REVIEW ARTICLE

Interaction between mRNA, ribosomes and the cytoskeleton

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INTRODUCTION

A major logistical problem for all cells is how newly synthesized proteins are directed to their appropriate subcellular location. Currently, little is known of how the majority of proteins are targeted to their site of function. In the case of membrane and secretory proteins it is known that the first step in such targeting is achieved by synthesis in a distinct polyribosome (polysome) compartment. It is well documented that membrane and secreted proteins are synthesized on polysomes bound to rough endoplasmic reticulum (ER) membranes (membrane-bound polysomes; MBP) and it has been proposed that the other cell proteins are made on the so-called 'free' cytosolic polysomes (FP) (see reviews by Shires & Pitot, 1973; Svoldal & Pryme, 1980). Thus, mRNAs were envisaged as being segregated into two distinct populations of polysomes depending on the ultimate cellular localization of the encoded protein (the signal hypothesis; see Blobel & Dobberstein, 1975). It has been calculated that some 500–2000 mRNA species, out of a total of 15000 (Adesnik & Maschio, 1981) that are released from the nucleus in liver cells, contain a signal sequence and are so selected for translation on MBP. It has been assumed until recently that the remaining mRNAs, which code for the bulk of cell proteins, are translated on FP. A simple division of protein synthesis between these two polysome populations has been questioned during the past 10 years, however, since evidence has accumulated which suggests that some mRNAs and polysomes are associated with the cytoskeleton (cytoskeletal-bound polysomes; CBP).

The cytoskeleton consists of cytoplasmic networks of microfilaments, intermediate filaments and microtubules together with the postulated microtrabecular lattice. The implications of interactions between the translational apparatus and the filament systems of the cytoskeleton are of considerable significance concerning the organization of protein synthesis. Thus if FP, CBP and MBP synthesize different proteins, then an association of a population of polysomes with the cytoskeleton could be a key feature in the way the cell is able to sort its newly synthesized proteins. Alternatively the association of mRNAs with the cytoskeleton might be important in the transport of mRNA from nucleus to cytoplasm (Agutter, 1988, 1990). The aims of the present review are to evaluate the evidence for the association of components of the translational apparatus with the cytoskeleton (reviewed earlier by Nielsen *et al.*, 1983) and then to discuss what is known about the biochemical nature and physiological significance of such interactions.

EVIDENCE FOR AN ASSOCIATION OF POLYSOMES, mRNA AND INITIATION FACTORS WITH THE CYTOSKELETON

Microscopy and immunohistochemistry

High-voltage techniques of electron microscopy have enabled the detailed structure of the cytoplasmic ground substance to be

visualized (Wolosewick & Porter, 1976) such that it is now appreciated that the cytoplasm is not merely a proteinaceous solution but contains a tight mass of cytoplasmic filaments with organelles packed in between. Examination of intact cells with these techniques often shows ribosomes and polysomes situated close to filaments or even present in pockets at points where filaments cross or join each other (Wolosewick & Porter, 1976, 1979). Such observations provided the first evidence that a class of polysomes appear to be attached to elements of the cytoskeleton.

Further evidence for polysome–cytoskeleton interactions has largely come from biochemical and histochemical studies using detergent-treated cells. Treatment of culture cells with low concentrations of non-ionic detergents such as Nonidet P40 or Triton X-100 causes solubilization of the plasma membrane and a release of cytoplasmic constituents and integral membrane proteins, but leaves the cytoskeleton as an insoluble matrix (Osborn & Weber, 1977). Electron microscopy of the non-ionic-detergent-insoluble material showed the presence of ribosomes (Lenk *et al.*, 1977) and this was confirmed histochemically using Acridine Orange (Fulton *et al.*, 1980). The association of ribosomal material with filamentous structures in such detergent-insoluble matrices was suggested by experiments in which auto-immune serum from lupus erythromatosus patients, which contains anti-ribosomal antibodies, showed aligned punctate labelling (Toh *et al.*, 1980). Electron microscopy of matrix material from lens cells (Ramaekers *et al.*, 1983) and from ascidian eggs (Moon *et al.*, 1983) has shown the presence of ribosomes either attached to, or in close proximity to, filaments; in the case of lens cells these were identified as microfilaments (Ramaekers *et al.*, 1983). Quick-freeze deep-etch techniques combined with electron microscopy have shown clusters of ribosomes attached to filaments in the cell matrix (Heuser & Kirschner, 1980). Staining of 3T3 fibroblasts with anti-ribosomal subunit antibodies (Hesketh *et al.*, 1991a) also shows linear arrays of beaded or punctate patterns of ribosome distribution in non-ionic-detergent-extracted cells, consistent with an association between ribosomes and filamentous structures. Antibodies which recognize either the 5' mRNA cap binding protein (Zumbe *et al.*, 1982) or initiation factor eIF-2 (Heuveljans *et al.*, 1989) label a filamentous component of the cytoskeletal network in detergent-extracted cells.

A comparable situation occurs in skeletal muscle where recent immunohistochemical data with anti-ribosomal subunit antibodies (Horne & Hesketh, 1990a) has shown ribosomes to be present in an organized and repetitive fashion along the myofibrils in a pattern consistent with ribosomes being associated with the myosin-containing A bands. These data are compatible with earlier histochemical studies of RNA distribution in muscle (Clavert *et al.*, 1949). There is thus considerable evidence from immunohistochemical studies with antibodies of widely different specificity that components of the protein-synthetic apparatus either appear to be distributed in a pattern consistent with an

Abbreviations used: ER, endoplasmic reticulum; MBP, membrane-bound polysomes; FP, free polysomes; CBP, cytoskeletal-bound polysomes.

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association with filaments or in some cases actually appear to co-localize with filament structures. This view is strongly supported by recent studies which, by combining high resolution electron microscopy with *in situ* hybridization (Singer *et al.*, 1989), have shown that mRNA species coding for actin, tubulin and vimentin appear to be clustered around filamentous structures in the non-ionic-detergent-insoluble matrix (referred to as the cell matrix throughout this review).

In the majority of the above studies, and in those which have attempted to isolate CBP (discussed below), non-ionic detergent treatment has been used to separate the soluble cell constituents from the insoluble cytoskeleton. A key question, therefore, is whether the retention of polysomes or mRNA within the cytoskeletal network in non-ionic-detergent-extracted cells represents the original distribution in the intact cell or whether it is an artefact induced by experimental procedure. In view of the fact that large amounts of cell protein (Lenk *et al.*, 1977) and virtually all lactate dehydrogenase, a soluble cytoplasmic enzyme (Vedeler *et al.*, 1991) are released by non-ionic-detergent treatment it is unlikely that the ribosomes are retained by some form of non-specific trapping. Gel filtration experiments have shown no evidence for non-specific polysome-protein interactions in the presence of Triton X-100 (Adams *et al.*, 1983) suggesting that artefactual ribosome-cytoskeleton interactions do not occur under such conditions. Furthermore, *in situ* hybridization studies using ascidian eggs have shown that non-ionic-detergent treatment does not affect the gross intracellular distribution of either total or specific mRNAs (Jeffery, 1984); similarly, ribosome distribution in HeLa cells is unaffected by such treatment (Lenk *et al.*, 1977). Studies of specific mRNAs have shown that there is no artefactual trapping of either endogenous mitochondrial

mRNAs or exogenous globin mRNA during the preparation of the cell matrix from *Xenopus* eggs (Pondel & King, 1988). It appears, therefore, that the presence of polysomes and associated factors in the cell matrix reflects the situation in the cell; microscopical and immunohistochemical studies have shown that this is due, in part at least, to an interaction with cytoskeletal structures.

Free, cytoskeletal-bound and membrane-bound polysomes

The first studies using detergent extraction to study so-called 'cytoskeletal-bound' polysomes (Lenk *et al.*, 1977; Cervera *et al.*, 1981; van Venrooij *et al.*, 1981) found that monosomes and ribosomal subunits but very few or no polysomes were released by non-ionic detergent alone. On the basis of these results it was suggested that polysomes do not exist 'free' in the cytosol and that cytoplasmic protein synthesis occurs on ribosomes attached either to the cytoskeleton or to membranes. Subsequent studies (for examples, see Table 1a), however, using similar procedures but for the most part either using different cell lines, or varying salt concentrations during the initial extraction, have shown that in some cases treatment of cells with non-ionic detergent releases up to 40 % of cellular polysomes. These results are also consistent with data both from the original subcellular fractionation experiments where polysomes were recovered in a cytosolic fraction without the use of non-ionic detergent (Blobel & Potter, 1967), and from experiments in which cells were disrupted by nitrogen cavitation (Svardal *et al.*, 1981) (see Table 1b). Furthermore, the polysome profile of the cytosolic fraction obtained from Krebs II ascites cells by nitrogen cavitation was essentially identical to that of the soluble fraction produced by non-ionic detergent

Table 1. Comparison of some techniques used for the isolation of free and 'cytoskeletal-bound' polysomes (a) with those used for separation of free and membrane-bound polysomes (b)

In several instances the combination of non-ionic detergent and deoxycholate used to release polysomes from the rough ER (b) has also been used to release polysomes apparently associated with the cell matrix (a). Free polysomes have been recovered from several cell types. Abbreviations: DOC, deoxycholate; NP-40, Nonidet P-40; KOAc, potassium acetate. In (a), values in parentheses indicate percentage of total cellular polysomes.

(a) Isolation of free and 'cytoskeletal-bound' polysomes

Treatment for release of polysomes in the presence of non-ionic detergent				
Cell type	Initial salt concn. for release of FP	Salt concn./detergent used for release of 'CBP'		Reference
HeLa S3	10 mM-NaCl	10 mM-NaCl/DOC	(100 %)	Lenk <i>et al.</i> (1977)
HeLa S3	10 mM-KCl	10 mM-NaCl/DOC	(> 97 %)	Ornelles <i>et al.</i> (1986)
HeLa S3	10 mM-KCl (20 %)	250 mM-(NH ₄) ₂ SO ₄	(80 %)	Bird & Sells (1986)
HeLa	100 mM-KCl (33 %)	10 mM-NaCl/DOC	(67 %)	Katze <i>et al.</i> (1989)
L6	10 mM-KCl (24 %)	250 mM-(NH ₄) ₂ SO ₄	(76 %)	Bagchi <i>et al.</i> (1987)
Brain	80 mM-KOAc (40 %)	300 mM-KOAc/DOC	(60 %)	Lequang & Gauthier (1989)
Lens	25 mM-KCl (40 %)	25 mM-KCl/ cytochalasin D	(35 %)	Ramaekers <i>et al.</i> (1983)

(b) Isolation of free and membrane-bound polysomes

Initial treatment to give free polysomes		Detergents used to isolate membrane-bound polysomes	Identification criteria	Reference
Method	Salt concn.			
Potter-Elvehjem	25 mM	DOC	Cytosol/RER membranes	Blobel & Potter (1966)
Thomas homogenizer	10 mM	DOC	Cytosol/RER membranes	Attardi <i>et al.</i> (1969)
N ₂ cavitation	25 mM	NP-40/DOC	mRNA/ <i>in vitro</i> translation	Pryme (1989a)
N ₂ cavitation	25 mM	NP-40/DOC	mRNA size differences	Pryme (1989b)
Non-ionic detergent	130 mM	Kyro EOB/DOC	Polysome profiles	Birckbichler & Pryme (1973)
Non-ionic detergent	130 mM	Kyro EOB/DOC	mRNA/ <i>in vitro</i> translation	Pryme <i>et al.</i> (1973)

treatment (Vedeler *et al.*, 1991), indicating that identical amounts of FP could be liberated with or without the use of non-ionic detergent. Finally, results from vaccinia-virus-infected cells have shown that up to 40% of viral mRNAs are associated with polysomes in the free fraction released by gentle treatment with non-ionic detergent (Lemieux & Beaud, 1982). The consensus of most results, therefore, is that free, cytosolic polysomes do exist. However, under some conditions it is possible that the FP fraction may be contaminated with polysomes removed from the cytoskeleton during the isolation procedure. For example, direct comparison of non-ionic detergent extraction and subcellular fractionation has shown that 54% of histone mRNA is recovered in the free (cytosolic) fraction obtained by subcellular fractionation but only 16% in the free (soluble) fraction released by non-ionic detergent (Zambetti *et al.*, 1985).

Using sucrose gradient analysis to separate monomeric ribosomes and subunits from polysomes, Lenk *et al.* (1977) showed that polysomes were retained in the cell matrix. Association with this matrix and subsequent release by a combination of 1% deoxycholate and non-ionic detergent was taken by these and many subsequent authors as sufficient evidence to define such material as being associated with the cytoskeleton (Table 1a). Unfortunately, however, in the majority of these studies the cytoskeletal fraction has been poorly characterized and, furthermore, this methodology is similar to that earlier established for the separation of FP and MBP (Birckbichler & Pryme, 1973; Pryme *et al.*, 1973; Table 1b). It has been known for many years that non-ionic detergents alone are insufficient to release MBP but that the ionic detergent deoxycholate effectively removes this class of polysomes from the ER (see Table 1b). This suggests that MBP are present in the cell matrix and are subsequently released upon treatment with deoxycholate.

Indeed, there is considerable evidence that the so-called 'cytoskeletal-bound' fraction obtained by deoxycholate treatment of the cell matrix contains MBP. This is illustrated for example by experiments in which polysomes were prepared by both subcellular fractionation following Dounce homogenization and by detergent extraction (Zambetti *et al.*, 1985). They showed that 96% of the mRNA for the membrane antigen HLA B7 was found in MBP prepared by subcellular fractionation, but in contrast 84–97% of the same mRNA species was found in the 'cytoskeletal-bound' fraction prepared using the procedure of

Penman and colleagues (Lenk *et al.*, 1977; Cervera *et al.*, 1981). The two different distributions of the HLA-B7 mRNA strongly suggest that the so-called 'cytoskeletal-bound' polysome fraction released by deoxycholate also contained the membrane-bound polysome population. Recent observations on release of specific mRNAs from the cell matrix (Zambetti *et al.*, 1990b) show that mRNAs for membrane-bound proteins are unaffected by cytochalasin D whilst those for histones and *c-fos* are released from the cytoskeleton; this again suggests both CBP and MBP are present in the cell matrix. Furthermore, the mRNA for the membrane protein β_2 -microglobulin is not found in the polysomes released by non-ionic detergent but in those solubilized by deoxycholate (Fig. 1). In addition, immunohistochemical studies using antibodies to ER components have shown that membrane material is retained in the cell matrix (Dang *et al.*, 1983; Mirande *et al.*, 1985) and the presence of ER residues has been confirmed by electron microscopy (Dang *et al.*, 1983; Ramaekers *et al.*, 1983). The presence of labelled phospholipids in the deoxycholate-solubilized cell matrix (Lenk *et al.*, 1977; Vedeler *et al.*, 1991) again demonstrates the presence of membrane material in the so-called 'cytoskeletal fraction'. There is thus considerable evidence that the polysomes released by double-detergent treatment following low salt extraction with non-ionic detergent (Lenk *et al.*, 1977) do not originate solely from the cytoskeleton, as assumed by most investigators, but also from the rough ER.

Fig. 1. Distribution of specific mRNAs between free, cytoskeletal-bound and membrane-bound polysomes isolated from 3T3 fibroblasts

Northern hybridization analysis of β -actin, *c-myc* and β_2 -microglobulin mRNAs isolated from free, cytoskeletal-bound and membrane-bound polysomes. Results are shown from a single experiment and are expressed in arbitrary absorbance units per μ g of RNA loaded. Each hybridization was carried out using the same nylon membrane.

Fig. 2. Polysome sedimentation profiles of fractions isolated from Krebs II ascites cells and 3T3 fibroblasts

Polysome-containing fractions were isolated from Krebs II ascites cells (a, b, c) and 3T3 fibroblasts (d, e, f). Free polysomes were released by NP-40 treatment, cytoskeletal-bound polysomes were extracted from the cell matrix with 130 mM-KCl and membrane-bound polysomes were released by combined treatment with Triton X-100 and deoxycholate. Reprinted from Vedeler *et al.* (1991) with the permission of Kluwer Academic Publishers.

Comparison of the extent of polysome release effected by various extraction conditions suggests that the degree of release during non-ionic detergent extraction is affected by ionic strength and cell type (Table 1a). Furthermore, in ascites cells 30% of polysomes were retained in the cell matrix when the extraction buffer contained 4 mM salt and 15% when the salt concentration was 150 mM (Traub & Nelson, 1982). In experiments using 3T3 fibroblasts it was shown that the extent of polysome release by non-ionic detergent was 40% higher in the presence of 130 mM-KCl compared to 25 mM-KCl (Hesketh & Pryme, 1988). Release of RNA from the cell matrix, as assessed by A_{280} , was shown to be similar at 10 and 20 mM salt but increased as the concentration was elevated to 100 mM (Bird & Sells, 1986). Taken together these data show that polysome release from the cell matrix is critically affected by salt concentration.

Since there is concomitant release of polysomes and actin (Hesketh & Pryme, 1988; Vedeler *et al.*, 1991) together with a loss of microfilaments (Hesketh *et al.*, 1991a) when non-ionic-detergent-treated Krebs II ascites cells or 3T3 fibroblasts are then extracted with 130 mM-KCl it would appear that the salt-extracted polysomes are initially present in a cytoskeletal-associated form. The large amounts of actin and cytokeratin in the salt extract show that this fraction is enriched in cytoskeletal components (Vedeler *et al.*, 1991). Furthermore, pre-treatment of cells with either cytochalasin B or D, which depolymerize microfilaments (Cooper, 1987), has been shown to increase the proportion of polysomes recovered in the non-ionic detergent soluble fraction whilst the proportion in the cell matrix was reduced (Ramaekers *et al.*, 1983; Ornelles *et al.*, 1986). The fact that cytochalasin B reduced the amount of polysomes recovered

Fig. 3. Scheme summarizing extraction procedures for the isolation of polysomes

in the 130 mM salt extract but had no effect on those in the deoxycholate-solubilized fraction indicates that polysomes released by salt originated from the cytoskeleton rather than ER membranes (Vedeler *et al.*, 1991). There is thus biochemical evidence which supports observations from both microscopy and histochemistry that CBP are present in the cell matrix.

Treatment of the salt-extracted cell matrix with deoxycholate and Nonidet P40 leads to further solubilization of membranous material and a simultaneous release of polysomes (Hesketh & Pryme, 1988; Vedeler *et al.*, 1991). Combination of detergent treatment and salt extraction in a sequential manner has allowed the preparation of fractions enriched in FP, CBP and MBP as judged by the distribution of markers such as lactate dehydrogenase (cytosol), actin, cytokeratins (cytoskeleton) and phospholipid content (membranes) (Vedeler *et al.*, 1991). The distinct nature of these fractions is further exemplified by both the different polysome profiles obtained from these fractions (Fig. 2) and by the detection of specific mRNA species in the cytoskeletal and membrane fractions (Fig. 1, and Hesketh *et al.*, 1991b).

The nature of the fractions obtained using different extraction procedures is summarized in Fig. 3. It is clear that interpretation of the identity of the polysomes extracted has often been confused. In particular, the presence of polysomes, mRNA etc. in the cell matrix and their subsequent release by deoxycholate cannot itself be taken as evidence for an association with the cytoskeleton. However, such deoxycholate-derived polysomes do not originate entirely from the ER and there is now strong evidence that a population of polysomes is indeed associated with the cytoskeleton. It is crucial that in future work different polysome fractions are well characterized either using markers for soluble, cytoskeletal and membrane compartments or, preferably, DNA probes for specific mRNA species.

Association of initiation factors and mRNAs with the cytoskeleton

Polysomes contain a number of protein factors which are intimately involved in the initiation of translation and subsequent elongation of the peptide being synthesized. Increased proportions of initiation factors eIF 2, 3, 4a and 4b were found in the soluble fraction after cells were treated with cytochalasin B, suggesting that these were initially associated with CBP (Howe & Hershey, 1984). As discussed by those authors, the remaining factors present in the deoxycholate fraction were probably derived from the rough ER. In addition, eIF1 has been shown by DNA sequencing and functional analysis to be an actin-binding protein (Yang *et al.*, 1990) and this is interesting in the light of an earlier, but unconfirmed, report that eIF2 kinase is a proteolytic product of spectrin, another cytoskeletal protein with actin-binding properties (Hardesty *et al.*, 1987).

Approx. 70 % of cellular mRNA has been recovered in a so-called 'cytoskeletal-bound' form either retained in the cell matrix or released from such structures by Triton X-100/deoxycholate treatment. This has been observed in a variety of cells such as HeLa cells (Lenk & Penman, 1979; van Venrooij *et al.*, 1981), myoblasts (Bag & Praminik, 1987; Meadus *et al.*, 1990), virus-infected cells (Lenk & Penman, 1979; Cervera *et al.*, 1981; van Venrooij *et al.*, 1981; Lemieux & Beaud, 1982; Bonneau *et al.*, 1985; Katze *et al.*, 1989) and oocytes (Moon *et al.*, 1983; Jeffery, 1984; Yisraeli & Melton, 1988). However, as with the analysis of polysome distribution, the main limitation of these experiments has been the definition of the insoluble cell matrix as a cytoskeletal fraction and any mRNAs associated with it or with material released by Triton X-100/deoxycholate treatment as cytoskeletal-bound. As discussed in the previous section, such fractions are heterogeneous and in many cases therefore it is not possible to

draw definite conclusions regarding an association of mRNA with the cytoskeleton.

The use of cytochalasin B or D to release microfilament-associated material from the cell matrix has provided one approach which allows one to distinguish cytoskeletal-associated material from that bound to the ER. Thus, using hybridization to poly(T) as an assay for total cellular mRNA, Ornelles *et al.* (1986) showed that pretreatment of cells with cytochalasin D released about 80 % of mRNA from the cell matrix. In HeLa cells such treatment causes release of *c-fos* and histone mRNA from the cell matrix (Zambetti *et al.*, 1990b). Similarly, cytochalasin B treatment of *Xenopus* oocytes caused the release of the specific Vg1 mRNA (Yisraeli & Melton, 1988), whilst in virus-infected cells it liberated polysomal-associated, i.e. actively translated, mRNA from the cell matrix (Lenk & Penman, 1979). Further evidence for the presence of specific mRNA species on the cytoskeleton has come from work in which fractions enriched in cytoskeletal components have been prepared from the cell matrix and shown to be highly enriched in mRNA coding for either Vg1 (Pondel & King, 1988) or *c-myc* (Hesketh *et al.*, 1991b; Fig. 1). Salt extracts of the cell matrix from HeLa cells and L6 myoblasts have been shown to contain specific mRNA species. Thus in HeLa cells considerably more actin mRNA is present in the matrix extract than in the non-ionic-detergent-soluble fraction, while the opposite is true for the histone mRNA (Bird & Sells, 1986); in myoblasts, mRNAs for actin, ribosomal protein L32, and histone were all recovered in the salt extract of the cell matrix rather than the soluble fraction (Bagchi *et al.*, 1987). Although no evidence was presented which indicated that these extracts contain cytoskeletal proteins, the known effect of salt on microfilaments in the cell matrix suggests that they may represent cytoskeletal fractions. This appears to be the case in HeLa cells where pretreatment with cytochalasin B was found to cause a redistribution of actin mRNA so that it was recovered in the soluble rather than in the 'cytoskeletal' fraction (Bird & Sells, 1986). The situation is less clear in myoblasts where cytochalasin had no effect on the distribution of several mRNAs although it caused a total loss of microfilaments (Bagchi *et al.*, 1987).

Important evidence for the presence of mRNAs on the cytoskeleton has recently come from *in situ* hybridization studies at the electron microscopy level; this work has shown that the mRNA for actin is localized in close proximity to filaments (Singer *et al.*, 1989). In conclusion, the results described above provide evidence for the presence of mRNAs in some form of association with the cytoskeleton. In some studies the associated mRNAs have been found to be present in polysomes and are therefore being actively translated (Bird & Sells, 1986; Bagchi *et al.*, 1987).

During virus infection the viral mRNAs appear to be associated with the cell matrix (Lenk & Penman, 1979; Cervera *et al.*, 1981; van Venrooij *et al.*, 1981) and Lenk & Penman (1979) suggested that attachment of mRNA to the cytoskeleton was a prerequisite for translation to occur; this hypothesis was based on the fact that in virus-infected guanidine-treated cells there was a release of uridine-labelled mRNA from the cell matrix and neither viral nor host mRNAs were translated. However, since the cell matrix also contains residual ER material, release from the cell matrix may be due to release of mRNAs for viral glycoproteins from ER membranes. Furthermore, since infection with cytomegalovirus causes dramatic changes in the organization of the cytoskeleton (Jones & Kilpatrick, 1987) it is possible that some of the observed effects of virus infection may reflect a release of mRNAs due to breakdown of the cytoskeleton rather than a regulatory mechanism involved in translation. This is supported by observations which show that in both adenovirus and influenza virus infection,

where disruption of the cytoskeleton does not occur, the host mRNAs are retained on the cytoskeleton (Katze *et al.*, 1989). There is also evidence to suggest that during viral infection there is retention of some host mRNAs, such as those for actin and tubulin, in the cell matrix fraction even though these are not translated (Lemieux & Beaud, 1982; Bonneau *et al.*, 1985; Katze *et al.*, 1989). It is likely that these two mRNAs are cytoskeletal-bound even during viral infection without being actively translated.

In studies on myocytes the mRNA for a P40 protein has been found to be present in the cell matrix isolated from both myocytes and myotubes, although it is only translated in the myoblasts (Meade *et al.*, 1990); however, such a mRNA species may be either cytoskeletal or membrane-bound. Further evidence for attachment of mRNA in the absence of ribosomes comes from experiments with inhibitors of protein synthesis such as fluoride and pactamycin which arrest translation and promote a release of ribosomes from mRNA. Both these compounds, and EDTA, which cause ribosomal subunits to dissociate and separate from the mRNA, do not release mRNA from the cell matrix (van Venrooij *et al.*, 1981; Bag & Pramanik, 1987); the total lack of mRNA release in these experiments suggests that the mRNAs are retained on both the cytoskeleton and ER membranes when not being translated. Thus it appears that certain mRNAs may be either cytoskeletal-bound in polysomes or associated with the cytoskeleton in some untranslated form. In this regard it is interesting to note that recent work has suggested that a class of ribonucleoprotein particles (prosomes) are associated with intermediate filaments (Grossi de Sa *et al.*, 1988).

Since inhibitors of translation failed to release polysomal mRNAs from the cell matrix (van Venrooij *et al.*, 1981; Bag & Pramanik, 1987) it would appear that the polysomes are attached to the cytoskeleton primarily through mRNA rather than through the ribosomes. Similar conclusions have been reached from studies which showed that ribosomes and initiation factors were lost from the cell matrix after heat shock or treatment of cells with pactamycin or fluoride (Howe & Hershey, 1984). However, in these experiments EDTA and ribonuclease caused no loss of ribosomes from the matrix although polysomes were dissociated; thus the continued presence of intact mRNA was not required for attachment of ribosomes to both the membrane and cytoskeletal elements in the deoxycholate fraction. 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 (Savitz & Meyer, 1990). The recent observation that elongation factor 1a may be an actin-binding protein (Yang *et al.*, 1990) further suggests that polysome-microfilament links may partly occur through protein-protein interaction.

Cytoskeletal structures with which mRNAs and polysomes are associated

Essentially four approaches have been used to investigate which cytoskeletal components interact with the protein synthetic apparatus; morphological analysis using electron microscopy; attempts to demonstrate co-localization using immunohistochemistry and *in situ* hybridization; studies of the effects of the pharmacological agents which affect the stability of the cytoskeleton; changes in extraction conditions so as to investigate which proteins are retained or released together with polysomes.

There is accumulating evidence for an association of polysomes or ribosomal material with the actin-containing microfilaments. Electron microscopy of lens cells showed polysomes to be associated with filaments which were of a size comparable to microfilaments (Ramaekers *et al.*, 1983) and in 3T3 cells immuno-

histochemical studies have shown ribosomal material to be distributed in a pattern which is similar to that of actin (Toh *et al.*, 1980). Double immunostaining experiments also suggested a localization of ribosomal material along and around actin-containing filaments (Hesketh *et al.*, 1991a). Similar immunostaining experiments, using an antibody against a nuclear envelope antigen which binds to mRNA and which is present in polysomes, also showed a staining pattern with some similarity to that of the microfilaments (Schroder *et al.*, 1988). Further evidence for an association with microfilaments has come from experiments in which either cytochalasin B or D have been shown to cause a release of polysomes or mRNAs from the cell matrix (Lenk *et al.*, 1977; Ramaekers *et al.*, 1983; Bird & Sells, 1986; Ornelles *et al.*, 1986; Bagchi *et al.*, 1987; Vedeler *et al.*, 1991); in HeLa cells the release of both mRNA and polysomes is paralleled by an inhibition of protein synthesis (Ornelles *et al.*, 1986). In myoblasts, cytochalasin D causes the release of polysomes containing nascent myosin heavy chains (Isaacs & Fulton, 1987). Release of polysomes is also induced by DNAase I, which also causes actin depolymerization (Adams *et al.*, 1983); in contrast, phalloidin, which stabilizes actin filaments (Dancker *et al.*, 1975), prevents the release of actin and polysomes (Adams *et al.*, 1983; Vedeler *et al.*, 1991). Extraction of the cell matrix with 130 mM-KCl leads to a release of polysomes and causes a simultaneous loss of microfilaments with a concomitant release of actin (Hesketh & Pryme, 1988; Hesketh *et al.*, 1991a; Vedeler *et al.*, 1991). This again suggests that polysomes are associated with actin filaments; importantly, 100–150 mM salt concentrations are known to affect the stability of actin filaments (Kasai, 1969) but not intermediate filaments (Steinart *et al.*, 1982). Further supporting evidence for a polysome-microfilament link comes from studies of cytomegalovirus infection (Jones & Kilpatrick, 1987); infection with this virus causes a rapid depolymerization of microfilaments followed later by a repolymerization and these changes are accompanied by a corresponding decrease and then an increase in the proportion of polysomes associated with the cell matrix. The observation that elongation factor 1a is an actin-binding protein (Yang *et al.*, 1990) again points to a direct interaction between components of the translatory apparatus and microfilaments. In conclusion, therefore, there is considerable evidence showing that a population of polysomes is associated with microfilaments; estimates of the proportion of the total cellular complement of polysomes which are linked to microfilaments vary between 25 and 40% (Ramaekers *et al.*, 1983; Hesketh & Pryme, 1988; Vedeler *et al.*, 1991).

Morphological examination of cultured cells has repeatedly shown polysomes to be present in areas of the cell cytoplasm which are free of intermediate filaments (Lenk *et al.*, 1977; Fulton *et al.*, 1980), suggesting that this class of filaments is not involved in any form of association with polysomes. This is supported by the observations that salt extracts polysomes but not the bulk of vimentin from the cell matrix of ascites cells (Traub & Nelson, 1982; Vedeler *et al.*, 1991) and that eIF2 is associated with filaments in extracts of three different cell lines which lack vimentin (Heuveljans *et al.*, 1989); furthermore, in these three cell lines both polysomes and specific mRNAs for actin and histone H2 are distributed between soluble and non-ionic-detergent-insoluble fractions in the same manner as in normal vimentin-containing HeLa cells. Colchicine has no effect on polysome distribution in cultured cells (Lenk *et al.*, 1977; Vedeler *et al.*, 1991), suggesting that CBP are not associated with microtubules. Microtubules and intermediate filaments appear to be linked, since microtubule depolymerization by colchicine causes collapse of the intermediate filament network (Hynes & Destree, 1978); therefore the fact that colchicine does not

promote a release of polysomes from the cell matrix also lends support to the idea that CBP are not associated with intermediate filaments.

Although there is little evidence for an association of polysomes with intermediate filaments, several studies have suggested that some mRNAs or ribonucleoprotein particles may be associated with them. Thus, in BHK cells the mRNA cap binding protein appears to co-distribute with intermediate filaments in double immunostaining experiments (Zumbe *et al.*, 1982), whilst in *Xenopus* oocytes Vg1 mRNA is recovered in a cytokeratin- and vimentin-enriched fraction (Pondel & King, 1988). Furthermore, after maturation of the oocyte the intermediate filaments disappear and there is a concomitant change in the distribution of the Vg1 mRNA so that it is found in similar concentrations in both soluble and cell matrix fractions. In contrast, Yisraeli *et al.* (1990) have found that Vg1 mRNA is released from the cell matrix by cytochalasin B, which suggests an association with microfilaments. It is not clear, however, in either of these studies if the Vg1 mRNA was present in polysomes or in some untranslated form. Monoclonal antibodies against protein components of prosomes (recently characterized ribonucleoprotein particles which contain untranslated mRNA), stain a fibrous network which coincides with the cytokeratin network in HeLa and PtK cells (Grossi de Sa *et al.*, 1988; Scherrer, 1990). Other large ribonucleoprotein particles called vaults have been reported to be concentrated in actin-rich areas of the cytoplasm (Kedersha & Rome, 1990), but in addition were observed in considerable amounts in the perinuclear region where intermediate filaments are also found, suggesting that they may be associated with the latter cytoskeletal component. It appears, therefore, that perhaps only untranslated mRNAs and ribonucleoprotein particles, but not polysomes, are associated with the intermediate filaments; this suggests that these filaments may play a role in mRNA transport (see Agutter, 1990) and/or that they are involved in some way in the sequestration of untranslated mRNA species.

PHYSIOLOGICAL SIGNIFICANCE OF CYTOSKELETAL-BOUND POLYSOMES

Translational control

The failure of some workers to extract polysomes using non-ionic detergents alone led to the suggestion that FP do not exist and, therefore, that attachment of polysomes to the cytoskeleton was an essential prerequisite for translation to occur (Lenk *et al.*, 1977; Cervera *et al.*, 1981; van Venrooij *et al.*, 1981). However, as discussed above, subsequent work has indicated that in many cell types a certain proportion of cellular polysomes does exist in a free state. It would thus appear that attachment to the cytoskeleton is not an absolute requirement for translation to occur. Furthermore, association of mRNAs with the cytoskeleton and their subsequent translation are not tightly coupled in that attachment to the cytoskeleton alone is not sufficient for translation. Thus in influenza- and vaccinia-virus-infected cells host mRNAs are associated with the cytoskeleton although they are not translated (Lemieux & Beaud, 1982; Katze *et al.*, 1989). This demonstrates that association of mRNA with the cytoskeleton and then its translation occur as independent events.

There is evidence, however, that the extent of polysome-cytoskeleton interaction does indeed vary according to different physiological conditions and that more mRNAs and polysomes are associated with the cytoskeleton under conditions of increased protein synthesis. Thus in the unfertilized ascidian egg, the bulk of cellular mRNA is present in small ribonucleoprotein particles in the soluble cytoplasmic fraction, whereas after fertilization there is an increase in the proportion of total mRNA recovered

in the cell matrix and this mRNA appears in polysomes (Moon *et al.*, 1983); this correlates with an observed increase in the number of polysomes. In fibroblasts the rapid stimulation of protein synthesis by insulin occurs by an activation of the existing protein synthetic machinery (Hesketh *et al.*, 1986). Under these conditions, insulin causes a 20% increase in the proportion of polysomes co-extracted with actin by 130 mM-KCl, and thus proposed to be associated with the microfilaments (Hesketh & Pryme, 1988). Similarly, in ascites cells insulin both stimulates protein synthesis and causes a redistribution of ribosomes between the FP, CBP and MBP populations (Vedeler *et al.*, 1990). 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, polyribosomes (Lenk & Penman, 1979). 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 (Horne & Hesketh, 1990a,b). These conditions are also associated with different rates of protein synthesis; for example, in the rat the proportion of ribosomes in the myofibrillar compartment falls between 14 and 51 days of age, 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. It would seem likely that an alteration in the distribution of polysomes between FP, CBP and MBP populations reflects a change in the pattern of proteins being synthesized.

Cytoskeletal-bound polysomes synthesize specific proteins

Several observations suggest that the increased association of polysomes with the cytoskeleton is due to an alteration in the types of proteins being synthesized rather than part of a mechanism causing an activation of protein synthesis. In ascites cells the proportion of CBP increases 1 h after insulin administration but then falls again after 2 h, although total protein synthesis has continued to increase (Vedeler *et al.*, 1990); there thus appears to be a continual redistribution of polysomes during the response and this is consistent with a continually changing pattern of proteins being synthesized. Some *in vitro* translation studies using mRNA isolated from polysomes extracted from the cell matrix failed to show the presence of a unique series of mRNAs in this class of polysomes (van Venrooij *et al.*, 1981; Moon *et al.*, 1983). On the other hand, Cervera *et al.* (1981) showed that the patterns of proteins synthesized by mRNA isolated from the soluble fraction and the cell matrix were not identical, suggesting differences in message populations. Interpretation of these data is difficult because of the presence of MBP in the so-called cytoskeletal fraction. Translation *in vitro* of poly(A)⁺ RNA from microfilament-associated polysomes which have been distinguished from membrane-bound by salt extraction suggests that there are some differences in the relative amounts of different mRNAs present on FP, CBP and MBP (Vedeler *et al.*, 1991).

Direct analysis of mRNAs using Northern hybridization techniques has provided a new approach to the problem of studying the physiological significance of CBP. Several studies have investigated whether or not the mRNAs for cytoskeletal proteins are found in association with the cytoskeleton. Thus in both HeLa cells and L6 myoblasts actin mRNA has been found largely on CBP (Bird & Sells, 1986; Bagchi *et al.*, 1987; Meadus *et al.*, 1990). Bagchi *et al.* (1987), however, were unable to release actin mRNA with cytochalasin B. As shown in Fig. 1, results from 3T3 fibroblasts (Hesketh *et al.*, 1991b) have shown the

presence of mRNA for actin both in FP and CBP in roughly equal concentrations but that in terms of overall quantities some 60 % of the actin mRNA was found in the cytoskeletal-bound fraction. *In situ* hybridization studies (Singer *et al.*, 1989) have shown that actin mRNA can be detected in close proximity to microfilaments. The majority of studies thus suggest that actin mRNA is primarily associated with CBP. Similar results were obtained with histone mRNAs which have been reported to be predominantly found in the cell matrix (Zambetti *et al.*, 1985; Bagchi *et al.*, 1987; Heuveljans *et al.*, 1989) and to be released by cytochalasin D (Zambetti *et al.*, 1990b). Although other conflicting data have shown an equal distribution between free and cytoskeletal-bound fractions (Bird & Sells, 1986; Pondel & King, 1988), in one case (Bird & Sells, 1986) the histone mRNA was released into the soluble fraction by pretreatment of cells with cytochalasin B. In *Xenopus* oocytes the Vg1 mRNA is highly enriched in a cytokeratin-rich cytoskeletal fraction (Pondel & King, 1988); this fraction contains some 35 % of the particular mRNA species. CBP isolated from 3T3 fibroblasts using the sequential extraction procedure of Vedeler *et al.* (1991) have recently been shown to be enriched in *c-myc* mRNA (Hesketh *et al.*, 1991b; see Fig. 1). The enrichment of specific mRNAs in the cytoskeletal-bound fraction is important because it indicates that these polysomes both represent a separate population distinct from the free and membrane-bound classes and that the CBP are involved in the synthesis of specific proteins.

Spatial distribution of mRNAs and ribosomes

Studies from several radically different and specialized biological systems suggest that ribosomes and mRNA can associate with cellular components in a spatially specific manner. This is well illustrated in certain amphibian eggs where a rare class of mRNAs are distributed within the oocyte cytoplasm in an anisotropic and structured manner (Jeffery, 1984; Rebagliati *et al.*, 1985). In *Xenopus*, for example, *in situ* hybridization studies

have shown that the Vg1 mRNA is localized in the cortical cytoplasm of the vegetal half of the egg (Weeks & Melton, 1987). Inhibitor studies suggest that in *Xenopus* at least this differential distribution of mRNA is dependent on an association with the cytoskeleton (Yisraeli *et al.*, 1990); thus cytochalasin B but not colchicine released Vg1 mRNA both from its normal tight distribution in the cortical shell of the cytoplasm and from the cell matrix, suggesting that its localization was dependent on microfilaments. At an earlier developmental stage, microtubule inhibitors prevented the translocation of the mRNA. It was thus proposed that mRNA translocation was effected by the microtubules but at a later stage mRNA was transferred to and then anchored on microfilaments. In view of the interdependence of microtubules and intermediate filaments (Hynes & Destree, 1978), the presence of intermediate filaments in the vegetal part of the egg and the recovery of the Vg1 mRNA in a cytokeratin-enriched fraction (Pondel & King, 1988), it is possible that in *Xenopus* it is the intermediate filaments which form the basis of the anisotropic distribution of Vg1 mRNA. Clearly further work is required to define the roles of the different filament systems in these processes. Importantly, however, these experiments do provide evidence that the distribution of mRNA in fertilized eggs depends on the integrity of the cytoskeleton. This concept is supported by observations on *Drosophila* eggs in which mRNA for the *bcd* gene is localized in the anterior end of the egg (Berleth *et al.*, 1988; MacDonald & Struhl, 1988). Evidence for a role of the cytoskeleton comes from genetic studies which have shown that two genes necessary for mRNA distribution in *Drosophila* eggs appear to code for cytoskeletal proteins (Berleth *et al.*, 1988). Furthermore, a gene whose mutant protein causes redistribution of the developmentally important *nanos* product has been shown to be a myosin-like protein (Wharton & Struhl, 1989).

Specific localization of mRNAs is obviously of critical importance in the developing egg or embryo, but spatial distribution

Fig. 4. Scheme illustrating the possible roles of the cytoskeleton in the organization of protein synthesis

of mRNAs is not restricted to oocytes and has been observed in other cell types. Thus in cultured fibroblasts, by using *in situ* hybridization, it has been shown that the mRNA for actin is localized in the leading lamellae, the mRNA for vimentin in the perinuclear region and the mRNA for tubulin in the peripheral cytoplasm (Lawrence & Singer, 1986; Singer *et al.*, 1989; Sundell & Singer, 1990); these studies thus illustrate how in one cell type three specific mRNAs for cytoplasmic proteins show different forms of spatial distribution through the cytoplasm. Furthermore, in the developing brain the mRNA for MAP2 has been found to be present in dendrites but not in neuronal cell bodies (Garner *et al.*, 1988); this would appear to be physiologically relevant since in neurones the MAP2 protein is localized primarily in dendrites.

Recent work suggests that not only do cells exhibit a specific subcellular distribution of mRNA but in addition their ribosomes appear to be organized in a spatial manner. This is evident for example in skeletal muscle where immunohistochemistry using anti-60 S antibodies has shown ribosomal material to be associated with the myofibrils in addition to being present in the subsarcolemmal cytoplasm (Horne & Hesketh, 1990a). These authors postulated that such ribosomes are involved in the synthesis of the myofibrillar proteins and this is supported by the observation that, in myoblasts, nascent myosin peptide chains being synthesized on polysomes are largely recovered in the cell matrix and released by pretreatment with cytochalasin D (Isaacs & Fulton, 1987). Furthermore, these latter authors pointed out that translation of myosin mRNA on the cytoskeleton may allow for the site of translation to dictate where the myosin heavy chains are to be inserted into the cytoskeleton or myofibril. A second example of such spatial organization is found in the neurone where polysomes have been found in dendrites but not in axons (Steward & Levy, 1982) and this would appear to be related to recent observations that have demonstrated transport of RNA from the cell body into the dendrites but not the axons of the hippocampal neurones (Davis *et al.*, 1987); the cytoskeleton appears involved in this transport since the radiolabelled RNA was visualized in a detergent-insoluble cytoskeletal fraction. These two sets of observations are important examples of the spatial organization of ribosomes within cells and in both cases it appears to depend on some form of association with structural filaments. Cells must therefore possess a mechanism for the organization of ribosomes so that they become associated with cytoskeletal microfilaments, myofibrils or dendritic structures. The spatial organization of ribosomes is at present best explained by assuming that the cytoskeletal-bound or myofibrillar ribosomes are involved in the synthesis of specific proteins.

The presence of specific mRNAs in CBP requires a mechanism which directs these RNA species to the cytoskeletal compartment rather than to the ER or the cytoplasmic ('free') compartment. Exogenous mRNA microinjected into oocytes becomes correctly localized, suggesting that the necessary directing information resides in the mRNA itself (Yisraeli & Melton, 1988). This is supported by recent observations indicating that translocation of actin mRNA to the cell periphery is not dependent on the presence of nascent polypeptides or an association with intact ribosomes (Sundell & Singer, 1990). Furthermore, in certain oocyte mRNAs it appears likely that this information is present in a sequence in the 3' untranslated region (MacDonald & Struhl, 1988; Yisraeli & Melton, 1988). Interestingly, this region may have a function in controlling mRNA stability (Bonnieu *et al.*, 1988) and it is possible that the subcellular localization of mRNAs and their stability are functionally related (Zambetti *et al.*, 1990a). In specialized cells such as neurones, muscle and the fertilized egg it is possible that information in the 3' untranslated region of certain mRNAs allows these specific mRNAs to be

translated into their corresponding proteins in certain localized areas of the cytoplasm. Furthermore, we propose a generalized model for all cells in which specific mRNAs are directed to be translated on the cytoskeleton by possessing a specific sequence in the 3' untranslated region (Fig. 4), which may interact with the cytoskeleton either directly or through a specific binding protein. This mechanism could therefore be involved in the spatial organization of protein synthesis in the cell.

CONCLUSIONS AND FUTURE PERSPECTIVES

Literature which has appeared since 1977 on the presence of polysomes associated with the cytoskeleton has been confusing because of the heterogeneous nature of the so-called 'cytoskeletal' fractions generally employed. An assessment of the different techniques employed is summarized in Fig. 3. However, there is now convincing evidence, largely from electron microscopy, immunohistochemistry and the biochemical manipulation of microfilament stability, which supports the concept that mRNAs and polysomes are associated with the cytoskeleton and that such interactions do not arise as a result of an artefact of isolation or fixation. Much of the data are morphological or merely correlative and do not provide evidence for a direct mRNA/polysome link with the cytoskeleton. Detailed biochemical studies, using cross-linking techniques or *in vitro* translation with polysome-cytoskeleton complexes, for example, will be necessary in order to provide such information.

At the present time it seems that mRNAs are associated with intermediate filaments when in an untranslated, sequestered form in a ribonucleoprotein particle such as a prosome, while most of the evidence suggests that CBP are associated with the actin-containing microfilaments, although it would appear that this is by no means exclusive. Clearly it will be important in future work to delineate both the relative importance and the different roles of these two filament systems. A scheme showing how the two filament systems may participate in the organization of protein synthesis is shown in Fig. 4.

It is now clear that the association of mRNAs/polysomes with the cytoskeleton has considerable physiological significance. Firstly, it forms the basis for the anisotropic spatial distribution of mRNAs and ribosomes observed in certain specific situations such as amphibian oocytes and skeletal muscle, and secondly the extent of polysome interaction with microfilaments or myofibrils changes under conditions where it is known that there are alterations in protein synthesis. Clearly the relative proportion of CBP to total cellular polysomes can change in response to physiological stimuli and to the specific needs of the cell. Both sets of observations are best explained by the cytoskeleton being involved in localization and translation of specific mRNAs. A major priority in this field is to determine to what extent CBP synthesize a specific set of proteins. This must be done with well-characterized fractions where CBP have been adequately separated from both FP and MBP. Present methods based on salt and detergent extraction need to be refined in order to obtain highly purified fractions. Using DNA probes it should then prove possible to study the distribution of specific mRNAs under different physiological conditions. Furthermore, molecular biological techniques will be useful to investigate the mechanisms by which mRNAs are directed to the cytoskeleton; critical parts of the message may be identified either by redirecting or misdirecting mRNAs using chimaeric gene constructs or by searching for common features such as consensus sequences and structural motifs in the mRNAs translated on CBP.

In conclusion, the picture has now emerged that a population of polysomes bound to elements of the cytoskeleton exists and that this polysome fraction is distinct from the FP and MBP

populations in that it contains specific mRNA species; the full implications of such an arrangement must await detailed knowledge of the types of proteins which are synthesized on CBP.

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