# Solutions Not Yet Polarized

THEORIES to explain polarity in bacterial operons, which is the topic of a contribution from Imamoto this week (page 232), have abounded since the phenomenon was first described in 1963. "Polarity" describes the observation that a nonsense mutation in one gene of a bacterial operon may not only inactivate the protein which that gene specifies, but may also reduce the amounts synthesized of the (normal) proteins coded by the subsequent genes in the operon. There is a gradient of polarity along the gene because the magnitude of the polar effect depends on the position of the mutation: the greater its distance from the end of the gene, the greater the reduction in synthesis of the subsequent proteins. Because polarity is caused only by nonsense codons—the signals for termination of protein synthesis—the most obvious explanation is that the premature termination of protein synthesis in one gene can in some way affect the ability of ribosomes to translate the messenger RNA corresponding to subsequent genes. "In some way" has led to complicated theories to explain how ribosomes translate polycistronic messengers, and although there is not yet any complete explanation, many polar effects can be explained at the level of translation.

But a bizarre effect which is more difficult to explain is how polar mutants interfere with messenger RNA synthesis. Strongly polar mutants of the tryptophan operon of E. coli (which has been extensively used to study polarity because of the development of a hybridization assay to measure its mRNA) contain trp messengers which are shorter than usual; these seem to contain only the RNA sequences corresponding to the region of the operon before the mutant site.

This is difficult to explain, because it implies that nonsense codons can affect transcription as well as translation. But two types of theory have been proposed to account for this. Morse and Yanofsky (Nature, 224, 329; 1969) have suggested that the RNA corresponding to the more distant regions of the operon is made, but is not usually detected because it is very unstable. They supported this contention by demonstrating that very short-lived trp mRNAs could be detected by hybridization only by using very short pulse doses of radioactivity. They suggested that its rapid degradation might be caused by the dissociation of ribosomes from the messenger at the nonsense mutation, which would leave the far regions of the messenger unprotected against nucleases. The gradient of polarity could be accounted for if ribosomes were able to reattach at the next cistron after the mutation; the probability of degradation would then depend on the distance unprotected between mutation and the end of the cistron.

An alternative idea is that transcription as well as translation come to an untimely end at the nonsense codon, and Imamoto presents evidence to support this concept (page 232). He too observes that polar mutants contain rapidly degraded trp mRNA, but differs from Morse and Yanofsky in finding that it represents the early part of the operon before the mutant site; mRNA for the genes beyond the mutation does not seem to be synthesized at all.

If transcription does stop at or near the nonsense mutation, the RNA synthesized so far could be released from the DNA prematurely, and might therefore become available to nucleolytic attack. But why should transcription stop at a nonsense codon? Imamoto suggests that RNA polymerase continues past the mutant site, but because the RNA which it synthesizes is not translated, enzyme and product may become entangled, jamming the polymerase in position on the DNA. This would prevent passage of any other polymerase molecules, and stop transcription. The probability of this interaction taking place would depend on the length of messenger left before the next cistron where it could be reloaded with ribosomes; this accounts for the gradient of polarity.

There is no obvious way to reconcile the different results obtained by Morse and Yanofsky and by Imamoto—they have, after all, been working with the same system. One possible way out is that they use different methods to de-repress the tryptophan operon; another is that there are slight differences in their hybridization assays and the ways in which the results are worked out. But for the while, at any rate, you reads your paper and you picks your theory.

## ENZYMES

# Dehydrogenases Disclosed
### from our Molecular Biology Correspondent

THE dehydrogenases are a ubiquitous family of enzymes which have in common their association with NAD or NADP as co-factors, and have provided a secure livelihood for countless biochemists over the years. More recently, as a consequence of some spectacular progress in their enzymology, sequences and crystallographic structure, they have formed a major growth point. In structural terms most is now known about glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and lactate dehydrogenase (LDH). Harris and his colleagues have determined the sequences of GAPDH from pig and from lobster muscle—no small task, for they contain 332 and 333 residues per chain, there being four identical chains in each native enzyme.

Now Jörnvall (Europ. J. Biochem., 16, 25; 1970) reports the sequence of the even longer chain of a horse liver alcohol dehydrogenase (LADH), which has 374 residues. The result establishes that the enzyme, which is a dimer, has identical subunits. There is perceptible homology with the GAPDH sequence only towards the N-terminal end of the chain. The essential, highly reactive, cysteine is residue 46, whereas in GAPDH it is at 149 (where indeed it also is in LDH). Positions 149 in LDH and 46 in GAPDH are both occupied by tyrosines, and it is an interesting speculation, if per-