Review Letter

THE PROTEIN MOIETIES OF ANIMAL MESSENGER RIBONUCLEOPROTEINS

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1. Introduction

The first reports identifying proteins associated with mRNA in animal cells appeared long before the conclusive identification of mRNA itself, in studies by Spirin and his colleagues on developing fish embryos [review: 1]. Because there is little synthesis of ribosomal RNA in early developmental stages, the newly synthesised RNA can be identified tentatively as mRNA. The sedimentation distribution, buoyant density after fixation and enzymatic sensitivity of the particles indicated their ribonucleoprotein nature. Monroy et al. [2] reported similar cytoplasmic mRNP particles in unfertilised sea urchin eggs. It is now clear that there are several classes of specific proteins associated with mRNA, and it is possible to suggest and evaluate possible roles for them.

Specific proteins have been found to be associated with messenger RNA in the nucleus [3], in free cytoplasmic particles [4], in polysomes [5] and bound to the polyA sequence at the 3′-terminus [6, 7]. The proteins in each case appear to be different. In some cases it is not clear whether the proteins are non-specifically bound contaminants; the molar ratios of protein:mRNA have not been determined. The proteins associated with polysomal mRNA do not enhance translation in heterologous cell-free systems [8]. No function has been proven for any of these proteins.

This review will not discuss the structural proteins of the ribosomes, although it is not yet known whether certain of these may be messenger-specific [e.g. 9, 10]. In general, it will also not review protein factors specifically involved in defined synthetic roles in translation, such as initiation factors or elongation factors, except insofar as they may be identical with messenger-associated proteins. A comprehensive list of papers on soluble protein factors involved in translation in animal cells has recently appeared [11].

2. Nuclear messenger RNP and informofers

Messenger ribonucleoprotein (mRNP), whether nuclear or cytoplasmic, can be distinguished from ribosomal ribonucleoprotein by its characteristic density of approximately 1.4 after formaldehyde fixation [12]. The nuclear mRNP can be isolated from a variety of sources, including rat liver, as high molecular weight complexes sedimenting as a broad band in the polysome region; these are broken down by mild RNAase digestion or other maltreatment to a fairly homogeneous band sedimenting at 30–40 S in sucrose gradients [3, 13]. After fixation with formaldehyde this component bands in caesium chloride at a density of 1.41. The protein:RNA ratio has been calculated to be 4:1 or 8:1 [3, 13].

The protein component of the nuclear mRNP can be dissociated from the RNA with 2 M NaCl. The aggregated free protein, or 'informofer', continues to sediment at approximately 30 S in 2 M NaCl although now free of RNA and banding at a lower density of 1.34 in CsCl gradients. Treatment with urea is necessary to disaggregate the informofers into monomer protein components of mol. wt. approx. 40 000 [3, 14]. The profile of these proteins is similar for mRNP isolated from different tissues (liver, ascites cells) or different species (rabbit, rat) [15]. There are characteristic interactions by S–S bridges which cause two further components to be seen in the absence of treatment with mercaptoethanol treatment. It has been suggested that the protein profile of nuclear mRNP is more complex than reported by Georgiev and his colleagues, and that differences occur between tissues

[16, 17]. However, since all of the isolation procedures involve massive destruction of the cells by homoginisation prior to isolation of the informofers, it is difficult to exclude non-specific binding of nuclear proteins which are not normally associated with mRNA.

Rather larger amounts of nuclear mRNP can be released by sonication, and the proteins from such particles (which closely resemble those of Lukanidin et al. [3] isolated from rat liver and hepatomas have been compared by SDS-gel electrophoresis [17]. Up to twenty bands are found in each case, and while the proteins are qualitatively similar from the various sources, quantitative differences are found. It is unclear to what extent these can be attributed to non-specific binding of soluble nuclear proteins. Two (rather than one) major components are evident, of mol. wt. approx. 40 000, in agreement with Georgiev. Another group describes multiple protein bands from rat liver-nuclear mRNP, with a major component of mol. wt. 36 000 [18, 19, cf. 22].

The evidence that the RNA associated with informofers is messenger RNA is rather tenuous. It is non-ribosomal in sedimentation coefficient and labelling behaviour, Informofer protein forms mRNP complexes with mRNA isolated from adenovirus-infected human cells [3]. Both the monomeric informofer and the 30 S aggregate can be isolated free of RNA from a rat liver nuclear extract [20].

3. Function of the proteins of nuclear mRNP

Monomeric 30 S RNP particles have been shown to contain two polyA-synthetase activities, one activated by Mn^{2+} and one by Mg^{2+} [21]. An endonucleolytic activity which cleaves RNA into smaller fragments has also been identified; this activity may be non-specific as nucleases are endemic in broken cell extracts [22]. It is still not proven that these activities are functionally related to the processing of heterogeneous nuclear RNA in vivo, although such a postulate appears likely, particularly in the case of polyA synthetase.

Isolated nuclear RNP particles do not bind to ribosomes in vitro, although the RNA isolated from them does [23]. This led Olsnes and Pihl to suggest that the function of the informofer protein is to protect the mRNA from non-specific binding to ribosomes. The nuclear mRNP co-sediments on sucrose gradients with the small ribosomal subunit after incubation with KB cell cytoplasm [24].

It is still unclear whether the protein(s) of the informofer moves with the mRNA to cytoplasm. Two groups working with rat liver both report the occurrence of a protein fraction in the cytoplasm similar in one-dimensional electrophoretic migration to informofer protein. In one case [25], the analogous cytoplasmic protein could be released from the polysomes by treatment with deoxycholate, implying that it is bound not to the polysomes but to the endoplasmic reticulum. Another group purified the cytoplasmic protein from 'rough microsomes' and 'free polysomes' and found that it was present in very much greater amounts in the former [26]. Liver is a heterogeneous tissue synthesising many proteins. Consequently it is not possible to isolate a discrete messenger RNP particle with EDTA or puromycin (see the following section). Nonetheless, proteins released from rat liver polysomes with EDTA have been separated from ribosomal subunits on Sephadex G-200 and co-electrophoresed with informofer protein, and a band of similar molecular weight is found [27]. Four main components are released from liver polysomes by deoxycholate, and one of these, of mol. wt. 160 000, co-sediments with rapidly labelled polysomal RNA after EDTA dissociation [28]. This protein also is found in the position occupied by rabbit reticulocyte 14 S mRNP in sucrose gradients (although the isolation of purified 14 S mRNP would be necessary to confirm this finding). It is not the same as the nuclear informofer protein [28]. On the other hand, for reticulocytes, one tissue with a well-characterised messenger RNP which can be isolated by EDTA, it has been shown that the polysomal proteins include one component of similar but not identical mobility to informofer protein. This is true for mouse, in which case the polysomal mRNP proteins from reticulocytes were compared with informofer protein from several tissues [15], and for duck, where a homologous reticulocyte system could be used [29]. Antibodies prepared to rat liver nuclear mRNP do not bind to the homologous polysomal mRNP [30].

In each case referred to above, and in those to follow, different techniques of protein isolation and gel electrophoresis (or even free-flow electrophoresis) have been used. Further advances in this field are par-

ticularly dependent upon a standardisation of technology. The results obtained for the migration of proteins with mRNA from nucleus to cytoplasm are not necessarily in disagreements; most of those claiming migration compare microsomal protein with informofers, while those denying migration use polysomal material.

4. Polysomal mRNP

If purified polysomes are treated with EDTA, pyrophosphate or other chelating agents in low salt, the ribosomes split into subunits and the messenger RNA is released, with an approximately equal amount of bound proteins [31]. Since most cells contain a spectrum of mRNA's of different sizes, the mRNP component is usually difficult to isolate. However, in the case of reticulocytes it sediments at 14 S in sucrose gradients, well separated both from the supernatant proteins (including those released from the ribosomes by the EDTA but not bound to mRNA) and the smaller ribosomal subunit. The proteins of this component have been isolated and characterised for rabbit [5], duck [29] and mouse [15]. In each case there are two major protein components, although careful examination of the gels or absorbance traces reveals other components at lower concentration. The duck mRNP profile is particularly complex. The molecular weights of the components from rabbit polysomal mRNP are 68 000 and 130 000 by SDS-gel electrophoresis; those of the duck proteins 49 000 and 73 000. The molecular weights of the mouse proteins were not determined. It is clear that these are not proteins which are also found in large amounts in the ribosomal subunits after EDTA treatment, although this may be thought to follow from the isolation procedure. From direct visualisation with the electron microscope it is thought that the major proteins occur at from four to seven distinct sites along the length of duck globin mRNA [32]. For duck globin mRNA, at least five minor components in addition to the two major ones are found to be associated with mRNP prepared with EDTA; the two major components, and one of the minor ones, are phosphorylated. The mRNP is stable in high ionic strength, suggesting that in this case the proteins are not fortuitously bound contaminants [33].

Good systems now exist for testing mRNA in cell-free systems, and the activity of reticulocyte mRNP obtained by EDTA treatment of polysomes has been compared with that of protein-free mRNA in heterologous reticulocyte lysates [8] and in liver cell-free systems [34]. In no case were there pronounced differences between 9 S mRNA and 14 S RNP, in ability to stimulate new protein synthesis or depress endogenous protein synthesis. If the proteins associated with the mRNA after EDTA treatment play a part in translation, it is a non-specific role. It would be useful to re-isolate heterologous mRNA from a cell-free system and determine whether it has become associated with the same two proteins during translation. The only suggestion of a functional difference is in the binding of 14 S mRNP to native 40 S ribosomal subunits which have been washed with deoxycholate, a binding which is not found with 9 S mRNA [5]. (The mRNP and mRNA bind equally well to unwashed native 40 S subunits [5, 35, 36].)

A more comprehensive recent study has compared the proteins of rabbit reticulocyte polysomal mRNP with initiation factors for protein synthesis [36]. The authors demonstrate that 0.5 m KCl wash factors contain all the initiation factors but that the mRNA remains bound to the ribosomes together with the proteins of the 14 S mRNP. They also show that the most mature reticulocytes, which have no active initiation factors, still have a normal amount of 14 S mRNP.

Puromycin also dissociates rabbit reticulocyte ribosomes with the release of a messenger RNP sedimenting at approx. 15 S, which is associated with two proteins of mol. wt. 78 000 and 52 000, remarkably close to those found for the duck (but not the rabbit) EDTA particle [37]. Blobel [37] states that he finds proteins of mol. wt. 78 000 and 52 000 associated with the EDTA-derived particle of rabbit reticulocyte as well, in clear disagreement with the results of Lebleu et al. [5].

There is a considerable body of previous work concerning the mRNP from polysomes of cultured cells or rat liver. Although often including much careful work, the results are hard to interpret because of the polydispersity of the mRNP obtained, which inevitably overlaps with the ribosomal components. After fixation in formaldehyde, the protein: RNA ratio of the mRNP is approx. 3:1. The sedementation coefficient of the mRNP in sucrose gradients can be as large as 1000 S [38]. Good examples of such ex-

periments have been described for rat liver [25,39], mouse L cells [40], and human KB cells [41]. Experiments on the release of mRNP during temperature shock of L cells demonstrates that under this non-physiological condition the mRNP is an intermediate in translation [42]. The authors do not feel that a similar physiological role can be postulated without evidence as to the function of the proteins. Olsnes [43] has shown that when cells are lysed at low ionic strengths and polysomes isolated with detergents, absorption of extraneous proteins is a serious problem, so much so that the buoyant density of the polysomes is reduced from 1.57 to 1.47, well into the mRNP range. Experiments by Mansbridge and his colleagues demonstrate a spurious mRNP component of an even lower density of 1.38 [44]. It is apparent that characterisations of mRNP which depend exclusively upon density gradient techniques may be in error, particularly when very complex systems with extensive membrane structures are studied.

5. 'Free' cytoplasmic mRNP

Cytoplasmic non-polysomal mRNP was identified before any of the other classes of mRNP, but its function is still poorly understood [1]. The 'informosomes' sediment at from 20 S–110 S at least, with some indication of discrete sedimentation classes. The protein to RNA ratio varies from approximately 1.2:1 for some of the fastest sedimenting particles to 4:1 for most of the material sedimenting from 50 S–110 S. Artefacts of absorption and formaldehyde fixation could arise in these earlier experiments. There is a free cytoplasmic protein of sedimentation coefficient 9 S which will form informosome-like particles with free RNA, but which is not specific for mRNA, since bacterial ribosomal RNA forms similar complexes [1,45]. Spirin [1] proposes three possible roles for informosome protein: transport of mRNA from nucleus to cytoplasm or from nuclear membrane to polysomes; stabilisation of mRNA and protection from RNAase action and modulation and repression of translation. There is as yet no proof for any of these roles, and it is not known if the protein associated with the non-translatable mRNA of unfertilised eggs [2] is similar to informosome protein.

The labelling kinetics of the mRNP argues for an intermediary role during transport of mRNA from the nucleus. The rapidly labelled polydisperse mRNP is not polysome-bound until approximately one hour after interperitoneal injection of isotope [46]. In rat liver no fewer than four newly synthesised cytoplasmic mRNP components were identified, and on the basis of their behaviour and shifts in density after treatment of the cells with actinomycin D, it is suggested that the lightest (density 1.40) represent unique associations of mRNA with informosome protein en route from nucleus to polysome [47]. Recent data indicating that actinomycin D interferes with translation as well as transcription suggests caution in interpreting these results. A longer paper reporting kinetic data (but also publishing some interesting electron micrographs of informosome-like particles which show a remarkable amount of regular substructure) was published by Scherrer and his colleagues [48]. Lable first appears in free mRNP and only after a time lag in polysomes. Although the controls are more extensive in this work, most labelling profiles are obtained after actinomycin D, and the cells used (HeLa) have no specifically identifiable mRNA's, but only a spectrum of rapidly labelled material. These results could well be repeated now that better methods for characterising specific mRNA's exist.

It is surprising that the only defined cytoplasmic non-polysomal mRNP, that occurring in reticulocytes and containing globin mRNA, has not been better characterised. It has been reported for rabbit [49–52], duck [53] and mouse [54]. It is evidently mRNP rather than mRNA, and in the case of rabbit and mouse sediments at approx. 15 S [52, 54]. Only in the case of duct has any analysis of the protein component been carried out; several proteins are present, some are phosphorylated and one may be similar to one of the two major proteins found associated with the polysome-derived EDTA particle [4]. Since the non-polysomal mRNA is enriched substantially in α-globin messenger activity, it is particularly interesting. It is not clear whether the enhanced α-globin synthesis activity is due to RNA components alone, as a protein factor which stimulated only α-chain synthesis has been identified in polysome-associated initiation factors [55].

6. Proteins associated with polyA

It is now known that polyA sequences are a characteristic of all mRNA's in animal cells with the exception of histone mRNA's. These sequences behave anomalously when extracted using phenol as a protein denaturant, and it was suspected that this might be due in part to a tightly bound protein component. This has now been shown by isolating an RNAase-resistant polyA-containing RNP fragment from mouse sarcoma polysomes [6]. An mRNP complex released with puromycin from mouse L cells contains proteins, including two major components; one, of molecular weight 78 000 was found to be associated with the polyA region [7]. This protein is ubiquitous but its function is unknown. It is not known whether proteins are specifically associated with histone mRNA, which contain no polyA sequences.

Conclusions

The refrain of 'unknown function' occurs at several points in this review. One cannot but conclude that a certain amount of old-fashioned protein chemistry, together with a concentration on a single messenger in nucleus, cytoplasm (free) and polysomes (as attempted by Scherrer and his collegues for duck globin mRNA) would be helpful. In the absence of definitive identification of mRNA, functions of associated proteins can only be surmised. Gradients now exist using new non-ionic materials which can band unfixed ribonucleoprotein, which is thus less likely to contain non-specifically bound proteins [56, 57]. Messenger RNA can be isolated using polyT cellulose, iodinated to specific activities of tens of millions, and added back to cell-free systems. Copies can be made with reverse transcriptase and copies of such copies transcribed with RNA polymerase. Such technical advances should allow a full analysis of the role of mRNA-associated proteins in the near future.

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