SUPPRESSION

Drosophila Mutations

from our Cell Biology Correspondent

BIOLOGISTS who enjoy vicarious pleasures should not miss reading the current issue of the *Journal of Molecular Biology* (**57**, 231; 1971), for in it Twardzik, Grell and Jacobson report that a suppressor mutation, which suppresses the vermilion eye colour mutation of *Drosophila melanogaster*, alters a species of the fly's tyrosine tRNAs.

Mention of genetic suppression to anybody reared on the biology of *Escherichia coli* conjours up, of course, pictures of mutated transfer RNA molecules which, either because they have a new anticodon, or as a result of some other structural modification, have gained the ability to read nonsense chain terminating codons or to rectify missense mutations. Fully aware that suppression in microorganisms is usually mediated by a structurally changed tRNA, Twardzik and his colleagues simply asked if the same might not be true of suppression in higher organisms, about the mechanism of which nothing is known; their hunch has paid off handsomely, albeit in an unexpected way.

Geneticists long since isolated a recessive sex-linked suppressor mutation, *su(s)*^{2}, which is non-allelic to the sex-linked vermilion eye colour mutation of *Drosophila*, which it suppresses. *Drosophila* carrying the vermilion mutation lack a brown eye pigment because they are unable to convert tryptophan to kynurenine, an intermediate step, catalysed by tryptophan pyrrolase, in the synthesis of the brown pigment. In these mutant flies tryptophan pyrrolase activity is never more than 25 per cent that in wild type flies but when the *su(s)*^{2} suppressor mutation is introduced the activity of this enzyme is partially restored, the large accumulation of non-protein tryptophan, which characterizes the vermilion mutants, is reduced, and eye colour is restored.

Taking the bull by the horns, Twardzik *et al.* screened extracts of flies, searching by reverse phase chromatography for a modified species of tRNA in the *su(s)*^{2} homozygotes; and sure enough they found that the *su(s)*^{2} locus controls directly the amount of a species of tyrosine tRNA in adult flies. Wild type *Drosophila* have three resolvable tyrosine tRNAs, two major species and a minor, and *su(s)*^{2} flies lack the second major species and have a larger than normal amount of tRNA chromatographing at the position of the first major species. Flies bearing a new suppressor mutation *su(s)*^{e1} isolated by Twardzik *et al.*, which maps at the same locus as *su(s)*^{2}, also lack the second major tyrosine tRNA. A series of genetic crosses established that although the vermilion and suppressor loci are both on the *X* chromosome, only the suppressor locus controls the loss of the tyrosine tRNA. The recessive nature of the suppressor mutations and the fact that tRNA structural genes are redundant in eukaryotes indicate that the suppressor locus is not the structural gene for the tRNA in question but the suppressor locus presumably specifies an enzyme which somehow modifies this tRNA during its maturation.

But how does the loss of the second tyrosine tRNA, $tRNA^{Tyr}_2$, suppress the vermilion mutation? At the time Twardzik and his colleagues wrote their report they were clearly thinking along conventional lines, speculating about possible ambiguous codon responses of the unmodified $tRNA^{Tyr}_2$, and suppression at the level of translation. But further experiments reported recently by Jacobson in *Nature New Biology* (**231**, 17; 1971) have changed all that. Jacobson has found that by treating homogenates of vermilion mutant flies with ribonuclease T1 he was able to restore their tryptophan pyrrolase activity. The obvious implication was that an RNA inhibits tryptophan pyrrolase in vermilion mutants. Pursuing this lead he has shown that the addition of uncharged $tRNA^{Tyr}_2$ to the tryptophan pyrrolase of vermilion mutants, activated by ribonuclease digestion, once more inhibits the enzyme's activity. This species of tRNA is therefore an inhibitor of the vermilion mutant enzyme but it does not inhibit wild type enzyme.

From all this Jacobson argues that the wild type enzyme is associated in some way with $tRNA^{Tyr}_2$ and is active whereas the tryptophan pyrrolase specified by the vermilion locus, presumably because of its mutated structure, is inhibited when it associates with this tRNA. Removal of the tRNA either by nuclease digestion or by introducing the suppressor *su(s)*^{2} mutation, which prevents the maturation of $tRNA^{Tyr}_2$, leads to the reactivation of the mutant enzyme. Clearly this is the first of a fascinating collection of stories. Why so many isoaccepting species of tRNAs exist has long been a puzzle but there is now a clue as to their function. They may well be more important for the control of enzyme activities—in other words the control of metabolism and differentiation—than the control of translation.

Intricacies of Starting a Protein

IN *Nature New Biology* next Wednesday, Rudland, Whybrow and Clark suggest that a protein called initiation factor F2 plays a crucial part in ensuring that the transfer RNA carrying the amino-acid which initiates the synthesis of all *Escherichia coli* proteins goes to the correct site in a ribosome.

During protein synthesis each transfer RNA molecule picks up a particular amino-acid, carries it to the ribosome and, if the codon for that amino-acid is waiting to be read, delivers it to the site at which amino-acids are added to a growing polypeptide chain. Clearly, ensuring that the charged tRNA is delivered to precisely the right site in the ribosome is of crucial importance and it is known that a protein called T factor promotes this reaction.

T factor will bind with a molecule of charged tRNA and a molecule of GTP to form a complex; this complex then delivers the tRNA complete with its specific amino-acid to what is called the A-site of a ribosome. The A-site is the reception centre for charged tRNA molecules which can donate the amino-acid they carry to the growing protein held in the so-called P-site.

But T factor only promotes the delivery of charged tRNAs to a ribosome which has already started to synthesize a protein. According to Rudland and his colleagues a different factor, the F2 initiation factor, is involved in the delivery of the very first charged tRNA which initiates protein synthesis. They have shown that F2 protein will form a complex with a molecule of GTP and a molecule of the initiator transfer RNA charged with the initiating amino-acid, formylmethionyl-$tRNA_f$. They envisage that F2, in this complex, plays a part analogous to T factor, but instead of delivering the formylmethionyl-$tRNA_f$ to the A-site of a ribosome it delivers the initiator to the P-site.

Once the formylmethionyl-$tRNA_f$ is bound to the P-site the second and subsequent amino-acids attached to their tRNAs can be delivered by the T factor to the A-site and added to the growing chain. And to ensure that only the initiator species of transfer RNA enters the P-site the two factors T and F2 have evolved mutually exclusive specificities. T factor will complex with any charged tRNA except the initiator species, while factor F2 will only complex with, and therefore deliver, the initiator formylmethionyl-$tRNA_f$. Having hit on a successful basic mechanism for the delivery of charged tRNAs to ribosomes, nature has evolved two neat variations to make certain that the processes of starting a protein and elongating a protein do not interfere with each other.