many previous models for achieving recombination by formation of hybrid DNA, it is based on the action of an enzyme which lambda mutants clearly implicate in recombination. It is appealing to suppose that a single enzyme creates hybrid DNA and yields a joint molecule, a structure which has been found *in vivo*. Although it is not certain what the β-protein does, and future research will doubtless complicate what is at present an elegant and simple model, this is at least a good starting point for working out the mechanisms of recombination.

Other problems remain to be solved, of course; the parental DNAs must be aligned with each other and broken in fairly close regions—the way this might happen is intriguing, but too little is known about how proteins could recognize nucleotide sequences to make speculation fruitful. Perhaps the only real drawback to the lambda system is that many systems for recombination seem to exist in bacteria and bacteriophages, and this particular mechanism may not necessarily apply in other instances. But such criticism can be made of virtually every available system, and phage lambda must rate as a promising candidate for the first to be worked out in detail.