MOLECULAR BIOLOGY

Nerve Fertilizer

from our Molecular Biology Correspondent

A PROTEIN factor which stimulates the growth of nerves and which was originally described by Levi-Montalcini and co-workers has been the subject of a series of interesting studies by Shooter and his associates. Two sources of the growth factor are at present available—snake venoms and mouse salivary glands—and it is the latter which have provided the material for the present work. The active component is found to have a sedimentation coefficient of 7*S*, which for a typical globular protein would correspond to a molecular weight of about 140,000. Outside the *p*H range 5–8, the protein dissociates reversibly into subunits with molecular weights of the order of 30,000, and electrophoretic or chromatographic analysis reveals the presence of three types of chain, differing widely in charge and termed α, β and γ. Dissociation is accompanied by loss of the greater part of the growth-stimulating activity (as well as diminished stability), but what remains is associated uniquely with the β subunits.

Greene, Shooter and Varon (*Proc. US Nat. Acad. Sci.*, **60**, 1383; 1968) have now discovered a quite different type of activity, residing in one of the other subunits, the γ chains. They observed that the intact 7*S* protein has a small but unmistakable proteolytic activity towards casein, and a large hydrolytic activity towards benzoylarginine ethyl ester, which is well known as a synthetic substrate for the assay of trypsin. The specific activity indeed is higher than that of trypsin, and when isolated γ subunits were assayed the specific activity was some seven times greater than that of trypsin. On recombination of all three subunits the original levels of both growth-stimulating and hydrolytic activity are regained. That there is some interaction between the subunits is clear: the nerve growth activity is much higher in the 7*S* protein than in the isolated β subunits, and the specific hydrolytic activity and its kinetic characteristics also change on dissociation. In the 7*S* protein there is a marked lag phase in the kinetics, which is absent in γ subunits alone. The co-existence of subunits with different activities in a single enzyme species has been observed before, notably in tryptophan synthetase, but it still represents a very unusual situation. The interrelation between the two activities, and the purpose of the α chains—whether enzymatic or regulatory for example—are at this stage still matters of conjecture.

A further feature of the growth-stimulating protein is the heterogeneity of the α and γ subunits, which is considered in detail in a further paper (Smith, Varon and Shooter, *Biochemistry*, **7**, 3259; 1968). In polyacrylamide gel electrophoresis the α chains show four components, one of them minor, and the γ chains three. The latter are all indistinguishable in esterase activity, and all of them, and the α components also, will recombine with β chains to form complexes of α with β, γ with β, or fully active $\alpha\beta\gamma$ molecules ($\alpha\gamma$ species and self-complexes are not formed). Because there can only be one chain of each kind in the 7*S* protein, the heterogeneity of the subunits should be reflected in a corresponding heterogeneity in the parent molecule, and this is indeed the case. The recovery of only one species of either chain on dissociating a 7*S* protein formed by combining single species shows that this is a true structural heterogeneity. Furthermore, protein prepared from one gland of a single animal exhibits the full range of heterogeneity. Evidently therefore all the molecular forms are present, probably in equilibrium, in each gland. Again, the relationship of these multiple combinations of different subunits in different aggregation states to the function remains obscure.

MOLECULAR BIOLOGY

Unused Codons

from our Cell Biology Correspondent

ALL sixty-four possible codons have now been assigned functions in the genetic code, largely as a result of Nirenberg's discovery of cell-free systems and trinucleotide binding techniques. Most codon amino-acid translations have been shown to be universal and the degeneracy of the code has become apparent. The degeneracy raises the question: to what extent are the *t*RNA-codon responses identical in different organisms and, if they differ, do factors affecting anti-codon–codon recognition serve as a mechanism for regulating protein synthesis? For example, are some codons not used and are some species of *t*RNA specific for any particular codon present in small amounts and therefore a rate limiting factor during translation?

In the latest issue of *J. Mol. Biol.* (**37**, 99; 1968) Caskey, Beaudet and Nirenberg report another step in their analysis of the RNA codons and protein synthesis which answers some of these questions. They isolated from *E. coli* and guinea-pig liver the *t*RNA species for seven amino-acids (arginine, isoleucine, serine, cysteine, lysine, methionine and threonine) and in trinucleotide binding experiments analysed the anticodon–codon relationships of all these *t*RNAs. Only seven species of *t*RNA in both guinea-pig liver and *E. coli* have the same anticodon–codon responses; for example, in both organisms there is a *t*RNA which translates AGU and AGC as serine, but the other serine codons fall into different sets. Thus in *E. coli* there is a serine *t*RNA which translates both UCU and UCC but not UCA, whereas in guinea-pig liver the *t*RNA which translates UCU and UCC also translates UCA. From results of this type Nirenberg's group concludes that, for the amino-acids they studied, there are only seven *t*RNAs, which have the same codon recognition patterns, common to both *E. coli* and guinea-pigs, while seven species of *t*RNA found in guinea-pig are not present in *E. coli* and conversely five *t*RNAs in *E. coli* are not present in guinea-pig.

The experiments also suggest that some codons are not used in one or other of these organisms. For example, the sole isoleucine *t*RNA from liver responds to the codon AUA whereas the sole isoleucine *t*RNA from *E. coli* gave no detectable response to this codon; a species of guinea-pig arginine *t*RNA responds well to AGG but poorly to AGA, whereas the two *E. coli* arginine *t*RNAs respond poorly to both these codons. Such results as these imply that during evolution, for some totally obscure reason, certain codons have been deleted from the genetic dictionary of some