overcome by assuming that actively multiplying cells possess a weak capacity to detect and relay cyclic AMP signals, or develop this capacity during the first few hours of starvation in the absence of external signals, and that the ability to signal spontaneously only arises later (Robertson *et al.*, *Science*, **175**, 333; 1972). The first cells to emit cyclic AMP are apparently incapable of sustained signalling (Durston, *Devl Biol.*, **37**, 225; 1974). But their spasmodic signals could be relayed over large distances, leading to increased responsiveness of the cells and to the gradual emergence of pacemakers able to emit regular signals. It is not yet known how the build-up of the components of the signalling system and of the cell contact mechanism is coupled to the signal response system, nor whether it involves an effect on gene expression. Coupling may occur by way of the intracellular oscillations of cyclic AMP levels (Goldbeter, *Nature*, **253**, 540; 1975) that probably accompany both spontaneous and induced signalling.

Eukaryote genes in *Escherichia coli*

from David Sherratt

SEQUENCE-SPECIFIC restriction endonucleases are now well established tools for inserting both eukaryote and prokaryote genes into plasmids, thus allowing gene organisation and expression to be analysed. The endonuclease *eco*RI cleaves DNA about once every five to ten genes, generating short, self-complementary, cohesive ends. A number of plasmids and derivatives of bacteriophage λ contain a single *eco*RI site; as they can still replicate in *E. coli* after other genes have been inserted at this site they provide suitable 'molecular vehicles' for cloning of inserted DNA sequences. The molecular vehicle is added to the required DNA and the mixture is cleaved with *eco*RI, annealed, sealed with ligase and used to transform *E. coli* (or in the case of λ, to transfect *E. coli*). With plasmid vehicles, plasmid-borne drug resistance is usually used to select transformants and these are then analysed for the presence and nature of inserted DNA.

Clearly then, in order to isolate hybrids (or 'chimaeras') containing specific inserted sequences, one needs either a source of DNA highly enriched in those sequences, or alternatively a means of selecting or identifying the few bacteria containing the required genes from within a heterogeneous bacterial population. For the first approach, the amplified ribosomal genes of *Xenopus laevis* provide an ideal source of specific DNA which can be separated from the bulk DNA by its distinct buoyant density. It is therefore not surprising that this DNA was used in the work that led to the first report of replication and transcription of eukaryote genes in *E. coli* (Morrow *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1743–1747; 1974). Another way of isolating specific eukaryote genes is to use the fact that some eukaryote mRNAs are relatively stable and are synthesised in large amounts by particular cells. Purification of these mRNA species followed by use of reverse transcriptase and DNA polymerase should allow synthesis of the relevant gene. Cohesive ends could be added *in vitro* using deoxynucleotidyl terminal transferase (see, for example, Jackson *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2904–2909; 1972). Possible candidates for such synthesis are globin, immunoglobulin and crystallin genes. Another possibility for small genes, for example, those specifying polypeptide hormones, is complete *in vitro* synthesis of a nucleic acid that should specify the known amino acid sequence.

Using the alternative approach, Kedes *et al.* (in this issue of *Nature*, page 533) have now described an elegant method for the identification of bacterial clones containing specific DNA sequences from within a hetero-

Control of ribosome synthesis

from A. A. Travers

A MAJOR activity of the bacterial cell is the synthesis of ribosomes. During normal exponential growth this process is finely regulated so that the rate of production of ribosomal RNA to a first approximation balances that of ribosomal proteins. Under these conditions the net synthesis of the principal macromolecular components of the ribosome is thus coordinated.

Another facet of the regulation of ribosome biosynthesis is the stringent response. When a stringent (*rel*$^+$) strain is functionally starved of an amino acid the extent of rRNA and tRNA synthesis is immediately curtailed. In contrast relaxed (*rel*$^-$) strains do not restrict stable RNA synthesis on amino acid deprivation. Another very early and apparently invariant manifestation of *rel*$^+$ gene function during the stringent response is the accumulation of the guanine nucleotide ppGpp, itself a metabolic product of the ribosome. Its discoverers, Cashel and Gallant, proposed that this unusual nucleotide might act as a direct inhibitor of rRNA (and tRNA) synthesis. Consistent with this hypothesis is the observation that under conditions of partial amino acid starvation in a *rel*$^+$ cell the rate of rRNA production is inversely correlated with the intracellular ppGpp concentration (Fiil, von Meyenburg and Friesen, *J. molec. Biol.*, **71**, 769; 1972).

Is the coordinate control of rRNA and r-protein synthesis that operates during normal growth maintained during the stringent response? Two recent complementary studies have produced strong evidence that to a large extent it is. Bennett and Maaloe (*J. molec. Biol.*, **90**, 541; 1974) showed that like rRNA synthesis, the rate of r-protein synthesis relative to total protein synthesis is inversely correlated with the intracellular level of ppGpp. Similarly Dennis and Nomura (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 3819; 1974) found that partial amino acid starvation resulted in the synthesis of rRNA and r-protein being more strongly inhibited than total protein synthesis. These same workers have now confirmed (*Nature*, **255**, 460; 1975) that this stringent control of r-protein synthesis reflects the regulation of the synthesis of mRNA coding for r-proteins. Thus as with rRNA synthesis stringency operates here at the level of transcription.

r-protein mRNA is the first class of messenger whose synthesis has been directely demonstrated to be under stringent control. Its regulation contrasts with that of φ80, *lac* and *trp* mRNA whose production is not inhibited in an amino acid starved *rel*$^+$ cell. The simplest model for stringent control is thus to consider the synthesis of one class of mRNA being regulated in concert with rRNA and tRNA while the synthesis of a second class of mRNA is relatively unaffected. Any other mRNA species that are wholly or partially subject to stringency remain to be identified.

Although stringency is an important mode of regulating ribosome biosynthesis Dennis and Nomura hint that other controls may be superimposed on this rather coarse control because different ribosomal proteins are synthesised in vastly disproportionate amounts when the amino acid starvation becomes more severe. The authors suggest that this perhaps reflects an autogenous control of r-protein production.