and is stable in the presence of streptomycin. Breakdown only occurs when the initiation complex is completed on the fMet tRNA entering the P site. Modolell and Davis also believe that the ribosomes released as a result of the breakdown of the abortive initiation complex may not be able to form further initiation complexes, although the reason for this is still unclear.

ANTIBODIES

Variations on a Theme

from our Molecular Biology Correspondent

EVEN such readers as have to make a conscious effort to recollect which is the antibody and which the antigen should find some interest in a number of elegant new uses to which antibodies can be put. Melchers and Messer (*Europ. J. Biochem.*, **17**, 267; 1970), for example, have developed a method, first contrived by Rotman and Celada, for the detection and isolation of defective enzymes produced by mutant cells. The β-galactosidase of *Escherichia coli* remains active when associated with its specific antibody, and can be caught on a column of 'Sepharose' to which the antibody has been covalently attached by methods that are now standard. Melchers and Messer have screened a series of *E. coli* mutants containing defective β-galactosidase, which in the purified state is essentially inactive. It is activated, however, by the antibodies to a level approaching the activity of the wild type enzyme. This is a remarkable though not unique phenomenon, the explanation of which is by no means obvious. Inasmuch as the antibodies recognize, bind to, and therefore stabilize the native conformation of the enzyme, one may envisage that a molecule containing an unfavourable amino-acid substitution that destabilizes the native conformation may still be trapped in that state in the antibody complex.

At all events, the mutant enzymes all cross-react with the antiserum to the wild type, and can be recognized in their antibody complexes by the appearance of the latent enzymic activity. After adsorption on the columns, the antigen–antibody complexes can be dissociated, and the enzyme recovered, by acid, urea or increased temperature. The molecular weights of the mutant β-galactosidases isolated in this way were in all cases the same as that of the wild type, and the defects therefore neither bring about nor are caused by failures in quaternary association. This method of extracting mutant proteins could clearly be of great value, especially perhaps when they are inactive, and cannot be easily, identified. It is necessary only that the antigenic determinants on the native protein should be present in the mutant, and that the wild type antigen be pure in the first place. It is also worth observing that when an antigen has an assayable enzymic activity, which is preserved in its complexes with antibody, the use of enzymic assay offers an amplification mechanism, whereby otherwise undetectably small amounts of the antigen can be determined.

A pretty variation on the same theme has been developed in Sela's laboratory, and involves chemical coupling of antigens to the surface of a bacteriophage. On addition of antibodies the phage is inactivated, and because of the sensitivity of essays of phage infectivity, miniscule amounts of antigen and of antibody can be detected by this means. Hurwitz, Dietrich and Sela (*ibid.*, 273) have now applied this strategy to the detection of an angiotensin. This is a peptide hormone, only fourteen residues long, and, like other such small molecules, exceedingly difficult to detect by ordinary means, for its complexes with antibodies do not precipitate. Antibodies can be produced by coupling the angiotensin as a hapten to a carrier protein. Coupling of the peptide to T4 bacteriophage by way of a bifunctional reagent, glutaraldehyde, presents no difficulties. The specific antibodies can be stripped out of the antiserum if required by passage through a column of angiotensin bound to 'Sepharose'. With unfractionated antiserum, however, very small antibody titres can be detected by the phage assay. Moreover, the phage inactivation is quantitatively inhibited by free angiotensin, and the most important upshot of this work therefore is that it allows the detection of as little as 10 pg or less of free angiotensin.

Another recent example of the use of specific antibodies is in the removal of a contaminant enzyme from the plant protease, phaseolain (Carey and Wells, *Biochem. Biophys. Res. Commun.*, **41**, 574; 1970). The contaminant, which is an endopeptidase, can be prepared in pure form more readily than it can be quantitatively removed from the phaseolain. It can therefore be used to stimulate antibodies, and phaseolain preparations extracted with the antiserum. The phaseolain could then be characterized as a true carboxypeptidase with no endopeptidase activity of its own.

Processing of tRNA Precursors

IT is well established that mature ribosomal RNA in bacteria arises by the processing of larger precursor molecules. Altman has now isolated tRNA precursors and he describes his method in next week's *Nature New Biology* (**229**, 19; 1971).

He took advantage of the invaluable system developed by J. D. Smith and his colleagues whereby the gene for tyrosine tRNA (either wild type or amber suppressing), or various mutants of this gene, can be transduced into *Escherichia coli* with the aid of the bacteriophage φ80. Large amounts of this tRNA are then synthesized in the post-infection period, in the wild type and suppressor tRNAs. Altman examined two mutants in which the tyrosine tRNA was synthesized at a much lower rate after infection. He found that when short pulses of ^{32}P (3.5 min) were administered to the infected cells, and the newly synthesized, labelled RNA was rapidly extracted and fractionated by polyacrylamide gel electrophoresis, a new prominent band appeared which was not present in extracts of cells infected with phage carrying the wild type or suppressor genes. From its mobility during gel electrophoresis, this material contains about 200 nucleotides. Fingerprints of this band revealed that all the oligonucleotides of the tyrosine tRNA were present within it, with the exception of the products from the 3′ and 5′ termini. There were also a number of additional products, among them an oligonucleotide which may be the 3′ terminal fragment without the $-CCA_{OH}$ terminus.

Altman suggests that this material is a precursor of the tyrosine tRNA, and he proposes that it is accumulated to a greater extent during short labelling times in the cases of the mutants (with consequently diminished amounts of mature tyrosine tRNA), because the processing of the precursor is less efficient than with the wild type or suppressor strains. This is confirmed by the finding that an additional band can also be isolated when cells infected with phage carrying the wild type or suppressor genes are pulse labelled at 25° C. This band (about 140 nucleotides or so in length) also contains the set of the tyrosine tRNA sequences. Presumably the processing of the larger precursor molecules is carried out more slowly at this temperature. Measurements of the half-lives of these precursors indicate a value of about 3 min for the mutant strains at 37° C and for the wild type or suppressor strains at 25° C.

The two mutants I have described were complex. But a further mutant with only a single defined nucleotide change also accumulated a precursor, slightly smaller than that accumulated with the wild type. This surprising result implies that the "tailoring" enzymes are sensitive to small changes even when well removed from the termini of the tRNA. All the precursors exhibited certain spots in common, in addition to the products from the tRNA itself. These other products totalled about forty nucleotides. This suggests that at least the later part of the biosynthetic process is likely to be common to all of them.