The regulation of protein synthesis in mammalian cells by amino acid supply

Sara A. AUSTIN and Michael J. CLEMENS

Department of Biochemistry, St. George's Hospital Medical School, Cranmer Terrace, London SW17 ORE, U.K.

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This review is concerned with the translational control of protein synthesis exhibited by a variety of mammalian cell types in response to changing nutritional conditions. The molecular basis of this phenomenon is now partially understood, and it has provided a number of insights into cytoplasmic control mechanisms in eukaryotes. We describe here the current state of knowledge about these mechanisms and, in particular, the role played by the polypeptide chain initiation factor eIF-2. We also briefly consider how cells may recognize changes in the availability of nutrients, especially essential amino acids, and how they adapt to short-term or long-term deprivation of these compounds which are needed for the accumulation of protein and for growth.

The Evidence for Translational Control

There is much evidence to indicate that protein synthesis in mammalian cells in tissue culture is closely regulated by the supply of essential nutrients (van Venrooij et al., 1970; Vaughan et al., 1971; Lee et al., 1971; van Venrooij et al., 1972; Henshaw, 1980). In such cells, deprivation of amino acids, glucose, or serum results in a reduction in the rate of protein synthesis. This occurs immediately in cells deprived of essential amino acids (van Venrooij et al., 1970; van Venrooij et al., 1972; Vaughan et al., 1971) and within 1 h in cells deprived of glucose (van Venrooij et al., 1972). The effect of serum starvation becomes apparent only at later times (Kaminskas, 1972). Refeeding amino acid-deprived cells with the missing amino acid results in a rapid recovery of the rate of protein synthesis (van Venrooij et al., 1970; Vaughan et al., 1971; van Venrooij et al., 1972; Sonenshein & Brawerman, 1977). These events occur even in the presence of sufficient actinomycin D to inhibit all new RNA synthesis (van Venrooij et al., 1970; Vaughan et al., 1971; Lee et al., 1971; Sonenshein & Brawerman, 1977), indicating that translational rather than transcriptional control mechanisms are operative. The rapidity of the response of cells to refeeding with the missing amino acid is in itself further evidence for translational control. Fig. 1 illustrates this point. The rates of incorporation of \( ^{14}C \)leucine into protein are shown for lysine-deprived Ehrlich ascites tumour cells and for such cells immediately after refeeding with the missing amino acid. Lysine deprivation results in a 60% reduction in the rate of protein synthesis (Pain et al., 1980), but on refeeding with lysine the rate recovers within 1 to 2 min. As this is approximately the length of time a cell takes to make a complete polypeptide chain, the response to the refeed amino acid is virtually instantaneous.
Fig. 1. Kinetics of recovery of cells from amino acid starvation. Ehrlich ascites tumour cells were incubated in lysine-free culture medium for 30 min at 37°C. Lysine was then added to one batch and protein synthesis measured from the incorporation of \([^{14}C]\)leucine (0.4 mM, 1.25 Ci/mol) into acid-insoluble material (Pain et al., 1980). Incorporation into 0.5-ml aliquots of cell suspension (1.15 x 10^6 cells) is shown for each time. (○), starved cells; (●), refed cells.

The Mechanism of Regulation

Associated with the reduction in protein synthetic rate in amino acid-starved cells there is marked polysome disaggregation and accumulation of inactive 80-S ribosomes (van Venrooij et al., 1970; Vaughan et al., 1971; Lee et al., 1971; van Venrooij et al., 1972). This indicates a blockage in ribosome attachment to mRNA during polypeptide-chain initiation. There may also be smaller effects on the rate of polypeptide-chain elongation (Vaughan et al., 1971; van Venrooij et al., 1972), but as initiation is the rate-limiting step for overall translation, these do not become apparent under normal circumstances.

In Ehrlich cells deprived of lysine there is a substantial reduction in the number of [40-S Met tRNAf] initiation complexes formed on native small ribosomal subunits (Pain & Henshaw, 1975), indicating that in starved cells initiation is controlled at this point. The effects of such starvation are preserved in cell-free extracts prepared from fed and lysine-deprived cells (Pain et al., 1980). In vitro the decrease in the level of 40-S initiation complexes can be reversed by the addition of the polypeptide-chain initiation factor eIF-2 (Pain et
al., 1980), as illustrated in Fig. 2. Interestingly, refeeding with the missing amino acid has no restorative effect \textit{in vitro} (Pain et al., 1980). It appears that the depression in the rate of initiation of polypeptide chains in amino acid-deprived cells is caused by inactivation of eIF-2, a control mechanism which is also seen in the reticulocyte system in its response to haem deficiency. It is well documented that protein synthesis in reticulocytes, and in lysates from these cells, is dependent upon the supply of haem (Austin & Clemens, 1980).

Fig. 2. Effect of initiation factor eIF-2 on the level of 40-S initiation complexes in extracts from fed and lysine-deprived Ehrlich ascites tumour cells. Extracts from fed cells and from cells starved of lysine for 30 min were incubated with $[^{35}\text{S}]$methionine (160 $\mu$Ci/ml) for 2 min in the presence and in the absence of 44 $\mu$g/ml eIF-2. The samples were fractionated by sucrose-density-gradient centrifugation (sedimentation was from right to left), and the radioactivity in 40-S initiation complexes was determined as described by Pain et al., (1980). -----, optical density at 260 nm; $\bullet$-$\bullet$, radioactivity (counts per minute per fraction). The positions of 40-S, 60-S, and 80-S ribosomal particles are indicated, and the radioactive methionine associated with 40-S-subunit initiation complexes is shown by the shaded areas.
1980b; Clemens, 1980; Ochoa & de Haro, 1979). In the absence of haem, synthesis stops, polysomes disaggregate, and the level of 40-S initiation complexes declines (Legon et al., 1973). Associated with this is the activation of a protein kinase, named the haem-controlled repressor (HCR) or haem-regulated inhibitor (HRI), which phosphorylates the small subunit of initiation factor eIF-2 (Ranu & London, 1976; Farrell et al., 1977). Phosphorylation of this factor has been closely correlated with its inactivation, although other interactions are now known to be necessary since phosphorylated eIF-2 itself is still active in fractionated cell-free systems (Farrell et al., 1977; Trachsel & Staehelin, 1978).

In cell-free extracts from fed and lysine-deprived Ehrlich cells, eIF-2 is also phosphorylated, but in this case we have been unable to detect any increase in phosphorylation in extracts prepared from starved cells (S.A. Austin & M.J. Clemens, manuscript in preparation). It is known that these cells possess a protein kinase which can phosphorylate the small subunit of eIF-2, as well as a phosphatase which dephosphorylates it (S.T. Wong and E.C. Henshaw, personal communication), but at present the role of these enzymes in the starvation-induced inactivation of eIF-2 remains unclear. It is possible that, as in the reticulocyte, phosphorylation of eIF-2 is a necessary prerequisite for its inactivation, but some further interactions may also be involved.

Studies of the subcellular distribution of eIF-2 in Ehrlich cells have revealed that there is less active factor on starved cell ribosomes than on fed cell ribosomes, at least in a form which can be washed off with 0.5 M KCl (Clemens & Bloyce, 1980). This correlates with the inability of starved cells to form 40-S initiation complexes efficiently. However, the interpretation of such data is complicated by the fact that when extracts are prepared from Ehrlich cells a high proportion of the total recoverable eIF-2 activity sediments with the nuclear and mitochondrial pellet, which is usually discarded (Clemens & Pain, 1981). It is not yet known whether this eIF-2 is functionally similar to that normally found associated with ribosomes.

Because of the obvious mechanistic similarities between the control of protein synthesis in Ehrlich cells by amino acid supply and the effects of haem on translation in reticulocytes, we have recently examined the effects of adding haem to Ehrlich-cell-free extracts (S.A. Austin & M.J. Clemens, manuscript in preparation). The results of this study have shown that in extracts from fed cells haem quite markedly stimulates protein synthesis and the level of 40-S initiation complexes. In extracts from starved cells, this stimulation appears to be blocked by the action of an inhibitor. Preliminary evidence also suggests that this inhibitor can partially abolish the haem stimulation of initiation when preparations from fed and starved cells are mixed together (S.A. Austin & M.J. Clemens, manuscript in preparation). However, the presence of the inhibitor is not always readily demonstrated in other types of assay. For example, extracts from both fed and starved cells, when added to haem-supplemented reticulocyte lysate, can inhibit initiation at the level of the 40-S initiation complex. A possible model for the relationship between this inhibition, the starvation-induced inhibition, and the phosphorylation of eIF-2, is summarized in Fig. 3. By analogy with the regulation of
eIF-2 activity in the reticulocyte system (Farrell et al., 1977), we propose that the addition of haem to extracts from Ehrlich cells inhibits a protein kinase with specificity for eIF-2, thus increasing the ratio of unphosphorylated to phosphorylated factor. This in itself may not stimulate initiation, since both forms of eIF-2 are active (at least \textit{in vitro}) (Farrell et al., 1977; Trachsel & Staehelin, 1978). However, we propose that eIF-2 P binds to inhibitors, depicted as I and X in Fig. 3, which inactivate it. Inhibitor I is believed to exist in both fed and amino acid-starved cells, and complexes of it with eIF-2 P are depicted as being in equilibrium with the free phosphorylated initiation factor. Thus when the level of the latter is lowered by the addition of haem, dissociation of the complex with I would be favoured and, in extracts from fed cells, this would lead to an increase in the level of active eIF-2. However, we postulate that starvation activates or causes the appearance of the inhibitor X, which does not dissociate from eIF-2 P on the addition of haem. Thus no further active eIF-2 is released for initiation and there is no effect of
haem. As yet we do not know the nature of I or X. They could be related, but need not be so. Alternatively, I or X may represent modifications to eIF-2, rather than discrete molecular entities. Further aspects of this model are currently being studied in our laboratory.

**Cellular Responses to Amino Acid Starvation**

It is of interest to consider the possible ways in which the lack of one essential amino acid in the extracellular medium results in an inhibition of polypeptide chain initiation inside the cell. In other words, what are the signals which the cell recognizes? One possible candidate for such a signal could be a change in the levels of endogenous guanine or adenine nucleotides in starved cells. It has been shown by others that intracellular pools of ATP and GTP decline in size following starvation for amino acids (van Venrooij et al., 1972; Grummt & Grummt, 1978).

However, in Ehrlich cells under the conditions we have used, the levels of ATP and GTP and their corresponding di- and monophosphates (measured by high-performance liquid chromatography) did not change significantly during amino acid starvation, at least at early times (S.A. Austin & D. Perrett, unpublished observations). The published studies (van Venrooij et al., 1972; Grummt & Grummt, 1978) have only looked at later times, i.e., 1-3 h after starvation was started, when a whole range of cellular functions in addition to protein synthesis may well have been affected (see below). Within the first 30 min of amino acid deprivation, when protein synthesis is rapidly and reversibly inhibited, a change in nucleotide levels sufficient to account for this response appears improbable.

It has frequently been suggested that it is the accumulation of uncharged tRNA in amino acid-starved cells that causes the inhibition of polypeptide-chain initiation (Vaughan & Hansen, 1973; Grummt & Grummt, 1978). We now no longer believe this to be the case, at least as far as the 40-S initiation complex is concerned. Several lines of evidence argue against uncharged tRNA being involved at this level. Firstly, if Ehrlich cells are treated with a low dose of cycloheximide and simultaneously starved for lysine, polypeptide-chain elongation is inhibited more than initiation, and polyribosomes are maintained in an undegraded state. Under these conditions all tRNAs will be fully charged, as the amino acids are being utilized for protein synthesis only very slowly. Sufficient lysine is provided from protein degradation to meet the requirement for protein synthesis. However, the reduction in the level of 40-S initiation complexes is still observed in extracts from such starved cells (Austin & Clemens, 1980a). The data are summarized in Table 1.

A second piece of evidence concerns the effects of deacylated tRNA which has been oxidized at the 3' end with periodate to prevent recharging with amino acids. Addition of such tRNA to cell-free extracts from fed cells has very little effect on the level of 40-S initiation complexes, although it is strongly inhibitory to 80-S complexes (Austin et al., 1980). Thirdly, we have found that extracts from both fed and starved cells have similar capacities to charge tRNA with amino acids, including the one of which the cells
Table 1. 40-S initiation complex formation in cell-free extracts from fed and lysine-deprived Ehrlich ascites tumour cells with or without exposure to a low concentration of cycloheximide

<table>
<thead>
<tr>
<th>Type of extract</th>
<th>[35S]Methionine bound to 40-S initiation complexes (pmol/A260 unit of extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fed</td>
<td>0.223</td>
</tr>
<tr>
<td>Starved</td>
<td>0.049</td>
</tr>
<tr>
<td>Fed + cycloheximide</td>
<td>0.142</td>
</tr>
<tr>
<td>Starved + cycloheximide</td>
<td>0.027</td>
</tr>
</tbody>
</table>

Two pairs of cell-free extracts from fed and lysine-deprived cells were prepared as described in Pain et al. (1980). One pair of fed and starved extracts was prepared by incubating the cells with 0.05 μg/ml cycloheximide (to partially inhibit elongation of polypeptide chains) during the 30-min starvation procedure. Cell-free extracts from these cells were then prepared as usual. 40-S initiation complex formation was measured in these extracts as described in Pain et al. (1980), and the amount of [35S]methionine bound to 40-S ribosomal subunits expressed as pmol/A260 unit of extract.

have been deprived. However, as stated earlier, under conditions where extracts from lysine-deprived cells are supplemented with lysine to equal or exceed the amount found in extracts from fed cells, the level of 40-S initiation complexes is still reduced (Pain et al., 1980; Austin & Clemens, 1980a). Taken together these various observations suggest that, in mammalian cells, the control of protein synthesis by amino acid supply does not depend critically on the accumulation of deacylated tRNA, at least with respect to the 40-S initiation complex. This lack of dependence is in contrast to the stringent response to amino acid starvation in bacteria, which is activated by ribosome-bound deacylated tRNA (Haseltine & Block, 1973).

The evidence against the involvement of either changes in nucleotide pool sizes or variations in levels of charging of tRNA in eliciting the effects of amino acid starvation on polypeptide-chain initiation therefore leaves unresolved the problem of how cells recognize their altered nutritional state. Whatever mechanism is involved works very rapidly in intact cells (Fig 1.). This instant response, contrasted with the complete refractoriness of cell-free systems, suggests a role for some cellular fraction which is discarded during the preparation of extracts from cells. The plasma membrane could be one candidate for such a fraction. Alternatively, a highly labile 'amino acid receptor' or similar intracellular component may be involved, but as yet we have no idea what this could be or how it might work.
Long-term Adaptations to Nutritional Deprivation

The majority of the changes described above are very rapid responses of cells in culture to amino acid starvation. However, prolonged exposure to nutritionally inadequate conditions results in several additional alterations in cellular metabolism. These may be interpreted as adaptive changes, enabling the cells to survive for a considerable time even though they can no longer proliferate. A slowing of the rate of synthesis of ribosomal RNA occurs within 30 to 40 min of amino acid deprivation (Grummt et al., 1976), consistent with a reduced cellular requirement for new ribosomes under conditions where growth does not occur and existing ribosomes are utilized with reduced efficiency. Nutritional limitations of many types also result in a series of co-ordinated metabolic changes termed the 'negative pleiotypic growth response' by Hershko et al. (1971). This is characterized by inhibition of DNA synthesis and reduced transport of several small precursors into cells, as well as by the translational and transcriptional effects already discussed. We also suggest that enhancement of protein breakdown should now be added to this list, since amino acid starvation stimulates proteolysis in several types of cell (Gunn et al., 1976; Neely et al., 1977; Fulks et al., 1975). In the absence of an exogenous essential amino acid, degradation of cellular proteins can provide a limited quantity of this compound sufficient to maintain a basal rate of protein synthesis (the starved cell rate shown in Fig. 1). This may be adequate for the survival of the cell — at least for a short time, until better nutritional conditions are encountered.

Conclusions

In this review we have concentrated on the biochemical behaviour of tumour cell lines grown in tissue culture. The implications of the findings which we have described may, however, be reasonably extended to normal tissues in intact animals. Despite homeostatic mechanisms which maintain nutrient concentrations in the blood at nearly constant values, an organ such as the liver has to cope with wide variations in the rate of influx of amino acids and other compounds from the portal vein, associated with changes in food intake. It may be expected that such metabolic challenges are dealt with by mechanisms at least related to those briefly described in this article.

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References