genetics of this species(s) than any other. But the real significance of house mouse biology is for biomedical workers: their inbred strains ought no longer to be regarded merely as biochemical artefacts, but as interacting feedback systems dependent on an evolutionary history of adaptation and opportunism. It is pertinent that 13 of the 22 speakers at the Zoo Symposium were employed in Medical Schools or by the MRC.

As medical resources become increasingly stretched, they will have to be applied more consciously at points of need rather than in blunderbuss endeavours to improve the 'environment' (such as water quality, toxicological and dietary hazards, or prenatal care). Wild house mice provide a logical step in interpreting results obtained in the artificiality of the laboratory environment to the confusing heterogeneity of human life. It is to be hoped that the symposium will stimulate both research and funding for work on naturally-living mice. □

Why ppGpp?

from Andrew Travers

THE ubiquity of purine nucleotides as regulators of macromolecular synthesis was highlighted last summer at a workshop most appropriately dedicated to Fritz Lipmann*. Historically the identification of regulators such as cyclic AMP, ppGpp, pppAppp, $A^{5'}$ $ppp^{5'}$ A and 2′5′ oligo-A has depended on their unusual and often bizarre structutes. Yet in evolution such control systems are unlikely to have arisen *de novo*. Rather it seems more probable that they initially evolved as more effective analogues of pre-existing systems. Such analogues should necessarily be chemically similar to the primordial regulators yet should possess some distinguishing structural features which would allow greater precision in the control of their intracellular concentration.

ppGpp may be considered as a paradigm of such an effector. Its accumulation in bacteria is elicited both by energy starvation and amino acid starvation and, in general, is accompanied by the reduction of protein and RNA synthesis to basal levels. Both genetic (A. Atherley, Iowa State University, Ames; M. Cashel, National Institutes of Health, Bethesda) and biochemical analysis (D. Richter, Universität Hamburg; J. Sy, Rockefeller University) indicate that there are two pathways for its synthesis, one dependent on ribosomes and activated by uncharged tRNA and a second whose activity is apparently independent of protein synthesis. Similarly the degradation of ppGpp to either ppGp or ppG may be equally complex (Richter).

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What is the role of ppGpp? The isolation of a viable mutant which lacks detectable ppGpp clearly demonstrates that the nucleotide is dispensable during normal growth (Cashel). However the ability of the mutant to undergo growth transitions is somewhat impaired. One correlation with the accumulation of ppGpp consequent on environmental stress is a change in transcriptional selectivity manifested as a preferential shut-off of stable RNA production. *In vitro* physiological concentrations of the nucleotide inhibit the initiation of rRNA synthesis with either promoter bound or free RNA polymerase as its target (M. Gruber, Rijiksuniversiteit, Groningen; A. Travers, MRC Laboratory of Molecular Biology Cambridge). Yet the extent of inhibition of rRNA synthesis *in vitro* is insufficient to account for the *in vivo* effect. Either the *in vitro* system lacks an activator normally present *in vivo* or ppGpp is not the immediate effector of transcriptional changes following amino acid starvation. Indeed a minor guanine nucleotide, ppGp, was proposed as a candidate true regulator (J. Gallant, University of Washington, Seattle).

Although most evidence favours the hypothesis that ppGpp is a major regulator of macromolecular synthesis during nitrogen starvation analogous changes in gene expression can occur without the concomitant accumulation of this nucleotide. This phenomenon is seen in strains containing mutations in the gene encoding fructose 1:6 diphosphate aldolase (Atherley; Bock & Neidhardt *J. Bact.*, **92**, 470; 1966). This observation again points to the possibility that the response of transcription to environmental stress depends on the balance between the levels of a number of effectors. Such a mechanism would allow diverse means of eliciting the same response. One example of this phenomenon is the process of sporulation in *Bacillus subtilis*. This can be induced by either nitrogen or energy starvation. The latter results in a substantial increase in the intracellular concentration of an adenine nucleotide tentatively identified as pppAppp (H. Rhaese, Universität Frankfurt). By contrast, on nitrogen starvation the GTP pool falls without a concomitant increase in pppAppp levels (E. Freese, National Institutes of Health, Bethesda). In both cases the correlation between the observed phenomena and the physiological response is strong and suggests a causal effect. To reconcile such apparently disparate observations it has to be argued that a fall in the GTP pool is equivalent to a rise in the pppAppp pool. This would be compatible with the opposing functional effects of adenine and guanine nucleotides on RNA polymerase.

Although the appearance and immediate effects of regulatory nucleotides such as ppGpp and pppAppp may be transient there is much evidence to suggest that their long term effects may also be important. The accumulation of ppGpp is accompanied by the preferential synthesis of two proteins, B56.5 and stringent starvation protein (Reeh *et al. Molec. gen. Genet.* **149**, 279; 1976). At least the latter binds to RNA polymerase and seems to alter its properties in a similar manner to ppGpp itself (Ishihama & Saitoh *J. molec. Biol.* **129**, 517; 1979). Thus ppGpp may induce the synthesis of proteins which act to stabilise the effect which the nucleotide itself elicits. Such a process would constitute a mechanism for changing the state of the cell. In a like manner the 37,000 molecular weight polymerase binding protein which appears in the initial stage of sporulation in *B. subtilis* (Haldewang & Losick *Nature*, **282**, 256; 1979) could stabilise the effects of the transient perturbation of the adenine and guanine nucleotide pools.

In eukaryotes, with the exception of cyclic AMP, there is little evidence for selective regulators of gene expression analogous to ppGpp. In yeast changes in nucleotide pools similar to those observed in *B. subtilis* are correlated with sporulation (Freese; Rhaese). Yet in higher eukaryotes ppGpp and similar compounds are apparently absent (R. Silverman, Iowa State University). Nevertheless highly phosphorylated compounds were reported to accumulate after heat shock in *Drosophila* (Travers) and immediately before sporulation in the water mould *Achyla* (H. Lejohn, University of Manitoba, Winnipeg). Although the appearance of these compounds correlates with changes in macromolecular synthesis there is as yet no conclusive evidence as to whether they have a regulatory role. However adenine nucleotides have been implicated as signals for the initiation of DNA replication. One proposed universal regulator is $A^{5'}$ $ppp^{5'}$ (P. Plesner, Odense University), which binds tightly to a single subunit of DNA polymerase α (F. Grummt, Max-Planck Institut für Biochemie, München). Alternatively, the effective signal could be the balance between ATP and ADP in the cell nucleus (E. Rapaport, Harvard University). Such a mechanism would utilise purine nucleotides required for essential intracellular processes, a characteristic which might also be expected to typify early stages in the evolution of regulatory processes.

If adenine nucleotides act as indicators of the energy balance within the cell what is the evolutionary origin of the guanine nucleotide regulators? In bacteria the major physiological role of guanine nucleotides is their involvement in protein synthesis which generates GDP from GTP. ppGpp and related nucleotides could thus be derived from a primitive regulatory system utilising the simple 5′ di and triphosphates for coupling transcription and translation. □

*Workshop on Low Molecular Weight Mediators of Macromolecular Metabolism was held on 24-27 July, 1979 in Hamburg and organised by G. Koch and D. Richter.

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