Review Letter

THE ROLE OF SOLUBLE PROTEIN FACTORS IN THE TRANSLATIONAL CONTROL OF PROTEIN SYNTHESIS IN EUKARYOTIC CELLS

Virginia M. PAIN
Department of Human Nutrition, London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1E 7HT, England

and

Michael J. CLEMENS
National Institute for Medical Research, Mill Hill, London, NW7 1AA, England

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1. Introduction

This review considers possible roles for soluble protein components of eukaryotic cells in the regulation of protein synthesis. We have chosen to treat the subject in this way because of the many recent reports of changes in the activity of supernatant fractions of cell extracts to support *in vitro* protein synthesis following alterations in cellular activity. To consider soluble components as a group is, of course, an arbitrary choice and we realise that molecules which exist free in solution in cell extracts may have particulate or membrane-bound forms *in vivo*. Our approach excludes consideration of other (non-protein) factors which may be involved in the regulation of translation since to describe all possible control elements in higher cells would make this review impossibly long.

Space does not permit a detailed account of the mechanism of protein synthesis. Excellent reviews have been written on this subject [1, 2] and we have assumed the reader has some knowledge in this area. The regulation of translation in eukaryotic cells is complex and involves many factors. Current evidence suggests that chain initiation is rate-limiting for translation in most cells and therefore controls the number of active ribosomes found in polysomes. This is demonstrated by the ability of cycloheximide to increase polysome size by slowing the rate of chain elongation relative to initiation [3—5]. Conversely, at elevated temperatures or during mitosis, when initiation occurs at a slower rate than normal, polysomes become smaller [4, 6, 7]. If initiation is rate-limiting for translation it is obviously a prime candidate for a regulatory role in this process. On the other hand, if initiation rates rise appreciably under certain conditions one might expect that some other step in protein synthesis (such as tRNA charging or chain elongation) could become limiting. It may be that specific mechanisms exist within the cell for increasing the rates of the other reactions as well as initiation. There are many examples in the literature where increased protein synthetic rate *in vivo* seems to be achieved by just such a multiple response to an environmental stimulus [8, 9].

In practical terms these phenomena are reflected in changes in the protein synthetic capacity of cell-free systems which are often ascribable to variations in both ribosomal and supernatant activities. Some examples include responses to altered nutritional conditions in the rat [10—14] or in isolated tissues [15, 16]; modula-

Abbreviations:
EF-1, elongation factor 1 (aminoacyl tRNA binding enzyme); EF-2, elongation factor 2 (translocation factor); IF-1,2,3, initiation factors 1, 2 and 3; EMC virus, encephalomyocarditis virus.

tion of protein synthesis in various pathological conditions [17–21]; effects of increased growth rate or cell proliferation [22] and responses to hormones such as ACTH [23], insulin [20] and oestradiol [24]. There are also a number of examples of changes which occur in the abilities of supernatant fractions to support cell-free protein synthesis during development. These include such diverse systems as developing mouse brain [25], tobacco horn worm pupae [26] and growing pea seedlings [27].

Other workers have compared *in vivo* rates of protein synthesis with *in vitro* polysomal activity and have concluded that soluble factors must affect translation in the cell under some conditions. Systems studied include fed versus fasted rats [28], Ehrlich ascites cells under various conditions of amino acid and glucose supply [29, 30] and slime-moulds at various stages throughout the mitotic cycle [31]. In this review we have analysed some of the possible soluble regulatory factors and have considered the mechanisms by which they may act.

2 Protein factors

2.1. *Initiation factors*

Protein factors required for initiation of polypeptide synthesis in cell-free systems are commonly prepared by washing ribosomes in buffers containing 0.5 M KCl [32–35]. Prichard et al. [36] have characterized the salt wash preparation from reticulocyte ribosomes and identified 3 components necessary for translation of exogenous natural mRNA *in vitro*. More detailed studies reported recently have defined further the individual roles of these factors in eukaryotic peptide chain initiation [37] and have shown that proteins purified from an extract of liver microsomes have very similar properties to the reticulocyte components [158]. The interchangeability of initiation factors from various mammalian sources is further emphasized by the demonstartion by Schreier and Staehelin [159] of the similarity in the properties of at least one factor purified from rabbit reticulocytes, mouse ascites cells and guinea-pig liver. The exact relationship of the factors described by these authors [159, 160] to those studied by Anderson and co-workers is not yet clear.

In addition to being associated with ribosomes, proteins required for initiation also exist in a soluble form. Thus appears to be the case in reticulocytes [38–40], rat liver [41–47], ascites cells [48, 49], wheat embryos [50] and brine shrimps [51, 52] and is probably a general phenomenon. Of particular interest is the observation of Bonanou-Tzedaki et al. [40] who showed that purified subunits derived from rat liver ribosomes could translate globin mRNA faithfully in the presence of reticulocyte supernatant without the need for any other added proteins. Thus reticulocyte cell sap must contain sufficient levels of all 3 factors for *in vitro* initiation. Cell sap from ascites cells also possesses all necessary factors whereas Leader et al. [49] conclude that rat liver and muscle supernatant each have only one. The report by Sampson and Borghetti [45] that a fractionated rat liver cell-free system will translate globin mRNA even when salt washed ribosomes are used suggests, however, that rat liver cell sap does contain all the components needed for *de novo* globin synthesis. Schreier and Staehelin [159] report that 3 out of 4 factors they have defined as being necessary for initiation can be found in a pH 5 fraction from rat liver cell sap but the remaining one is associated with native 40 S subunits. The distribution of factors between ribosomes and cytoplasm may be a function of the physiological state of the cell and its protein synthetic activity at any one moment.

There is now considerable evidence that some aspect of the initiation process is rate-limiting for overall protein synthesis in many (possibly all) cells under most conditions, and initiation factors may play an important role as regulatory elements. In the model proposed by Kaempfer [53] the level of subunits available for initiation is controlled by the availability of IF-3 which acts as a "dissociation" or "anti-association" factor. Examples of regulation of initiation are to be found in cases such as control of globin synthesis by haem [54], embryo development [55, 56], the action of anabolic hormones [57–59], effects of alterations in nutritional conditions [8, 60] and translational changes occurring during ageing and maturation of differentiated cells [61]. Whether IF-3 is the crucial factor involved in all these situations remains to be investigated. Kaempfer's model further proposes that, following formation of the initiation complex, IF-3 dissociates from the ribosome. This is entirely compatible with its existence in both ribosome bound and soluble forms.

In addition to the role of the initiation factors in the quantitative regulation of protein synthesis there

may be some qualitative control exerted by these proteins at the translational level although the concept of messenger-specific initiation factors in eukaryotic cells is at present a very controversial subject. Evidence for [62–66] and against [67–84] factor specificity was accumulated recently. In addition to studies using ribosomal salt-washes as sources of initiation factors it has been shown [85, 86] that there is an absolute requirement for ascites cell sap for translation of EMC virus RNA by ascites or reticulocyte ribosomes, and Clemens and Tata [87] reported a specific *Xenopus* liver supernatant requirement for initiation on endogenous *Xenopus* liver polysomes which was not met by rat liver supernatant. However, the possibility of *in vitro* artifacts due to differing ion requirements for translation of different mRNA's has been pointed out [86, 88].

The final truth may lie somewhere between the 2 presently conflicting sets of results. Thus factors specific for rather broad groups of mRNA molecules may exist, discriminating between these groups on the basis of secondary structure or conformational differences. Such a mechanism may be the basis of the observed differences in initiation rates between α and β globin chains in extracts of normal reticulocytes [89, 90] or reticulocytes from thalassaemic subjects [91]. In cell-free systems it is possible that factor specificity may be exerted only by altering relative efficiencies of translation of different messages and may not be apparent when mRNA is present in large excess.

Finally, consideration of regulation of initiation should take into account the possibility of tissue specific inhibitory factors acting at this level [92]. Levy et al. [93] have described proteins isolated from the soluble fraction of reticulocytes and liver which inhibit the heterologous but not the homologous systems *in vitro*. The proposed existence of such substances requires closer investigation.

2.2. *Elongation factors*

Elongation factors are found both in the cell sap and bound to ribosomes, as evidenced by their presence in 0.5 M KCl or NH_4Cl wash fractions from these particles [22, 94, 95]. EF-1 and EF-2 are soluble at pH 5 and their activities are commonly studied in whole cell sap or in the supernatant remaining after precipitation of tRNA and aminoacyl-tRNA synthetases. However, some elongation factor activity is found in pH 5 precipitates of cell sap, probably owing to co-precipitation, and results obtained with this fraction appear to reflect the situation in whole sap [22].

The presence of elongation factors bound to ribosomes can influence the behaviour of these particles in cell-free systems. Hence salt washing removes the differences in activity between muscle ribosomes from normal and protein deficient rats [14], between spleen ribosomes from control and immunized mice [22] and between ribosomes from fertilized and unfertilized sea urchin eggs [96]. Young and co-workers, extending these studies [97], have found that the salt wash fractions from muscle ribosomes of protein deprived animals show lower activity of both EF-1 and EF-2 than those from normal rats. The pH 5 enzyme preparation from the sap of protein depleted rats is also defective in its capacity to support incorporation by isolated ribosomes, the step affected being subsequent to aminoacylation of tRNA [14]. Young (personal communication) finds that the total cellular EF-2 concentration is lowered in muscle from protein deficient rats, but suggests that this does not necessarily represent a situation where EF-2 becomes rate-limiting for protein synthesis since there is a commensurate fall in the concentration of ribosomes *in vivo*.

In HeLa cells, too, there is a rise in cellular EF-2 content during periods of rapid growth, and fall when growth slows [98]. In addition, this factor, as assayed by diptheria toxin catalysed ADP-ribosylation [99], varies in its distribution in the cell. When conditions favour a high growth rate, much of the cellular EF-2 is found in the cell sap, whereas when protein synthesis is restricted a large proportion of the factor is found in association with 80 S ribosomes [98, 100, 102]. These results tend to imply that the EF-2 in the cell sap has functional significance, in contrast to the suggestion of Gill and co-workers that it represents a surplus [99].

Studies of the elongation promoting activity of cell sap fractions from rat liver, brain and kidney show that the rate-limiting component in these preparations is EF-1 [102]. Increased EF-1 activity probably has regulatory significance in the hypertrophic response of the nephrotic kidney [18]. Another example of the role of EF-1 in a growth response is in the proliferation of mouse spleen cells following immunisation. Stimulation of antibody synthesis results in a large increase in EF-1 activity from initially very low levels [22]. Such

an increase probably occurs under conditions where the rate of peptide chain initiation is also elevated.

2.3. *Ribosomal proteins*

So far we have only considered factors present in the cell sap or bound to ribosomes by salt sensitive links. A recent report by Dice and Schimke [103] however has called into question the concept of "ribosomal" and "supernatant" proteins as distinct categories. These workers, having observed that ribosomal proteins have heterogeneous rates of turnover, found that during incubation *in vitro* considerable exchange of proteins took place between rat liver ribosomes and cell sap. This was true even of proteins which are not dissociated from ribosomes when they are washed in high ionic strength media. Such exchange could provide the basis of a mechanism for regulating ribosomal activity and indeed, Garrison et al. [104] have recently shown that incubation of the relatively inactive ribosomes from *Xenopus* eggs in the presence of cell sap from *Xenopus* liver results in improvement in their subsequent activity in translating poly(U) in a cell-free assay. They propose that the ribosomes acquire a factor from the liver supernatant which assists in the binding of initiation factors.

In yeast it appears that the exchange between ribosomal and sap proteins may be more limited since only 3 out of 80 proteins found in highly purified ribosomes become labelled when yeast cells are incubated in the presence of radioactive amino acids under conditions when no new ribosomal RNA is being made [105].

2.4. *Aminoacyl tRNA synthetases*

These enzymes are normally found in the cell sap as prepared by standard fractionation procedures and are therefor commonly regarded as soluble proteins. However, they can be recovered in salt wash fractions from crude ribosomes [106, 107] and there have been recent demonstrations of their presence, together with tRNA, in a pellet obtained when cell sap is centrifuged at high speed for very long periods [108–111]. These findings suggest the possibility of multi-molecular organization to form aggregates [112]. The involvement of these proteins in regulation of translation appears to be of 3 main types:

i) Changes in the patterns of protein synthesis during growth and development are accompanied by changes in the spectrum of activity of synthetases specific for different amino acids. An example of such an effect is the increased level of seryl-tRNA synthetase in the liver of laying hens compared to immature birds [113]. It is probable that this response is an adaptation to the synthesis of the serine-rich egg-yolk protein, phosvitin. There is evidence that multiple tRNA synthetases corresponding to different isoaccepting forms of tRNA exist in differentiated tissues, with a different spectrum in each tissue [114]. High activity of most synthetases, together with a different spectrum has been observed in a fast growing ascites hepatoma in comparison with slower growing Morris hepatomas or normal liver [115]. tRNA and synthetase fractions from late stage pupae, but not from early stage pupae, will support synthesis of adult cuticular protein by ribosomes from *Tenebrio* pupae of either stage [106]. There appear to be changes in species of tRNA and the specific synthetase which develop in this insect as pupation proceeds.

ii) Activity of synthetases varies in concert with the general rate of protein synthesis in response to changes in metabolic state. Diabetes, which lowers the rate of protein synthesis *in vivo* in muscle [116, 117] also lowers the ability of rat muscle cell sap to charge exogenous tRNA with phenylalanine or with an amino acid mixture [20, 21] and reduces the activity of several synthetases in rabbit liver [118]. Muscle cell sap from potassium depleted rats has a reduced ability to charge tRNA with leucine [13]. Conversely the increase in protein synthesis in *Xenopus* liver following oestrogen induction of egg yolk proteins is accompanied by increased activity of at least 4 aminoacyl tRNA synthetases [119].

iii) Synthetase activity rises in order to converse amino acids for protein synthesis during periods of protein of amino acid deficiency. In *E. coli*, starvation for certain amino acids results in derepression of synthesis of the corresponding synthetases [120]. A similar phenomenon has been observed in exponentially growing yeast for 12 out of 19 synthetases [121] and, for valyl-tRNA synthetase at least, appears to be due to regulation of synthesis of this enzyme [122]. Protein deficiency in the intact rat likewise results in an increased activity of hepatic aminoacyl tRNA synthetases [123, 124] but this does not occur in muscle [124, 125]. This may be correlated with the fact that muscle, unlike liver, exhibits a pronounced decrease in protein

synthesis rate in response to protein deficiency [126, 127].

2.5. *Ribonuclease and ribonucleare inhibitor*

Ribonucleases occur in all cells Endonuclease activity appears to be of 2 types, known as acid ribonuclease (active at pH 5.8) and alkaline ribonuclease (active at pH 7.3) [128]. Most studies have been carried out on the latter type, the activity of which is to some extent counteracted in the cell by the existence of a protein inhibitor [128, 129]. The assayable ribonuclease in cell extracts is therefore greater if the inhibitor present in the homogenate if first inactivated by *p*-chloromercuribenzoate to reveal the "latent" activity.

During fractionation of liver cells it has been shown that most of the inhibitor is recovered in the cell sap and the ribonuclease remaining bound to the microsomal and ribosomal fractions is no longer inactivated by it [130]. In other tissues where ribonuclease is especially active this often leads to difficulties in the isolation of intact polysomes [131].

Activities of both ribonuclease and its inhibitor have been shown to respond to various changes in cellular physiology and it is probable that the balance between these opposing factors is an important influence in controlling levels of cytoplasmic RNA *in vivo* [132]. Increases in cellular protein synthesis or in activity of subcellular components are often associated with decreased ribonuclease activity and/or increased ribonuclease inhibitor activity, and vice versa, as is seen from examples of responses to changing nutritional state [133–136], hormones [137–142], partial hepatectomy [128, 143, 144], hepatic carcinogenesis [145] and other conditions [146–150].

Hunter and Korner [151] have found that, in addition to the endonuclease which is counteracted by the inhibitor, rat liver contains an exonuclease, which attacks RNA from the 5′ end liberating nucleoside 3′ triphosphates. Messenger RNA may be vulnerable to this kind of degradation, and it is probable that the action of this enzyme imposes a severe limit on the activity of cell-free systems from both liver [151] and brain [152] by removing 5′ initiation sequences *in vitro*. It is possible that this exonuclease activity may be analogous to the ribonuclease V of *E. coli* [153].

3. Concluding comments

Considering the diverse changes in the activities of the various soluble proteins which we have described here it is at present difficult to assess the relative importance of all these factors in regulation of cellular function. Furthermore we have not considered the roles which non-protein components of eukaryotic cells may play in translational control. Both tRNA and mRNA occur in the cytoplasm in soluble forms and the concentrations of these molecules of other kinds of RNA [5, 6] may have regulatory significance for either specific or general protein synthesis under some conditions. Additional factors which have been implicated in the control of protein synthesis include amino acids [8], ATP [30, 154], haemin [54, 155], cyclic AMP [156] and glutathione [157]. A more precise knowledge of the functions which all these molecules perform and the mechanisms by which they act awaits further development of *in vitro* systems which reflect as closely as possible the behaviour of the intact cell. Such systems will help to elucidate the numerous interactions which undoubtedly occur between soluble components and the ribosome located protein synthetic machinery.

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