ADENOVIRUSES

Endonuclease Activity

from our Cell Biology Correspondent

REPORTS of the discovery of this or that enzyme in particles of one or another of the enveloped viruses are these days mundane events, unless, of course, the enzyme happens to be as unusual as reverse transcriptase. By contrast the discovery of enzyme activities associated with the virions of encapsidated viruses is still a comparatively rare event. One such is Burlingham, Doerfler, Pettersson and Philipson's report (*J. Mol. Biol.*, **60**, 45 ; 1971) that the endonuclease activity which appears in cells infected with adenovirus 2 or 12 and is also associated with purified virions seems to reside in the penton base subunits of the capsids of these viruses.

This new endonuclease activity first came to light when Burlingham and Doerfler (*J. Virol.*, **7**, 707 ; 1971) were studying the replication of adenovirus 2 and 12 DNA in KB cells and a variety of other permissive and nonpermissive hosts. Burlingham and Doerfler were able to identify three classes of viral DNA in infected cells; molecules which cosediment with DNA extracted from virions and appear to be uncoated parental viral genomes; molecules of adenovirus DNA which are considerably smaller than intact genomes; and finally DNA molecules which are much larger than genomes and may well be parts of the host genome carrying integrated adenovirus DNA. Kinetic studies of the appearance of these three classes of DNA during the course of adenovirus replication lead Burlingham and Doerfler to the conclusion that many adenovirus genomes are cleaved by an endonuclease which appears in the cells after infection and that the cleaved molecules may well be those which are integrated into the host genome. Furthermore, although the experimental details have yet to be published, Burlingham and Doerfler have apparently obtained evidence that the endonuclease is not only a virus-coded protein but also remains associated with pure virions.

Collaborating with Pettersson and Philipson, who with their colleagues at Uppsala have extensively analysed the polypeptides in the three structural units of adenovirus capsids (the hexons, penton bases and penton fibres), Burlingham and Doerfler have tested the capsid subunits for endonuclease activity. Only the penton bases, purified from infected KB cells rather than from pure virions for technical reasons, have this activity, and both selective digestion of the penton bases with trypsin and antiserum to penton base inactivates it. The endonuclease activity is specific for DNA; its activity is twenty-fold greater with native DNA than with denatured DNA as substrate and it cleaves both strands of double-stranded DNA by some "double-hit" mechanism. Moreover, the homogeneity of the cleavage products produced by the endonuclease with adenovirus DNA as substrate suggests it attacks specific sites which are probably rich in G.C base pairs because poly dG.poly dC is a strong competitive inhibitor whereas poly d(AT) has little effect, and actinomycin D partially inhibits the activity. Assuming that penton bases in capsids have the same activity as those extractable from infected cells, Doerfler and Burlingham have provided an interesting example of a viral protein which as well as forming part of a capsid also plays a part in the replication of the viral genome.

Also in the current issue of the *Journal of Molecular Biology* (**60**, 185 ; 1971) Landgraf-Leurs and Green report their various attempts to exploit the differential binding of synthetic RNAs by the DNA of adenoviruses 2, 7 and 12 for the separation of the complementary strands of these genomes. Apparently poly(U.G) molecules with judiciously selected ratios of U and G bind to different extents to the two strands of these three genomes and separation of complementary strands is possible. To prove their point Landgraf-Leurs and Green report the separation on a preparative scale of the strands of adenovirus 2 DNA and self annealing and hybridization experiments show there is no detectable cross contamination.

PHOTOMORPHOGENESIS

Ach Implicated Again

Gressel, Strausbauch and Galun from the Weizmann Institute provide on page 648 of this issue of *Nature* what may be the first evidence that acetylcholine has a part to play not only in plant photoresponses initiated by red light, but also where blue light provides the stimulus. They have shown that sporulation in the fungus *Trichoderma viride*, which is induced normally

Drug Resistance and Ribosomal RNA

ANTIBIOTICS such as streptomycin, spectinomycin, erythromycin and fusidic acid all impair to greater or lesser extents the faithful translation of messenger RNAs in *Escherichia coli*. Resistant mutants can, however, be isolated and, as the pioneering work of Nomura and his associates first established, the loci controlling such resistance specify structural proteins of ribosomes or, in the case of fusidic acid resistance, as other groups have shown, the locus specifying the translocation factor G. But it would be wrong to run away with the idea that mutations leading to resistance to antibiotics which affect translation are confined to genes that specify proteins which are part of the translation machinery. For Helser, Davies and Dahlberg report in next Wednesday's *Nature New Biology* that a mutation which results in resistance to the drug kasugamycin causes a failure in the methylation of 16S ribosomal RNA.

Sparling has shown that kasugamycin interacts with the 30S ribosomal subunit of wild type *E. coli* ribosomes. With the expertise required for the reconstruction of ribosomes from their constituent RNAs and proteins, which has been chiefly developed by Nomura, Helser and his colleagues, at hand at the University of Wisconsin, set about reconstructing 30S ribosomes with proteins and RNAs from either wild type of kasugamycin resistant cells. They quickly discovered that resistance to this drug is not the consequence of a mutated 30S ribosomal protein. They therefore compared fingerprints of the 16S ribosomal RNAs of resistant and sensitive strains, and sure enough found a difference. An oligonucleotide sequence dimethyl-AACCUG present in digests of wild type 16S RNA is replaced by the unmethylated oligonucleotide AACCUG in digests of the 16S RNA from resistant strains. Using RNAs labelled in their methyl groups they confirmed this difference, and further reconstruction experiments proved that resistance or sensitivity to kasugamycin depends on the source of the 16S ribosomal RNA and not on the source of ribosomal proteins.

Apparently therefore the mutation to kasugamycin resistance results in the failure to dimethylate an adenine residue near the 3′ terminus of the 16S ribosomal RNA. Helser *et al.* favour the idea that the mutated locus specifies a methylase responsible for adding the two methyl groups to this adenine residue, although alternative explanations of their findings have not been rigorously excluded. As they note, if the kasugamycin resistance locus does specify a methylase probably more than one species of methylase is involved in methylating ribosomal RNA. For even in the kasugamycin resistant strains the 23S RNA of the large ribosomal subunit is methylated normally.

In short, Helser *et al.* have proved that the function of ribosomes can be altered by mutations which affect ribosomal RNA and they have provided circumstantial evidence suggesting that more than one methylase may be involved in the specific methylation of ribosomal RNA.