Minor tyrosine genes of major importance

THE structure and function of transfer RNA are so well understood that it is surprising how little is known of the transcription of this RNA and of the genes from which it is transcribed. This is partly due to the difficulties involved in isolating the tRNA genes which are scattered through the genome in small clusters.

Unless the tDNA is chemically synthesised, it is only possible to obtain DNA enriched in tDNA genes, rather than pure tDNA. For example, using the DNA of a species of mycoplasma enriched so that the tDNA is 10% of the total, it has been possible to show that uridine is converted to pseudouridine post-transcriptionally. Forming hybrids between total cell DNA and tRNA has given some information about the size of the genes and supports the idea that the genes for tRNA are grouped together in twos or threes. But by far the most informative method has made use of the transducing bacteriophage φ80. This phase has an attachment site (in *Escherichia coli*) near the two minor tyrosine genes which together contribute 30% of the total tyrosine tRNA. The two genes are indistinguishable except in su^+_{III} cells where one of them—the suppressor gene—has a single base change in the anticodon region, which allows tyrosine to be read instead of the terminator codon UAG.

When the phage carrying one or other of the two minor tyrosine tRNA genes is transduced into *E. coli*, large amounts of tyr tRNA can be detected in the post-infection phase. In mutants in which this amount of RNA is reduced, it was found that this was because the processing of the RNA was less efficient, leading to an accumulation of the first transcription product. Using φ80 carrying one of the minor *tyr* tRNA genes (the suppressor mutant), the accumulated RNA was examined by Altman and was shown to contain some 40 nucleotides more than the mature tyr tRNA (*Nature new Biol.*, **229,** 19; 1971). Subsequent sequencing of the molecule by Altman and Smith (*Nature new Biol.*, **233,** 35; 1971) revealed several interesting features of this tyr tRNA precursor. It seems that, like the mature tRNA, the precursor also has a 'clover-leaf' secondary structure but differs from it in that bases modified on the active tRNA are unmodified in the precursor. The authors also present evidence that mutations which alter the amount of mature RNA produced are close to the point at which the precursor molecule is cleaved.

Chysen and Celis have used one of these mutations—one which reduces the rate of synthesis of su^+_{III} tyr tRNA—to determine whether the two minor *tyr* tRNA genes are transcribed into one large precursor or two separate ones (their results are described in *Nature*, **249,** 418; 1974). In this case it was necessary to infect *E. coli* with a transducing phage carrying both su^+_{III} and wild type *tyr* genes. They reasoned that if the two genes are jointly transcribed then a mutation which affects the rate of transcription of the su^+_{III} gene should also affect the rate of transcription of the wild type gene.

The experimental approach made use of the separation of tyrosine tRNA from bulk tRNA obtained by polyacrylamide gel electrophoresis. Once eluted from the gel the ^{32}P-labelled tyrosine tRNA was subjected to RNase T_1 digestion. Characteristic T_1 fragments identified tyr_{II} tRNA (the major species) and the two minor species tyr_I su^+_{III} and tyr_I wild type. By comparing the relative amounts of tyr tRNA I and II mutations which reduced the amount of tyr_I tRNA were identified, and those which reduced the level of su^+_{III} tRNA also reduced the level of wild-type tRNA. It seems therefore that the two genes are transcribed into one single precursor tRNA. Confirmatory evidence of this was obtained from an analysis of the RNA synthesised, after infection with φ80 carrying both minor *tyr* genes in cells deficient in RNase P (RNase P is the enzyme which cleaves the precursor to form the mature tRNA). The bands obtained by electrophoresis of the RNA were eluted and digested with RNase T_1. Of the fingerprints obtained there was none characteristic for a 'singlet' precursor of the wild-type tRNA.

Knowing that the two minor tyrosine tRNAs are transcribed together, it becomes particularly interesting to have some information concerning the structure of the genes. This is provided in an article in page 738 of this issue of *Nature* by Landy, Foeller and Ross, although at the time of writing the existence of the 'doublet' precursor was not fully established. Their approach was to compare the DNase digestion pattern of phage φ80 (parental) with φ80 su^+_{III} (singlet—the suppressor gene) and with φ80 su^+_{III} (doublet—the suppressor gene and the wild type). Limited digestion with site-specific nuclease endo R. *Hin*d, followed by electrophesis under different conditions, led to complete resolution of all the fragments. Most of the fragments were the same for all three RNAs of course, but three fragments were unique to the parental φ80; one fragment was unique to the singlet, one to the doublet, and five fragments were found in both singlet and doublet but not in the parental phage. These last fragments did not form hybrids with mature tyr tRNA and so their transcript may be that part of the precursor which is cleaved during tRNA biosynthesis. It seems that the size of one tRNA gene plus any unduplicated intergenic sequence is approximately 200-260 base pairs—about two or three times the size of tyr tRNA. The authors suggest a maximum of 125 base pairs for the intergenic sequence between the duplicated genes. Further sequencing of the DNase fragments will allow these figures to be more accurately assigned and provide a complete analysis of the *tyr* tRNA gene(s).

PAMELA HAMLYN

Alpine tundra change after road building

from Peter D. Moore
Plant Ecology Correspondent

ALPINE tundra habitats are simple in structure, yet often diverse in species. The species which can survive under such rigorous climatic conditions are of considerable biological interest because of their elaborate phenological and physiological adaptations (see Billings and Mooney, *Biol. Rev.*, **43,** 481; 1968). Their extreme adaptation to climatic stress may render them particularly susceptible to hardships of other kinds, such as human interference. A short growing season results in low productivity and this means that recovery following disturbance is bound to be slow.

Bell and Bliss (*Biol. Conserv.*, **5,** 25; 1973) studied regeneration of vegetation on areas laid bare by road construction in Olympic National Park, Washington, and found that after more than 30 years plant cover was still very low. Greller (*ibid.*, **6,** 84; 1974) has now produced similar data from Rocky Mountain National Park, Colorado. In this park two roads provide easy access to an open expanse of alpine tundra; one of the roads was constructed in 1920 and the other in 1932, but recolonisation of the verges, laid bare by construction activities, has been very slow.

Greller found that north-facing slopes had become dominated by *Poa fendleriana*, and south-facing slopes by *Agropyron scribneri*, both of which are tussock-grass species, well adapted to growth on unstable soils. It is a matter for concern that these slope communities are low in diversity and lack many of the more interesting