BACTERIOPHAGES

Peaceful Coexistence

from our Cell Biology Correspondent

COLIPHAGES which peacefully coexist with, rather than kill, *Escherichia coli* cells seem to be comparatively rare—although, of course, this may well reflect nothing more significant than the selective sampling of molecular biologists—and they are comparatively poorly understood. Such phages, and fd is the most familiar, are, however, attracting increasing attention. For just as the lytic phages have proved to be ideal tools for probing many of the basic processes of gene expression—the discovery of messenger RNA and much that has followed depended on their availability—so such coexisting phages as fd may prove equally useful for probing second order biological processes. Particles of fd, for example, are apparently assembled as they pass across the bacterial cell envelope rather than in the infected cell's cytoplasm; the secretion of fd particles may well therefore be a useful model system for studying the movement of large molecules across membranes. Cells infected with fd also provide a system for investigating the continued replication of two replicons, the bacterial and viral chromosomes, in one cell.

Marvin and his colleagues (Hohn, Lechner and Marvin, *J. Mol. Biol.*, **56**, 143; 1971) have been looking at the latter problem and have come up with a model for the replication of fd, which, although similar to that proposed by Sinsheimer to account for the replication of ϕ X 174, has some unique features. The pattern of incorporation of 1 minute pulses of thymidine into fd and bacterial DNA during the first hour after infection is consistent with the model of the replication of ϕ X 174 DNA which fd DNA closely resembles. The single stranded and circular fd DNA is first converted to a closed, circular and double stranded replicative form (RF1). This, attached to some membrane site, replicates to produce progeny RF1 molecules. The model for ϕ X 174 replication envisaged that only the RF1 molecules, retaining a parental strand of DNA from the infecting particle, remain attached to membrane and able to replicate. Hohn *et al.* suggest, however, that in the case of fd replication all the RF1 molecules are able to replicate semiconservatively because they observe an exponential increase in the rate of DNA synthesis at this stage in the replication cycle. Eventually the RF1 molecules, instead of replicating semiconservatively, begin to act as templates for the asymmetric synthesis of single strands of progeny viral DNA which can be incorporated into progeny virus. Hohn *et al.* propose that this switch from double to single stranded DNA synthesis is brought about by the

product of fd gene 5, which is known to be essential for the synthesis of progeny single strands but not RF1 molecules. They suggest that this gene product somehow specifically inhibits the replication of one of the two strands of fd DNA, thereby generating single stranded molecules and preventing these from being converted into double stranded RF1 molecules.

Hohn, von Schutz and Marvin (*ibid.*, 155) have also examined what happens in cells infected and killed by fd because the secretion of progeny particles is inhibited. In such a situation, which they call abortive infection, the input fd genomes are converted to RF1 molecules but by the end of this latent period bacterial DNA synthesis, RNA synthesis, protein synthesis and ATP generation are inhibited although the synthesis of bac-

terial membrane seems to continue. The product of the fd gene 2 plays a part in this killing because fd mutants with an amber mutation in this gene do not kill *E. coli* which are prevented by one means or another from secreting progeny phage. The gene 2 product is also required for the replication of double stranded viral DNA and it is probably bound to the bacterial membrane. Hohn *et al.* suggest from all this that in abortive infection the gene 2 product interacts with the bacterial membrane in such a way as to shut off respiration, believed to be a membrane associated function, and kill the cell. Killing by fd, as well as the secretion of fd in normal infections, may therefore provide new experimental approaches to the structure and function of the bacterial envelope.

Methionine initiates Adenovirus Proteins

THIS time last year few biochemists would have readily agreed that methionine is the first amino-acid laid down at the start of synthesis of proteins in the cells of higher organisms as well as in bacteria. But in the past year it has been conclusively shown that haemoglobin, protamines in the cells of trout testis and polypeptides synthesized in an ascites cell-free system programmed with EMC virus or synthetic messenger RNAs are all initiated with a methionine residue. Methionine seems indeed to be the universal initiating amino-acid and the report by Caffier, Raskas, Parsons and Green, in next Wednesday's *Nature New Biology*, that eight or nine adenovirus proteins are initiated with this amino-acid should prove that this generalization is not over-optimistic.

Caffier *et al.* prepared cytoplasmic extracts of KB cells (a line of human carcinoma cells) infected with adenovirus type 2. These extracts support the synthesis of eight structural proteins of adenovirus particles and possibly a ninth adenovirus protein which is not part of the virion. The first amino-acid of the eight species of structural proteins, as isolated from virus particles, is either alanine or glycine but in no case is the first, N-terminal, amino-acid methionine. To test whether alanine or glycine rather than methionine are the amino-acids which actually initiate the synthesis of these proteins in their cell free system, Caffier *et al.* assayed the incorporation of methionine donated by an initiating methionine transfer RNA isolated from yeast and added to the cell free system. At least 60 per cent of the methionine incorporated into nascent adenovirus proteins, and 35 per cent of the methionine found in completed and released proteins was at the N-terminal position. Clearly in this artificial situation methionine initiator

transfer RNA from yeast can initiate the synthesis of adenovirus proteins. But is methionine the initiator in intact KB cells not supplemented with yeast transfer RNA?

To answer that question Caffier and his colleagues compared the uptake of methionine by adenovirus 2-infected KB cells exposed to a 5 minute pulse of H³ methionine and by their cell free system exposed to a 20 minute pulse of H³ methionine. In both cases, 25 per cent of all the methionine found in nascent adenovirus proteins was at the N-terminal initiating position whereas in completed and released proteins 5 per cent of the methionine was found to be N-terminal. These results strongly suggest that in KB cells adenovirus proteins are initiated with methionine. On average there are fourteen methionine residues in these eight adenovirus proteins. If the synthesis of each began with methionine and if the methionine was not cleaved from the chain until after it was completed and released, about 7 per cent of all incorporated methionine residues would be N-terminal. This is close to the observed 5 per cent, which suggests not only that all these adenovirus proteins are probably initiated with methionine but also that many of the initiating methionine residues are not cleaved until after the protein has been completely synthesized.

One other interesting detail emerges from this work; yeast initiator transfer RNA carrying a methionine residue which is formylated (with a formyl group on the amino-group of methionine) does not initiate adenovirus proteins. This result, in keeping with those of Smith and Marcker and others, indicates that it is a methionine residue, rather than an amino-acid with a blocked amino-group, which is universal to chain initiation.