for the samples incubated with and without Glc6P) makes it tedious and slow. Moreover the response to different concentrations of the material retarded on the Sepharose CL-6B columns was very non-linear. However, at present this approach seems the only certain way forward towards identifying the site of action of hexose phosphates. Although the identity of the protein remains unknown, we can at least eliminate the possibility that it is eIF-2. As might be expected, addition of eIF-2 to the assay of gel-filtered ribosomes does result in a stimulation of the binding of Met-tRNA to 40S subunits but it does not restore the response to Glc6P.

This work was supported by a grant from the Medical Research Council. We thank Drs. H. B. F. Dixon and J. Siekierka for the gift of materials used in these experiments.


**Translational control by amino acids and aminoacyl-tRNA synthetases in cultured mammalian cells**

**MICHAEL J. CLEMENS,* SARA A. AUSTIN,**† ANGELA R. GALPINE* and JEFFREY W. POLLARD‡

* Cancer Research Campaign Mammalian Protein Synthesis and Interferon Research Group, Department of Biochemistry, St. George's Hospital Medical School, Cranmer Terrace, London SW17 ORE, U.K., and ‡ M.R.C. Group in Human Genetic Disease, Department of Biochemistry, Queen Elizabeth College, Campden Hill, London W8 7AH, U.K.

Considerable attention has been focused on translational control mechanisms in reticulocytes and on the roles played by the initiation factors elf-2 and elf-2B (GEF) in this system (Clemens et al., 1982; Siekierka et al., 1982; Konieczny & Safer, 1983). Much less emphasis has been placed on non-erythroid cell systems in which translational regulation can be effected by changes in the cellular environment. There are numerous examples of control of protein synthesis at the translation level, including the effects of heat shock, amino acid starvation, serum deprivation, changes in availability of growth factors and hypertonic salt treatment of cultured cells (Pain et al., 1980; Panniers & Henshaw, 1984; Krupp & Clemens, 1984). In the case of starvation and diabetes in wild-type animals (Harmon et al., 1984). However, it is not clear to what extent the mechanisms which exist in reticulocytes are also responsible for such control. Our interests concern particularly the effects of amino acid starvation and changes in aminoacyl-tRNA synthetase activity on the initiation of protein synthesis in cultured animal cells.

It has been known for many years that when cells are deprived of a single essential amino acid protein synthesis is rapidly inhibited by 50–60% (van Venrooij et al., 1970; Vaughan et al., 1971). Polysomes disaggregate, indicating a block in chain initiation, but the mRNA remains intact and is potentially translatable. When 40S ribosomal initiation complexes are labelled by brief incubation of intact cells with [35S]methionine or of cell extracts with [35S]methionyl-tRNA, a large inhibition is observed as a result of amino acid starvation (Pain & Henshaw, 1975; Pain et al., 1980). The available evidence suggests that the inhibition is associated with a defect in activity of initiation factor elf-2 and/or elf-2B. However, it is not clear how amino acid starvation generates a signal inside the cell which leads to the shut down of initiation activity. We have examined the possible role of aminoacyl-tRNA synthetases in this regulation by utilizing temperature-sensitive mutants of Chinese hamster ovary (CHO) cells. We have mostly used the mutant tsH1, which has a temperature-sensitive leucyl-tRNA synthetase (Thompson et al., 1973). These cells grow at the permissive temperature of 34°C but cease to proliferate at 39–40°C (the non-permissive temperature). Under the latter conditions there is substantial inhibition of protein synthesis, polysome disaggregation (Stanners & Thompson, 1974) and inhibition of 40S initiation complex formation in extracts prepared from these cells (Austin et al., 1984, 1985; Clemens et al., 1984). Thus these responses are very similar to those observed after amino acid starvation. The effect of the elevated temperature is not seen when wild-type cells are incubated at 39°C, and is therefore not a heat-shock response, but it is seen with other synthetase mutants such as Leu-21 or Arg-1 (Austin et al., 1985).

Because initiation is inhibited so severely at the level of 40S complex formation, we examined whether elf-2 activity was responsible by assaying its ability to form ternary complexes in postribosomal supernatants. Using this assay we could observe 2–4-fold differences in ratio of ternary complex formation between extracts from tsH1 cells cultured at 34°C and at 39.5°C. Again amino acid starvation at the permissive temperature had a similar effect.

The initiation ability of extracts from both tsH1 and wild-type CHO cells is limited in vitro by the availability of elf-2 activity, as shown by the effects of adding purified elf-2 to the systems. Added elf-2 is utilized stoichiometrically under the conditions of the assay. It appears that there is a more marked deficiency of elf-2 activity in extracts from cells exposed to the non-permissive temperature than in control extracts. We have investigated whether this may be a consequence of sequestration of this initiation factor into an inactive complex with elf-2B. Such an event occurs in haem-deficient reticulocyte lysates due to the phosphorylation of elf-2 (Siekierka et al., 1982, 1983, 1984) and it results in a blockage of GDP/GTP exchange (Clemens et al., 1982; Pain & Clemens, 1983). This in turn prevents the recycling of elf-2 between successive rounds of protein synthesis. It is possible to test the ability of cell-free systems to catalyse guanine nucleotide exchange on elf-2 by adding a complex of the purified factor with [3H]GDP. The kinetics of displacement of this labelled nucleotide in the presence of excess GTP are then followed using a cellulose nitrate filter binding assay.
We have carried out such experiments on cells exposed to the non-permissive temperature relative to the controls. This result strongly suggests a temperature-induced defect in the eIF-2-catalysed exchange reaction, for which a number of mechanisms are possible.

In agreement with this, preliminary data suggest that initiation in CHO cell extracts is limited by eIF-2B activity, which in turn controls the availability of eIF-2. We have added eIF-2B, purified from rat liver, to postribosomal supernatants from tsH-1 cells and then measured the formation of eIF-2-dependent ternary complexes. The added eIF-2B alone has little endogenous eIF-2 in it. Both extracts from 34°C and 39°C cultured cells are stimulated by eIF-2B but the latter extracts need approximately twice as much of the factor to produce the same level of stimulation. This could suggest that eIF-2B is titrating out an inhibitor which is present at a higher level in the 39°C extracts. Such an inhibitor could be phosphorylated eIF-2 itself or some other as yet unknown component.

These results raise a number of interesting questions. For example, what is the mechanism of inactivation of eIF-2B in tsH-1 cells at the non-permissive temperature, and in cells subjected to amino acid starvation? Is there increased phosphorylation of eIF-2 or some other mechanism about which we know nothing as yet? The phosphorylation question is currently of great interest, although to date we have found no evidence for any increased eIF-2 kinase activity in extracts from amino acid-starved compared with fed cells (Clemens et al., 1984). Nevertheless non-erythroid cell types clearly do contain eIF-2 kinase and phosphatase activities (Wong et al., 1982; Duncan & Hershey, 1984) and one must recognize the possibility that only a small change in the state of phosphorylation of eIF-2 could result in the sequestering of a large proportion of the available eIF-2B. The latter may be present in limiting amounts in the cell. An additional question concerns the mechanism by which a change in function of a component involved at the elongation stage of protein synthesis, namely an aminocacyl-tRNA synthetase, can influence the initiation machinery. Our data at present cannot answer this question, except to rule out effects of changes in the ratio of charged to uncharged tRNA molecules (Austin et al., 1982). However, whatever the mechanism involved the present data suggest a novel regulatory feedback system operating between polypeptide-chain elongation and initiation which clearly merits further investigation.

We thank Vivienne Tillers and Margaret Seagrave for skilful technical assistance and Dr. Chris Proud (University of Kent) for gifts of rat liver eIF-2 and eIF-2B. This research is supported by the Cancer Research Campaign and the M.R.C. M.J.C. holds a Cancer Research Campaign Career Development Award.