Regulatory genes in fungi

from a Correspondent

THE paper by Arst in this issue of *Nature* (page 231) marks another step in our understanding of regulation of gene action in the mould *Aspergillus nidulans*. In 1970, Hynes and Pateman (*Molec. gen. Genet.*, **108**, 97–106, 107–116) reported mutants which identified a gene whose product seemed to act as a positive regulator of the structural gene for amidase (*amdS*). Now Arst reports that this regulatory gene—now called *intA* for integrator—also regulates expression of two genes concerned with ω-amino acid metabolism. These latter two genes, *gatA* and *gabA*, code, respectively, for an inducible transaminase acting on β-alanine, γ-aminobutyrate or δ-aminovalerate and a permease for the same ω-amino acids. The three genes under control are all unlinked to *intA* and to each other. It seems that various mutations in *intA* can affect the control of *amdS*, *gatA* and *gabA* differentially, and can also be associated with changes in the relative responses to γ-aminobutyrate and β-alanine as effectors. The conclusion is that the *intA* product interacts with each of the three genes (or with controlling regions associated with them) and also with various small molecular effectors, and that these specificities can be altered by mutation. Arst draws attention to the similarity between *intA* and the type of integrator gene proposed by Britten and Davidson in 1969 (*Science*, **165**, 349–357) as part of an influential general model for regulation of gene action in eukaryotes.

Arst's report, together with earlier work, shows that sets of unlinked genes can be simultaneously subject to more than one system of integrative regulation. Arst and Cove and their colleages (for example, *Molec. gen. Genet.*, **126**, 111–141; 1973) had already described in *Aspergillus* a regulator gene, called *areA*, which is concerned with nitrogen catabolite repression, and which regulates the action of several other genes with functions in the generation of ammonium ion. A type of mutant allele, called $areA^r$, represses these other genes (including the amidase gene) even in the absence of ammonia. "Constitutive" alleles of *intA* can nullify the repressing effect of $areA^r$, the positive integrative control in this case over-riding the negative. In addition, one can have positive gene-specific regulation, acting in parallel with the "integrator" regulation. Thus, another regulatory gene, *amdA*, is found by Arst to act positively on *amdS* (more or less additively with *intA*) with no effect on *gabA* and *gatA*.

The regulation of nitrogen metabolism in *Aspergillus*, thanks mainly to the work of J. A. Pateman in Glasgow, Arst (with D. J. Cove and C. Scazzocchio) in Cambridge, M. J. Hynes in Australia and P. Weglenski in Warsaw, is becoming an extremely complex story—excessively so, perhaps, in the opinion of some. But the complexity is there in the eukaryotic cell. Attempts to unravel the network of gene interactions are bound to produce something of a tangle at the outset, but perhaps some general features are beginning to emerge which will eventually fall into place in a higher-order pattern.

Grosjean *et al.* were able to monitor in temperature jump relaxation experiments. This absorbance change shows the right spectral distribution to be expected from the base pairs presumed to be formed, and is not manifest when the two anticodons are not complementary. Moreover, the three complementary bases must be centred in the middle of each seven-membered anticodon loop for the reaction to be appreciably manifest. Thus, for interaction, the anticodons *per se* must be complementary, and complementarity between other bits of the anticodon loops does not help.

Returning to the temperature jump results, the authors found that the rate constant for the association reaction was close to that expected for the association of complementary trinucleotides, and, furthermore, virtually temperature independent. The surprise comes in the rate constant for the back dissociation reaction which was six orders of magnitude slower than expected. It follows that the equilibrium constant is 10^6 times higher than expected.

More detailed analysis showed that the entropy of interaction was not very different from that expected for trinucleotide interaction, but the enthalpy change was about −25 kcalories, which is about 10 kcalories larger than expected. This difference of 10 kcalories is sufficient to account for the factor at 10^6 and is at the kernel of the enhanced anticodon–anticodon interaction. This in itself suggested that stacking interactions were involved.

Pushing the investigation further, Grosjean *et al.* studied the interaction of *Escherichia coli* $tRNA^{Gln}$ with yeast $tRNA^{Phe}$ in the presence of EDTA, in which conditions the former species is in the denatured conformational state. The association was still observed, suggesting that the structural features which distinguish the two conformer states are not important to the association. Similarly the removal by specific cleavage of the first 16 nucleotides from the 5′ end of the $tRNA^{Phe}$ did not alter the interaction. On the other hand, fragments obtained from both these species such that anticodon helical arms could not form, still associated but with a constant reduced by about two orders of magnitude—leaving an enhancement of four orders still to be accounted for.

The remaining factors were deemed to reside in the "dangling ends" or the residual four bases in the anticodon loop that are not involved in the anticodon itself. Grosjean *et al.* point out that the sixth base in the anticodon loop (that is the one at the 3′ end of the anticodon) is frequently modified. They go on to show that the association constant varies for species with similar anticodons but differs in the nature of the sixth base, and, in particular, that excision of the Y base from this position in $tRNA^{Phe}$ leads to a substantial reduction in the enthalpy of association.

Grosjean *et al.* tie all these observations together by postulating that the enhanced association constant and the increased enthalpy of association originates from the stacking of the two anticodon arm helices on either side of the helical region formed by the anticodon–anticodon interaction, with the 3′ dangling ends stacked up as jam in this three layer cake. I choose this analogy carefully since jam is well known to be sticky.

We are finally left with the thought that something similar happens in the codon–anticodon interaction. Maybe the ribosome holds the relevant portion of the messenger in a configuration similar to that in the tRNA and allows the interaction to be similarly stabilised by stacking.

These results also imply that the anticodon helical arm exists in both the native and denatured forms of *E. coli* $tRNA^{Glu}$. Further evidence of the similarities and differences between these two forms of tRNA comes from a paper by Jones, Kearns and Muench (*J. molec. Biol.*, **103**, 747: 1976) who worked with *E. coli* $tRNA^{Trp}$. They measured the low field nuclear magnetic resonance (NMR) spectrum of the native form of this species and assigned to their satisfaction all parts of the 11.5–14.5 p.p.m. region to one or other structural feature expected of the clover leaf model in its surmised tertiary structure. There were thus 19 base pairs, a protected U or G and two tertiary base pairs including A_{14}–s^4U_8. The agreement between their experimental spectrum and that computed from these assignments was very reasonable. They then repeated the process for the denatured form and considered in detail the difference NMR spectrum between the two. They