Which Way for Replication?

ONE of the few facts to have been established about the mechanism of DNA replication is that the process is semiconservative. Each daughter molecule contains one of the strands of the parental duplex and, because evidence suggesting that replicating DNA molecules include extensive regions of single-stranded DNA has never been obtained, it seems clear that the possibility that DNA replication proceeds in two distinct stages, the complete separation of the parental strands followed by the synthesis of complementary daughter strands, can be ignored. The new daughter strands must be made as the parental strands separate. It is, of course, easy enough to envisage the unwinding of the two strands of a linear DNA molecule, even if we do not know how a cell manages to achieve this at the rates demanded by the rate of DNA replication, without tying its DNA in knots, but the chromosomes of many bacteria, bacteriophages and at least some animal viruses are not linear molecules but are covalently closed, circular duplexes. If the two strands of such molecules are to unwind, at least one phosphodiester bond in at least one of the two DNA chains must be broken to allow freedom of rotation.

But, having introduced at least one nick into a double helical, circular DNA, how does the replication proceed? For the past several years this question has been the subject of a continuing and often heated debate which has crystallized around two conflicting models, that proposed by Cairns and the rolling circle model notably championed by Gilbert and Dressler. The Cairns model envisages that replication starts at a fixed origin from which it proceeds in one or both directions as the parental strands are rotated and separated by some swivel mechanism, and a θ-shaped replicating form is generated. This model predicts that none of the DNA strands in this complex is longer than the parental strands and the two daughter strands are the same length. The rolling circle model, by contrast, envisages that one parental strand is nicked to expose a 3′ hydroxyl and 5′ phosphate group ; nucleotides are then polymerized at the 3′ end, giving rise to a DNA chain longer than either parental chain and simultaneously displacing the 5′ end of the nicked parental strand which can thus act as a template for synthesis of a complementary daughter strand. As replication proceeds, the complex assumes a p-shaped structure.

To date, the most compelling evidence for the rolling circle model has come almost exclusively from studies of replicating ϕX 174 DNA, while investigations of replicating *Escherichia coli* chromosomes and the chromosomes of certain bacteriophages have in general provided ammunition for the Cairns camp which has now apparently been reinforced by the DNA tumour virologists. For, as Jaenisch, Mayer and Levine report on page 72 of this issue of *Nature New Biology*, and as Saltzman and his colleagues have by all accounts related at meetings this summer in the US, the form assumed by the covalently closed, circular and supercoiled genomes of the small DNA tumour viruses as they replicate is precisely that predicted by the Cairns model. Under the electron microscope, for example, as Jaenisch *et al.* show, the SV40 genome at all stages of replication appears as a θ-shaped structure ; ^{3}H-thymidine incorporated during short pulses is not covalently linked to parental DNA strands and the DNA molecules containing label are shorter than a single strand of SV40 DNA. Furthermore, the changes in sedimentation properties of SV40 genomes as they replicate can all be accounted for by assuming that the supercoiled DNA is first nicked and then replicates as Cairns suggested. The unexpectedly fast rate of sedimentation of only partially replicated SV40 genomes proves simply to be a consequence of supercoiling generated in the unreplicated portion of the parental molecule.

And it comes as no surprise to read the claim Jaenisch *et al.* make that under the electron microscope discontinuities are sometimes seen at both, rather than at one of the forks of the θ-shaped complex. Two such discontinuities have been observed in replicating molecules of phage λ DNA, for example, and they are believed to be regions of single stranded DNA at active replication sites. It appears likely, therefore, that the replication of the DNA of papova viruses, like that of several phages and of *E. coli* itself, as Masters and Broda have recently shown by genetic experiments (*Nature New Biology*, **232**, 129 ; 1971), sometimes occurs in both directions round the circle.

Where Repressor Goes

IT has seemed almost certain for quite some time that the repressor protein of the lactose operon works merely by the act of binding to the operator site which is located between the promoter (where RNA polymerase binds) and the first structural gene. The enzyme could then only proceed past this block when a β-galactoside is added to release the repressor protein from DNA.

But it now seems that there may be more than one way to repress an operon. Pastan and his colleagues at the US National Institutes of Health have devoted much effort recently to developing a system in which the DNA of the lactose operon can be transcribed into RNA and then translated, *in vitro*, all under proper control of repressor protein, β-galactoside co-inducer, cyclic AMP and cyclic AMP binding protein. One use to which they put this system was to study the action of the cyclic AMP system, which it seems may act at a new regulator site on the DNA of the operon (see *Nature New Biology*, **231**, 129 ; 1971).