from substrates of GTP and ATP; and ribosomes of relaxed cells are unable to perform this reaction, but can do so when extracts are supplemented with a 'stringent factor' extracted from wild type cells. Formation of the magic spot compounds takes place *in vitro* when the A site of the ribosome is occupied by an uncharged transfer RNA; this evidently acts as a signal to the ribosome that protein synthesis is limited and allows the cell to titrate its amount of uncharged tRNA.

The protein responsible for responding to the blocked A site has been studied by Ramagopal and Davis (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 820; 1974). Examining *rel*$^+$ ribosomes showed that the protein is easily extracted from them and so may be only loosely bound to the ribosome. Using an assay in which protein extracted from *rel*$^+$ ribosomes was measured for its ability to stimulate the reaction *in vitro* of unwashed *rel*$^-$ ribosomes (the lack of washing means that these ribosomes possess all other necessary components), Ramagopal and Davis found that 50S subunits, 70S ribosomes of polysomes, and 70S ribosomes which have runoff polysomes all possess *rel*$^+$ protein activity. The protein activity is not present on 30S subunits. These results suggest that the stringent factor is a ribosomal protein as such, rather than a factor which associates with the ribosome during only part of its protein synthetic cycle, and is a loosely bound component of the 50S subunit. Since it is present on both free and polysomal 70S ribosomes, these results cast no light on the conditions necessary for formation of magic spots. But they offer the prospect that further characterisation of the protein may allow both its stringent activity and also whatever other functions it may have to be defined.

Although both genetic analysis and biochemical characterisation of the stringent system suggest the outlines of its operation, many important questions remain to be answered. The first class concerns the formation of ppGpp and pppGpp. Clearly, they are formed on the ribosome in response to inhibition of protein synthesis by entry of uncharged tRNA to the A site. Although the exact mechanisms of this reaction remain to be elucidated, the *rel*$^+$ protein seems to mediate this response. One discrepancy which remains to be resolved is that ppGpp can be formed *in vitro* from GDP and ATP, whereas pppGpp can be synthesised from GTP and ATP; but *in vivo* the *spoT* gene product has been implicated in converting ppGpp to pppGpp. Another critical reaction, so far investigated scarcely at all, is how these components are formed in response to conditions other than amino acid starvation—isolation of mutants in these processes must provide an essential first step in analysis.

The second set of problems, which at the present seems more difficult to approach, concerns the reactions by which ppGpp and pppGpp mediate the inhibition of rRNA synthesis. One particularly interesting problem is why there are two magic spot compounds and what difference there may be in their functions. But attempts to show *in vitro* that ppGpp influences rRNA synthesis at the level of RNA polymerase activity have not so far proved very satisfactory. Yet the nature of this general coordinating system may prove to throw an important new light on the control of transcription in bacteria.

Control of 3T3 cell growth

from Brigid Hogan

THE mouse 3T3 line—so-called because it was derived from embryo cells transferred every 3 days at an inoculum of 3×10^5—since it was established in 1963 has been widely used in studies of the control of proliferation of mammalian cells in culture. As long as the strict transfer regime is maintained, the cells consistently show distinct growth properties. First, they are very sensitive to a reduction in serum concentration in the medium and divide about five times slower in 1% than in 10% serum. Second, even if the cells are regularly replenished with 10% serum medium, they only grow very slowly after reaching a monolayer density of about 5×10^4 cells per cm^2. Finally, they will not grow in suspension in agar or methylcellulose. It is, however, well established that 3T3 cells transformed by the oncogenic viruses SV40 or polyoma exhibit very different properties; they divide rapidly in 1% serum, reach far higher densities and form colonies in agar.

During the past few years Pollack's group in Cold Spring Harbor has been investigating the complex interrelation between these three parameters and the way in which they are modified by tumour viruses, by selecting sublines of SV3T3 which have reverted back to more normal behaviour. The first lines were selected for a reduction in growth at high cell density by exposing dense cultures to 5-fluorodeoxyuridine for 2 days so that cells synthesising DNA were killed. Clones which survived this treatment had a much more 'normal' morphology and were therefore called "flat" revertants, and reached a saturation density of only about 8×10^4 per cm^2, although the shutoff in division at this density is not as complete as in normal cells.

More recently similar 'density revertants' have been selected by killing growing cells with 5-bromodeoxyuridine or colchicine (Vogel *et al.*, *J. cell. Physiol.*, **82**, 181-188; 1974). When their other properties were tested, the density revertants fell into two classes; one which grew well in 1% serum and one which did not, but neither class would grow in agar. Using similar techniques Pollack's group have also selected 'serum revertants' which do not grow rapidly in low serum (Vogel and Pollack, *ibid.*, 189–198); these also show low saturation density but may or may not grow in suspension. Thus it has been possible to select variants of SV3T3 which have reverted in either of two, or in all three aspects of transformation. All classes of revertants have more chromosomes per cell than the parent, synthesise SV40 Ts antigen, and in some cases have been shown to contain intact virus genomes, so that their altered behaviour is likely to arise from a change in the expression of some cellular gene(s).

Pollack's group have not been slow to take advantage of the properties of their mutants to throw light on the role of cyclic AMP in the control of 3T3 cell proliferation (Oey *et al.*, *Proc. natn. Acad. Sci., USA.*, **71**, 694–698; 1974). In agreement with many other groups they found that sparse cultures of 3T3 in 10% serum contain twice as much intracellular cyclic AMP as SV3T3s. In the same conditions most revertant lines returned to having the higher levels of cyclic AMP characteristic of 3T3s, but two retained the low concentration found in SV3T3s. These two are the only revertants able to form colonies in methylcellulose so that there may be a correlation between low amounts of cyclic AMP and ability to grow in suspension. Only those revertants which are unable to grow in low serum share the property with 3T3s of rapidly and extensively increasing their concentrations of cyclic AMP when the serum concentration is reduced to 1%, and density revertants which can grow in low serum maintain the lower concentrations of cyclic AMP found in SV3T3s.

Finally, Oey *et al.* allowed normal 3T3s and two serum revertants to attain their low saturation density in conditions in which the 10% serum medium was regularly replaced, even after cell growth had slowed down. In all three lines they failed to find an increase in cyclic AMP as the cells reached their saturation densities. Taken together with the other findings, this suggests that density restriction and serum restriction of growth are not identical phenomena, as some workers have postulated, but are mediated by two different, but probably interrelated, mechanisms.