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Translation and the cytoskeleton: a mechanism for targeted protein synthesis

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Abstract

This review describes the critical evidence that in eukaryotic cells polyribosomes, mRNAs and components of the protein synthetic machinery are associated with the cytoskeleton. The role of microtubules, intermediate filaments and microfilaments are discussed; at present most evidence suggests that polyribosomes interact with the actin filaments. The use of non-ionic detergent/deoxycholate treatment in the isolation of cytoskeletal-bound polysomes is described and the conclusion reached that at low salt concentrations this leads to mixed preparations of polysomes derived from both the cytoskeleton and the endoplasmic reticulum. At present the best approach for isolation of cytoskeletal-bound polysomes appears to involve extraction with salt concentrations greater than 130 mM after an initial non-ionic detergent treatment. Such polysomes appear to be enriched in certain mRNAs and thus it is suggested that they are involved in translation of a unique set of proteins. The evidence for mRNA localisation is presented and the role of the cytoskeleton in transport and localisation of RNA discussed. Recent data on the role of the 3′ untranslated region in the targeting of mRNAs both to particular regions of the cell and for translation on cytoskeletal-bound polysomes is described. The hypothesis is developed that the association of polysomes with the cytoskeleton is the basis of a mechanism for the targeting of mRNAs and the compartmentalizaton of protein synthesis.

Abbreviations: CBP = cytoskeletal-bound polysomes; FP = free polysomes; MBP = membrane-bound polysomes; ER = endoplasmic reticulum

Introduction

The targeting of newly synthesized proteins to their site of function plays a major role in cell organization. This is partly achieved by signals within the polypeptide chains [1] and in the case of membrane and secreted proteins the first step in targeting involves direction of mRNA-ribosome complexes to the endoplasmic reticulum and synthesis of the proteins on a specific class of membrane-bound polyribosomes [2]. However, in addition the asymmetric distribution of non-membrane protein mRNAs has been shown to occur in a variety of situations [3, 4] and this specific location of mRNAs may provide a further mechanism for protein targeting. Such site-specific translation requires mechanisms for selective segregation and targeting of specific mRNAs to particular compartments. In addition the highly organised nature and high protein content of the cytoplasm restrict diffusion of large molecular complexes such as polyribosomes [5] and therefore there is also a requirement for a mechanism to transport the mRNAs as ribonucleoprotein complexes or as polyribosomes (polysomes). It has been suggested for a number of years that mRNAs and polysomes are associated with the cytoskeleton (a complicated network consisting of microfilaments, intermediate filaments, microtubules and the microtrabecular lattice), and this association could provide the biochemical basis of mechanisms for the transport and targeting of mRNAs to different subcellular sites by providing the transport mechanism and/or localised attachment sites which could be the basis of a variety of different 'compartments' of polysomes.

The aims of this review are threefold: to describe

the evidence for an association of the translational apparatus with components of the cytoskeleton, to discuss the nature of the interaction and to describe possible physiological functions of such an association, particularly in relation to the spatial organization of translation.

Polysomes in the cell matrix

Treatment of cultured eukaryotic cells with low concentrations of non-ionic detergents such as Nonidet-P40 or Triton X-100 causes release of soluble cellular components and plasma membrane components but leaves the cytoskeleton as an insoluble matrix, commonly referred to as the cell matrix [6]. A great many studies have shown that components of the translational apparatus are retained in the cell matrix. Electron microscopy and sucrose gradient analysis have shown that such cell matrix material from a variety of cell lines contains polysomes, translation initiation factors and approximately 70% of the cellular mRNA [7–9] and on the basis of such data it was suggested that the majority of cell polysomes were associated with the cytoskeleton. Evidence that such retention of polysomes in the cell matrix is at least partly due to an association with the cytoskeleton has been obtained by a series of immunohistochemical and histochemical studies showing ribosomal material [10, 11], mRNA 5′ cap binding protein [12], or initiation factors [13–15] to be localised within cells in a pattern which closely resembles that of the cytoskeletal network; for example, as shown in Fig. 1, staining of the cell matrix from 3T3 fibroblasts with antibodies recognizing the 60S ribosomal subunit shows linear arrays of punctate or beaded patterns consistent with a ribosome-cytoskeleton interaction. Furthermore, the combination of *in situ* hybridisation and electron microscopy has shown mRNA species coding for actin, tubulin and vimentin to be clustered around filaments in the cell matrix [16]. There is thus considerable evidence that mRNA, initiation factors and other polysome components are retained in the cell matrix; furthermore at least a proportion of the polysome complexes appear associated or co-localised with cytoskeletal components.

It is important to note that in many studies it has been assumed that all the polysomes in the cell matrix are associated with the cytoskeleton and those polysomes released from the matrix by deoxycholate have been refered to as cytoskeleton-associated polysomes. However it is now apparent that the non-ionic detergent insoluble cell matrix contains membranous fragments of the unsolubilised endoplasmic reticulum [17,18] and therefore it is probable that in addition to cytoskeletal-bound polysomes the matrix also contains membrane-bound polysomes associated with the endoplasmic reticulum. Since it is known from the classical studies of Blobel and colleagues that deoxycholate causes release of polysomes from the endoplasmic reticulum it is likely that the so-called cytoskeletal polysome fractions produced by treatment of the cell matrix with deoxycholate produces a mixed population of cytoskeletal and membrane-bound polysomes [3]. There are several pieces of experimental evidence which indicate that this is indeed the case; firstly, the cell matrix has been shown to contain mRNAs for membrane proteins [19], which would be expected to be translated on the endoplasmic reticulum; secondly depolymerization of actin filaments with cytochalasin D causes release from the cell matrix of some mRNAs but not of those for membrane proteins [20]; thirdly, the mRNA for the membrane protein β2-microglobulin is present in polysomes released from the microfilament-depleted cell matrix by deoxycholate [21]. Therefore, as discussed in detail elsewhere [3], it appears that polysomes released by double-detergent treatment following non-ionic detergent treatment at low salt do not originate solely from the cytoskeleton, as assumed by most investigators, but also from the rough ER.

Critical evidence for an association of polysomes with the cytoskeleton

Although the early experiments on polysomes released from the cell matrix were seminal in suggesting that some of the cell's polysomes were associated with the cytoskeleton [7–9], as discussed above such data, on their own, do not provide conclusive evidence for an association of polysomes with the cytoskeleton. However data from *in situ* hybridisation and histochemistry showing co-localisation of ribosomes and other components of the translational apparatus with cytoskeletal components in the cell matrix provides strong evidence for an association of some polysomes with the cytoskeleton. Although this co-localisation could theoretically be due to an artefact of fixation or detergent treatment, several lines of evidence suggest that this is not the case: the large amount of the soluble enzyme lactic dehydrogenase released by non-ionic detergent [18] suggests that non-specific trapping of mRNA

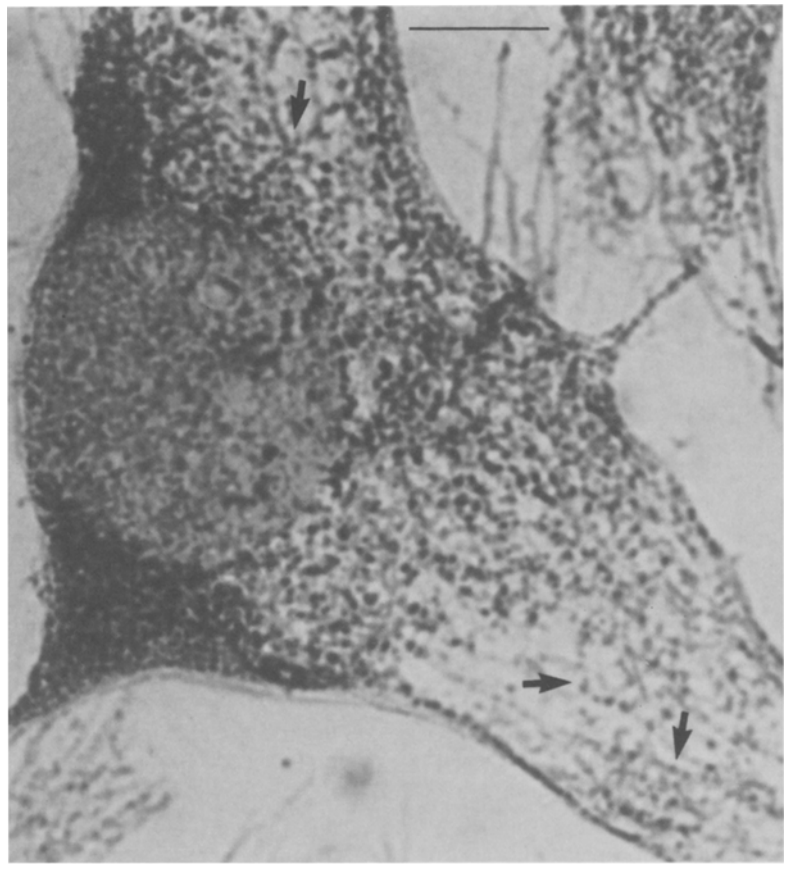

Fig. 1. Ribosome distribution in the cell matrix of 3T3 fibroblasts. 3T3 fibroblasts were treated with buffer containing 0.05% Nonidet before fixation and subsequent incubation with antibodies raised against 60S ribosomal subunits [11]. The distribution of ribosomes is shown by the peroxidase staining; note the linear arrays of punctate labelling (→). Bar, 10μm.

or ribosomes during extraction is unlikely and this is supported by both gel fitration experiments which showed no evidence of cytoskeleton-polysome interactions in the presence of non-ionic detergents [22] and by the lack of trapping of exogenous mRNA during cell matrix separation from *Xenopus* oocytes [23]. Furthermore, *in situ* hybridisation studies of ascidian eggs have shown that non-ionic detergent treatment does not affect the gross intracellular distribution of either total or specific mRNAs [24]. Critically, recent experiments using *in situ* hybridisation techniques have shown that polyA-containing mRNA co-localises with cytoskeletal elements in fixed, unextracted cells [25]. Further important data has come from high resolution structural studies using high-voltage and deep-etch electron microscopy which show the presence of polysome-like structures close to or surrounding filamentous structures [26,27].

Critical evidence for an association of polysomes with the cytoskeleton has also come from experimental treatments which alter cytoskeletal integrity. Pretreatment of cells with cytochalasins produces depolymerization of actin and a collapse of the microfilament network. These compounds also cause a redistribution of the protein synthetic apparatus such that there is a loss of polysomes from the cell matrix and an increase in the proportion of polysomes recovered in the soluble fraction [18,28-30], thus indicating that actin depolymerization is associated with polysome redistribution. Similar effects have been observed after actin depolymerization induced by DNAase I [22], and after treatment of the cell matrix with 130 mM KCl, which causes actin depolymerization and also redistribution of polysomes so that there is loss from the cell matrix and increased recovery in the soluble fraction [18, 31].

Taken together these biochemical, immunohis-

tochemical and morphological observations strongly indicate an association of polysomes with the cytoskeleton. However estimates of the extent of cytoskeleton-polysome interactions vary greatly. Originally it was reported that 70% of cellular mRNA was present in the cell matrix [7–9] and recent data using *in situ* hybridisation has shown as much as 85% of polyA-containing mRNA to be retained after Triton treatment [25]. Since, as discussed above, the polysomes or mRNA retained in the cell matrix are associated with both the endoplasmic reticulum and the cytoskeleton, it is likely that these figures reflect RNA associated with both structures. However, using sucrose gradient analysis after salt [18, 31] or cytochalasin [29] treatment to release specific cytoskeletal-bound polysomes it has been estimated that some 35–45% of polysomes are associated with microfilaments. This proportion changes under different physiological conditions [31, 32]. In some studies cytochalasins have been shown to cause release of over 80% of the cell matrix mRNAs [25, 28] and presumably this reflects either a small ER compartment or an effect of cytochalasins on the integrity of the ER; since these mRNAs were not necessarily in polysome complexes it may also relect release of untranslated mRNAs. On the other hand salt or cytochalasin treatment may give an underestimate of the proportion bound to the cytoskeleton because it will not take into account any polysomes that may be associated with microtubules or intermediate filaments. Bearing in mind such caveats, the best estimate at present available is that approximately 35–45% of polysomes are associated with the cytoskeleton.

Isolation of cytoskeletal-bound polysomes

Detailed study of the nature and function of the polysome-cytoskeleton interactions requires the isolation of specific cytoskeletal-bound polysomes (CBP); for example analysis of the mRNAs in CBP may define whether CBP represent a distinct polysome compartment or a transport phenomenon, and studies with specific mRNAs will allow dissection of the molecular mechanisms involved in the interaction. As discussed above and in [3] the majority of attempts to isolate such a fraction has involved release of polysomes from the cell matrix with Triton/deoxycholate and this produces a fraction which includes polysomes derived from the endoplasmic reticulum.

Some success at releasing cytoskeletal-bound polysomes from the cell matrix has been achieved using 100–200 mM KCl. Salt concentrations above 100 mM have been shown to cause both cytoskeletal disorganization [7], actin depolymerization [31], progressive loss of RNA [30] and release of polysomes [31]. In myoblasts the salt treatment produced a fraction which contained specific mRNAs [30]. 130 mM KCl treatment of the cell matrix was shown to release a fraction which differed in polysome profile and which was enriched in actin and thus appeared to be a cytoskeletal fraction [18, 31]; furthermore the polysome content of this fraction was depleted by pretreatment of the cells with cytochalasin. Thus salt treatment of the cell matrix appears to produce a fraction enriched in microfilament-associated polysomes; nonionic detergent treatment followed by 130 mM KCl treatment of the cell matrix and the extraction with deoxycholate produces fractions of free, cytoskeletal-bound and membrane-bound polysomes. At present such procedures, based on the KCl-induced cytoskeleton disorganization, provide the best approach to isolate CBP; it should be noted however that optimal salt and detergent concentrations for separation of the polysome fractions vary between cell lines and it is therefore vital to fully characterize a putative CBP fraction. However this method has limitations, particularly since the precise compartment from which these polysomes are released has not been well-defined and it is not clear if such fractions represent polysomes from a distinct microfilament-associated compartment, from several such compartments or from a number of heterogeneous cytoskeletal-bound polysome populations; salt extraction may not release all the actin linked polysomes or any polysomes associated with other cytoskeletal filaments. Another potential problem is that of cell damage during the initial stages of fractionation, particularly if attached cells are removed by scraping; under such circumstances damage to the cytoskeleton (particularly in the cell periphery) could lead to altered polysome and mRNA distribution. Recently an attempt has been made to isolate polysome fractions by treatment of the cells *in situ* [33]; such an approach, if combined with the sequential non-ionic detergent, salt and deoxycholate extraction procedure [18] may improve the degree of fractionation.

Which cytoskeletal components are associated with polysomes?

The majority of experimental evidence indicates an association of polysomes or mRNAs with the actin-

containing microfilaments. Electron microscopy of lens cells shows polysomes associated with microfilaments [29] and in fibroblasts ribosomes [10, 11], mRNA-binding proteins [34] and initiation factors [14, 15] co-localise with actin filaments. These studies show close co-localisation with part of the actin network. However, not all filaments have polysomes associated with them [15] and this is also evident in *in situ* hybridisation studies of total mRNA distribution where digital imaging microscopy shows close association of mRNAs with some but not all actin filaments [25]; it appears that it is the finer actin filaments, not the stress fibres, that are associated with mRNA.

Further evidence for microfilament-polysome/mRNA association has also come from the use of agents which induce filament depolymerisation or stabilise microfilaments: thus cytochalasins B or D, which induce depolymerization, release polysomes and mRNAs from the cell matrix; DNAase I, which also induces depolymerization, also causes release of polysomes [22] whilst phalloidin, which stabilises actin filaments, prevents the loss of actin and polysomes [18, 22]. Finally, the fact that extraction of the cell matrix with 130 mM salt (known to affect microfilament stability but not that of intermediate filaments) leads to concomitant loss of actin and polysomes from the cell matrix [11, 18, 31] also indicates the association of polysomes with the microfilament network.

Since most biochemical studies of polysome-cytoskeleton interactions have been done under conditions (4 °C) when many, but not all, microtubules depolymerize such procedures would be unlikely to detect polysomes associated with microtubules; thus it is unlikely that polysomes found in the cell matrix are associated with microtubules and the lack of effect of colchicine on polysome distribution in cultured cells [7, 18] may reflect the fact that the majority of microtubules would have been depolymerised by cold in these experiments.

However, there is evidence from electron microscopy [35] and *in vitro* observations for an association of ribosomes and RNA with microtubules, particularly with the mitotic spindle and microtubule organizing centres (reviewed in [36]). Furthermore the microtubule-associated protein tau has been reported to be present in brain polysomes [37] but the significance of this and other *in vitro* observations is unclear since the specificity of the interactions between polysomes and microtubule proteins remains to be established [36]. Importantly, insect nutritive tubes have been shown by electron microscopy to contain large numbers of ribosomes associated with microtubules [38]. These cells are carrying out a highly specialised transport function and it may be that the ribosome-microtubule interaction is due to a microtubule role in transport of the ribosomes. Interestingly a role of microtubules in mRNA transport has also been suggested by inhibitor studies in *Xenopus* oocytes which indicate that microtubules are required for translocation of the Vg1 mRNA to the cortical cytoplasm of the vegetal half of the egg but microfilaments are involved in anchoring the mRNA in some way, so that its distribution is restricted to this specific part of the cytoplasm [39]. In *Drosophilia* oocytes there is also good evidence that localisation of specific mRNAs depends upon microtubules [40, 41].

At present there is no evidence for any association of polysomes with intermediate filaments. Indeed the available experimental data suggests no relationship between intermediate filaments and translation: polysomes are found in areas of the cytoplasm which are free of intermediate filaments [4, 7] and in cells which lack the intermediate filament protein vimentin, initiation factor eIF2 is still associated with filaments [13]. However there is evidence for an association of mRNA with such filaments; the 50 kDa cap-binding protein appears to co-localise with IF [12]; in oocytes the Vg1 mRNA is recovered in a cytokeratin/vimentin-enriched fraction [23] and ribonucleoprotein particles termed prosomes are co-localised with the IF network in cultured cells [42]. It is possible, therefore, that untranslated mRNAs, as opposed to polysomes, may be associated with IFs.

In conclusion there is a strong body of evidence which indicates that polysomes are associated with microfilaments. It is possible, although more speculative, that intermediate filaments interact with untranslated mRNAs, that some polysomes may be associated with microtubules, particularly the mitotic spindle, and that microtubules may be involved in ribosome/mRNA transport (see Fig. 2).

What is the function of cytoskeletal-bound polysomes?

The failure in early experiments to extract polysomes using non-ionic detergents at low salt concentrations led to the suggestion that free polysomes (FP) do not exist and that an association of the ribosome-mRNA complex with the cytoskeleton was essential for trans-

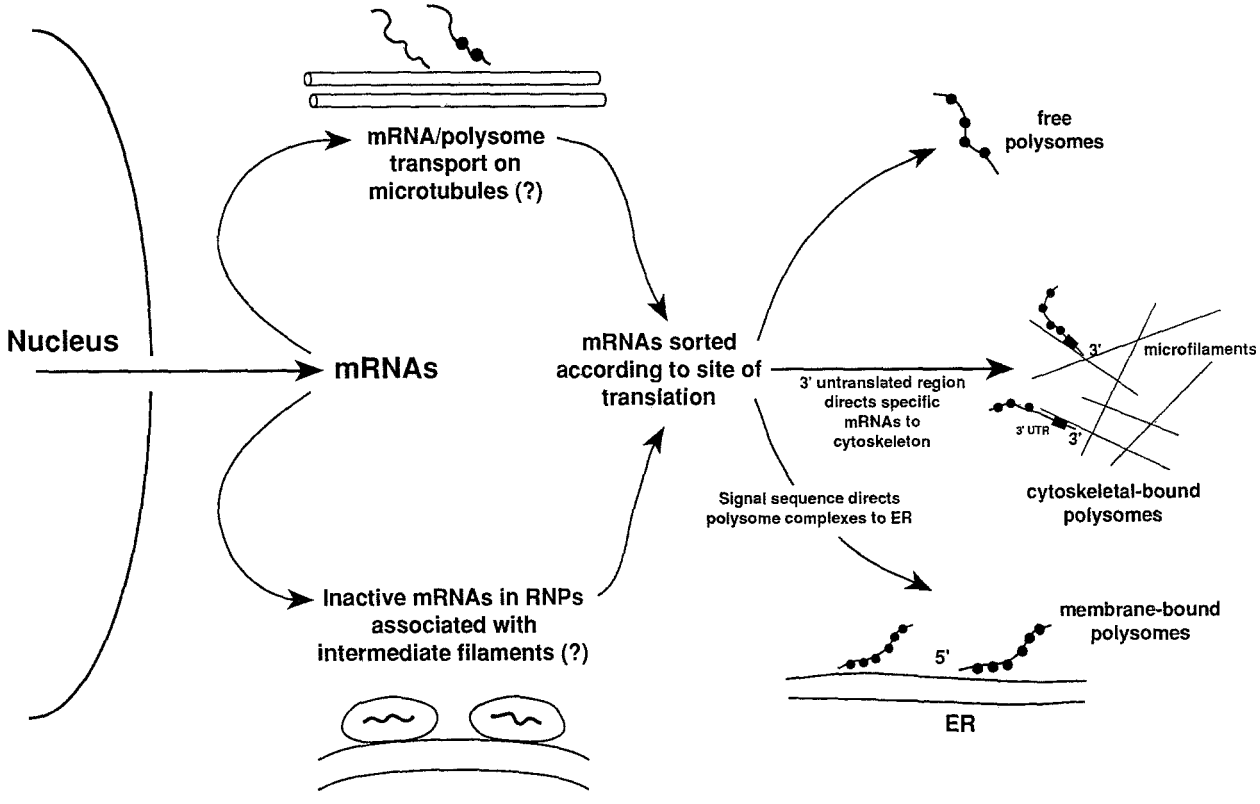

Fig. 2. A scheme illustrating the possible roles of the cytoskeleton in the transport and localisation of mRNAs, particularly targeting of mRNAs to the cytoskeleton by the 3′UTR. ER, endoplasmic reticulum; 3′UTR, 3′ untranslated region; RNP, ribonucleoprotein particle.

lation to occur [7–9]. However further experiments on a variety of different cell lines has shown that non-ionic detergent treatment does release soluble components such as lactic dehydrogenase and polysomes [3, 18, 29, 30, 43–45]. Analysis of polysome profiles suggests that some 20–40% of polysomes are in the 'free' cytosolic fraction and, furthermore, these polysomes have a different profile from those retained in the cell matrix; *in vitro* translation data suggests that they synthesise different proteins [18]. More recently in transfected fibroblasts we have found polysomes released by non-ionic detergent to be enriched in the mRNA for β-globin suggesting that in these cells at least the free polysomes are involved in the translation of specific mRNAs [46]. The accumulated data suggests that a proportion of polysomes are recovered in the cytosolic fraction and that these are distinct from those retained in the cell matrix; therefore the association of the translational complex with the cytoskeleton is not a prerequisite for translation.

However the association seems to be of physiological significance because the extent of interaction, as judged by the proportion of polysomes associated with the cytoskeleton, varies with physiological conditions. Thus in ascidians oocyte fertilisation is associated with an increase in the proportion of polysomes/mRNA recovered in the cell matrix [47]. In fibroblasts the rapid stimulation of protein synthesis by insulin occurs concomitantly with a 20% increase in the proportion of polysomes which are co-extracted with actin by 130 mM KCl, and thus proposed to be associated with the microfilaments [31]; this redistribution occurs under conditions where it is known that the stimulation of protein synthesis occurs by an activation of the existing protein synthetic machinery rather than by synthesis of new RNA [48]. Similarly in ascites cells insulin both stimulates protein synthesis and causes a redistribution of ribosomes between the FP, CBP and MBP populations [32]. In contrast, in virus infected cells where host protein synthesis is shut down, viral but not the host mRNAs are associated with cytochalasin B releasable, i.e. microfilament-associated, polyribo-

somes [49]. Following attachment of Krebs II ascites cells to the substratum there is both a reorganization of the cytoskeleton and an increase in the proportion of polysomes recovered in the CBP fraction [50]. In skeletal muscle there is evidence for an association of ribosomes with the myofibrillar apparatus and the distribution of ribosomes between the myofibrillar and subsarcolemmal compartments changes with age and during hypertrophy [51, 52]. These conditions are also associated with different rates of protein synthesis; for example the proportion of ribosomes in the myofibrillar compartment falls between 14 and 51 days of age in the rat, a period during which it is known that both total muscle protein synthesis and actomyosin synthesis also decrease. We can conclude, therefore, that changes in protein synthesis are associated with parallel changes in the extent that ribosomes/polysomes are associated with microfilaments or myofibrils. During activation of protein synthesis in ascites cells the proportion of polysomes associated with the cytoskeleton increases after 1 hour but then decreases after 2 hours, although total protein synthesis has continued to increase [32]. It would appear therefore that increased polysome-cytoskeleton interaction is not part of a mechanism involving activation of synthesis but plays a role in the continual change in the pattern of proteins being synthesised. This would imply that the cytoskeletal-bound polysomes synthesize a distinct set of proteins.

Direct analysis of mRNAs using hybridization techniques, together with the use of salt or cytochalasins to separate CBP from membrane-bound polysomes (MBP), has provided the possibility of analysing the mRNA complement of CBP and thus directly addressing the question of whether CBPs contain different mRNAs and are thus involved in the synthesis of a distinct set of proteins. Results from these types of experiments suggest that polysomes isolated from cytoskeletal fractions are enriched in certain specific mRNAs. Thus in fibroblasts and myoblasts actin mRNA is recovered largely on CBP [21, 30, 43, 53] and in several of these studies the CBP show an enrichment in actin mRNA; in 3T3 fibroblasts and ascites cells this was not the case [21; Campbell, Vedeler, Pryme & Hesketh, unpublished data] and the actin mRNA appeared equally distributed between FP and CBP. Since some actin mRNA is found in the cell periphery [54] this distribution may reflect loss of actin mRNA from the cytoskeleton during cell fractionation; alternatively it may reflect the β and γ actin mRNAs being present in different fractions, a possibility which appears likely in view of recent data showing the mRNAs for the two isoforms to be localised in different areas of the cytoplasm [55]. Analysis of actin isoform mRNAs in FP and CBP may allow a start to be made in defining the relationship between recovery of an mRNA in CBP and its spatial localisation (see below). Histone mRNAs have also been reported to be present in the cell matrix and to be released by cytochalasins [13, 19, 30, 43]. Using sequential extraction with non-ionic detergent at low salt concentration (FP), 130 mM KCl (CBP) and deoxycholate (MBP) c-myc mRNA was found present at greatest enrichment in the CBP [21]. More recent data show that the c-myc mRNA is associated with CBP not only in 3T3 fibroblasts but also in a number of other cell lines such as ascites, MPC11, HepG2 [Campbell, Hovland, Vedeler, Pryme & Hesketh, unpublished observations] and also in L9 fibroblasts transfected with the c-myc gene [46]. It appears that c-myc mRNA is a useful marker for CBP in a variety of cell lines. Interestingly the mRNAs for other nuclear proteins such as fos [19], histones [30] and cyclinA [Campbell, Henglein & Hesketh, unpublished data] also appear to be associated with CBP; the significance of this is unclear but it may be related to a need to retain these mRNAs in the perinuclear cytoplasm.

In summary the bulk of evidence suggests that CBP are enriched in certain specific mRNAs and thus that they are involved in the translation of specific mRNAs. This requires some mechanism to segregate such mRNAs from those translated on the ER (i.e the mRNAs for membrane proteins such as β2-microglobulin and glucose transporter 1) or FP. Since, as described below, there is evidence for spatial segregation of mRNAs within the cell it is possible that there is some functional link between mRNA localisation and cytoskeleton-polysome interaction.

Cytoskeleton, CBP and mRNA localisation

An increasing number of studies indicate that certain mRNAs are localised to specific cytoplasmic regions. Thus in muscle, myosin heavy chain mRNA and ribosomes are associated with the myofibrils [51, 56, 57] and during muscle stretch there is relocalisation of the mRNA to the myotendinous junction [58]. Localisation of mRNAs to specific intracellular sites is also evident in neurones (see [59]) and is particularly well documented in amphibian eggs where, for example, the Vg1 mRNA is localised in the cortical cytoplasm of the vegetal half of the egg [60]. Furthermore the localisa-

tion of this mRNA depends upon the integrity of the cytoskeleton [39] and in *Drosophila* oocytes the *bicoid* and cyclin mRNAs also show specific cytoskeletal-dependent localisation [40, 41]. Using probes which identify all actin isoform mRNAs the actin mRNA has been found to be situated in the motile periphery of the cytoplasm and this localisation is not dependent on nascent peptide chains but on microfilaments [61]. Recent data show that the mRNAs coding for the β and γ actin isoforms are differentially located in myoblasts with only the β isoform mRNA being located peripherally [55]. It would appear therefore that both localisation of specific mRNAs in developing oocytes and the peripheral location of the β-actin mRNA in cultured cells is dependent on information within the mRNA and upon the cytoskeleton.

Recent data from cells transfected with either normal c-myc or chimaeric myc-βglobin gene constructs indicate that both the intracellular localisation of the mRNA and the association of the c-myc mRNA with cytoskeletal-bound polysomes is dependent on the 3′UTR of the mRNA [46]; replacement of the 3′UTR by that of globin results in both targeting of the chimaeric mRNA to free, rather than cytoskeletal-bound, polysomes and relocalisation so that the mRNA is no longer found to be tightly located to the perinuclear cytoplasm. Since these data demonstrate a link between the specific intracellular localisation of the c-myc mRNA to the perinuclear cytoplasm and association with cytoskeletal-bound polysomes they support and extend the above observations on oocytes and on actin mRNA in indicating that not only does the cytoskeleton play a role in mRNA localisation but that the localised mRNA is probably being translated.

The information for correct localisation of certain mRNAs in developing oocytes of amphibians and *Drosophila* is present within the 3′UTR of those mRNAs [62–64] and it has been suggested that there is a consensus sequence which is involved in targeting of a set of mRNAs to a particular location [63]. Experiments with cells transfected with chimaeric constructs in which the β-globin coding sequences are linked to the the c-myc 3′UTR show that the c-myc 3′UTR is sufficient to target the β-globin coding sequences to CBP [46]. Similar experiments with actin isoform 3′UTRs show that the 3′UTR regions of these mRNAs are also capable of directing a reporter sequence to specific locations within fibroblasts or myoblasts [65]. Data on the targeting of reporter sequences by 3′UTR regions, together with that on c-myc localisation discussed above, provides the first evidence that a mRNA targeting mechanism involving the 3′UTR occurs not only in oocytes but also in mature mammalian cells [3, 46]. Thus the picture emerging from these studies is that polysome-cytoskeleton association plays an important role in the compartmentation of translation (see Fig. 2) by targeting of mRNAs to specific intracellular locations so that translation occurs in particular sites. The functional significance of such mechanisms may be obvious in certain specific cases such as oocyte development and the neurone but in other cases such as actin isoform distribution and function it remains to be elucidated.

Nature of the interaction between cytoskeleton and polysomes

Inhibitors of translation such as pactamycin or fluoride, which arrest translation and promote a release of ribosomes from mRNA, fail to release mRNAs from the cell matrix [9, 66, 67] suggesting that mRNAs are retained on the cytoskeleton and on the ER when not being translated and in the absence of intact polysomes. Such data, together with the similar lack of mRNA released after EDTA treatment, suggests that the major polysome-cytoskeleton interaction is through the mRNA. However in the experiments of Howe and Hershey [67] EDTA and ribonuclease caused no loss of ribosomes from the matrix although polysomes were dissociated; thus ribosome association with both the membrane and cytoskeletal elements in the deoxycholate fraction did not require the continued presence of intact mRNA. As depicted in the model shown in Fig. 3, it seems likely, therefore, that after the initial interaction of the mRNA itself with the cytoskeleton there is subsequent binding of the ribosomes, perhaps to a 'receptor' on the cytoskeleton, in a similar manner to that which occurs in the attachment of MBP to ER membranes [68]. Loss of mRNA from the cell matrix after puromycin treatment [25] has also been interpreted as evidence for ribosomes or nascent peptide chains stabilising the mRNA-cytoskeleton interaction. The recent observation that elongation factor 1a may be an actin-binding protein [69] further suggests that polysome-microfilament links may partly occur through interactions which do not directly involve the mRNA.

Not only is it clear that the major interaction between polysomes and the cytoskeleton is through the mRNAs involved but both from oocyte studies and from recent experiments with cells transfected

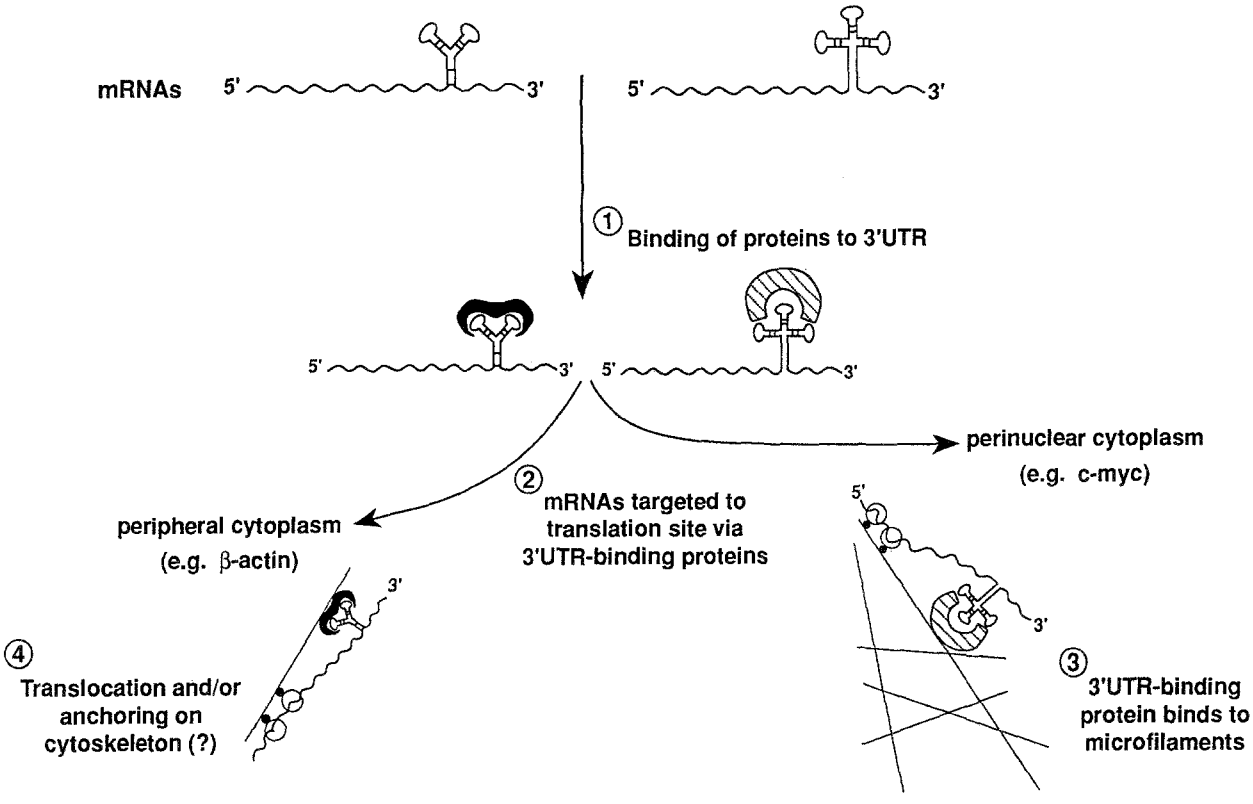

Fig. 3. Cytoskeleton-mRNA interactions: a hypothetical scheme illustrating how specific 3′UTR sequences and different 3′UTR binding proteins could sort mRNAs and target them to different subcellular locations. The two hypothetical mRNAs illustrated are shown to have different localisation sequences in their 3′ untranslated regions (3′UTR); these are shown for simplicity as different secondary structures but there is no evidence for a role of secondary structure. These sequences are shown to bind different proteins (■ and ▨. Different interactions between the 3′UTR-binding proteins and cytoskeletal components are proposed to generate sorting of the mRNAs and targeting to different subcellular locations. In addition polysome-cytoskeleton interactions may be stabilised through the ribosomes, possibly by a putative receptor protein (•).

with chimaeric myc, β-globin or actin gene constructs [46, 65] it appears that the cytoskeleton-polysome or cytoskeleton-mRNA interaction depends on sequences within the 3′UTR of certain mRNAs. The nature of the interaction is not understood at present but it seems likely that it will involve specific sequences and that these will be recognised, either directly or through formation of secondary RNA structure, by specific proteins which bind to the cytoskeleton. Interestingly, recent studies have shown that the cell matrix contains proteins which bind to the β-actin mRNA 3′UTR [70] and such proteins are clearly prime candidates for playing a targeting role. The binding of different proteins to different sequences in mRNAs could provide the basis of the spatial segregation and specific localisation of mRNAs (see Fig. 3). For example, c-myc mRNA is associated with the cytoskeleton and is perinuclear [21, 46] while β-actin mRNA is found in the peripheral cytoplasm and this localisation requires the cytoskeleton [30, 53]; presumably, a mechanism is required which will produce differential mRNA-cytoskeleton interactions and thus specific localisation.

Although the available data indicates that mRNA localisation depends upon both the cytoskeleton and 3′UTR sequences, the details of the transport and localisation mechanisms remain to be defined. It is not clear for example if the mRNA is transported in a RNP particle or in a mRNA-ribosome or polysome complex. At what stage of the transport pathway does polysome formation occur? If transport occurs as polysomes then is translation repressed during transport so as to achieve localised synthesis?

Future perspectives

Further understanding of the physiologcal significance of CBP requires further classification of the mRNAs translated on these polysomes so as to define which proteins are synthesised there. It is essential to improve fractionation techniques and fraction characterization so that the relationship between isolated fractions, different cytoskeletal components and spatial location can be defined. It is important to ascertain whether fractions of CBP are derived wholly from microfilaments or whether other cytoskeletal components are involved and whether CBP represents a spatially restricted compartment(s). Whatever procedures are developed it is crucial that fractions are fully characterised using appropriate protein (actin, cytokeratins, and vimentin for cytoskeleton, lactic dehydrogenase for cytosol), phospholipid (for ER) and mRNA (β2-microglobulin and glucose transporter 1 for ER and c-myc for CBP) markers.

The association of specific mRNAs with the cytoskeleton requires a targeting mechanism. Data from oocytes suggests that this may involve the 3′UTR and recent data from fibroblasts indicates that targeting of specific mRNAs to CBP and to a spatially distinct site both involve the 3′UTR. Thus it appears that translation on CBP may be an important universal mechanism which allows for synthesis to occur in specific locations within the cell; actin in the cell periphery, c-myc in the perinuclear region and other proteins in other specific locations. Such mechanisms are likely to be important in cell organization and metabolic compartmentation but may be particularly relevant in specialised situations such as the myofibril, the neurone or the developing oocyte. The next phase of research will be to identify the 3′UTR sequence(s) involved in targeting and the proteins involving in linking the 3′UTR to the cytoskeleton. The association of specific mRNAs with CBP, particularly under conditions where they are introduced by transfection, will provide excellent model systems to study the interaction of mRNA-polysome complexes with the cytoskeleton. When combined with *in situ* hybridization techniques this approach will allow the relationship between cytoskeleton-polysome interactions and the spatial organization of mRNA translation to be described in greater detail.

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