THE SPECIFICITY OF INTERACTION BETWEEN mRNP PROTEINS AND GLOBIN mRNA IN POLYRIBOSOMAL AND CYTOPLASMIC FREE mRNP

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1. Introduction

In eukaryotic cells, mRNA is associated with proteins in messenger ribonucleoprotein complexes (mRNP). In duck erythroblasts, two types of globin mRNP particles have been isolated under low ionic strength conditions: the actively translatable 15 S mRNP isolated from polyribosomes and the in vivo and in vitro translationally repressed 20 S free cytoplasmic mRNP [1–4]. A specific group of proteins (possibly equivalent to initiation factor eIF-3) is removed from 15 S polyribosomal mRNP when purified in a 0.5 M KCl containing buffer. Furthermore, the 20 S free mRNP is split into several sub-particles at this ionic concentration. Two of these still translationally repressed subparticles, namely the 16 S and 13 S mRNPs, contain both α- and β-globin mRNA [1] and differ partially in their protein composition [2]. The protein composition of these two free globin mRNP particles is also clearly different from that of the translatable 15 S polyribosomal globin mRNP, suggesting a relationship between protein composition of mRNP and mRNA function [3,5,6]. We have shown that proteins do not interact at random with mRNA within mRNP particles, but rather specifically. Indeed, specific segments of the mRNA are protected in the RNP against staphylococcal nuclease action [7,8]. To gain further insight into the structure/function relationship of the active and repressed globin mRNP complexes, we pursued our comparative studies on the structure of cytoplasmic-free and polyribosomal duck globin mRNPs and investigated:

(i) Whether in the different forms of mRNP particles, i.e., 16 S, 13 S and 15 S, different proteins interact with the same region of the mRNA sequence;
(ii) Whether the group of loosely bound proteins removed from 15 S mRNP at 0.5 M KCl interact with globin mRNA in the mRNP.

We show here that the specific sets of mRNP proteins induce specific structural alterations in the globin mRNA chain which may be related to translational activity or repression.

2. Materials and methods

RNase-free sucrose was from Merck. [γ-^{32}P]ATP (3000 Ci/mmol) was from the Radiochemical Center (Amersham). *Staphylococcus aureus* (micrococcal) nuclease was from Worthington, polynucleotide kinase from PL Biochemicals and RNase T1 from Sankyo. Cellulose acetate was from Schleicher-Schuell and DEAE-cellulose HR 2-15 thin-layer plates were from Machery-Nagel. For autoradiography, Fuji R-X films (Japan) and Du-Pont Cronex 'Light Plus' intensifying screens were used.

Polyribosomal 15 S and cytoplasmic free 20 S duck globin mRNP were isolated from duck erythroblasts as in [4]. 16 S, 13 S and 15 S 'core' particles were obtained by sedimentating 20 S and 15 S mRNPs through 5–21% sucrose gradients in the presence of 0.5 M KCl (16 h, 2°C, 41 000 rev./min, Beckman SW-41 rotor). The different pools from the sucrose gradient dialysed against 5 mM phosphate buffer (pH 6.8), 50 μM $CaCl_2$ were digested with micrococcal nuclease for 2 h at 37°C at 1:20 enzyme/RNA (w/w); this reaction was stopped by the addition of EGTA.

Proteins were extracted with phenol/chloroform and analysed on 13% polyacrylamide gels containing SDS [9]. The residual RNA from the digested mRNP labelled in vitro with [γ-^{32}P]ATP by the polynucleotide kinase [10] was then digested with RNase T1 (1 h at 37°C, 1:20 enzyme/RNA), and analysed by fingerprinting [7] according to [11].

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3. Results

As described [3], treatment of duck globin mRNP particles with buffers of high ionic strength results in modification of the physiological complexes: Two partially distinct free globin mRNP core complexes can be separated, whereas a specific group of proteins is removed from the 15 S polyribosomal mRNP giving rise to the 15 S 'core' particles. The zones of sedimentation containing 16 S, 13 S and 15 S mRNPs which, as we know from previous studies [1,2,12] contain exclusively 9 S globin mRNA, were collected and dialysed against phosphate buffer and digested with micrococcal nuclease as in section 2. As a control, the protein composition of these mRNP was analysed by SDS gel electrophoresis (fig.1) and was found identical to that in [4].

The residual RNA extracted from the nuclease-digested mRNPs was 5′-terminally labelled with [γ-^{32}P]ATP, digested with RNase T1 and analysed by *fingerprinting* (fig.2). Fig.2A shows the fingerprint pattern of the RNA extracted from the nuclease-digested 15 S mRNP isolated in physiological conditions (50 mM KCl). Comparison with fig.2B shows that some of these protected oligoribonucleotides are no more detected in the fingerprint of the RNA isolated from the same mRNP washed in 0.5 M KCl. Since such washing of polyribosomal 15 S mRNP removes a specific group of proteins (comprising polypeptides of 105 000, 86 000, 67 000, 47 000, 40 000, 38 000 and 34 000 M_r) [4], the less complex fingerprint pattern of the washed 15 S mRNP (fig.2B) relative to that of the physiological particle in fig.2A, can be most easily interpreted as indicating that this group of proteins interacts within the 15 S mRNP holo-complex directly with globin mRNA sequences. It can, however, not be excluded that the protein complex removed from the 15 S mRNP induces profound changes in the tertiary structure of the mRNA exposing more or less the RNA. The interaction observed here is particularly interesting since a relationship of these loosely bound mRNP proteins with the pleiotropic initiation factor eIF-3 has been suggested [3,4]. A further step in our analysis was the comparison by fingerprinting of the residual RNAs of nuclease digested polyribosomal and free globin mRNPs. Fig.2C,D show the T1-fingerprint pattern of the residual RNAs extracted from digested 13 S and 16 S free cytoplasmic mRNP particles, respectively; it is evident that the two patterns are not identical.

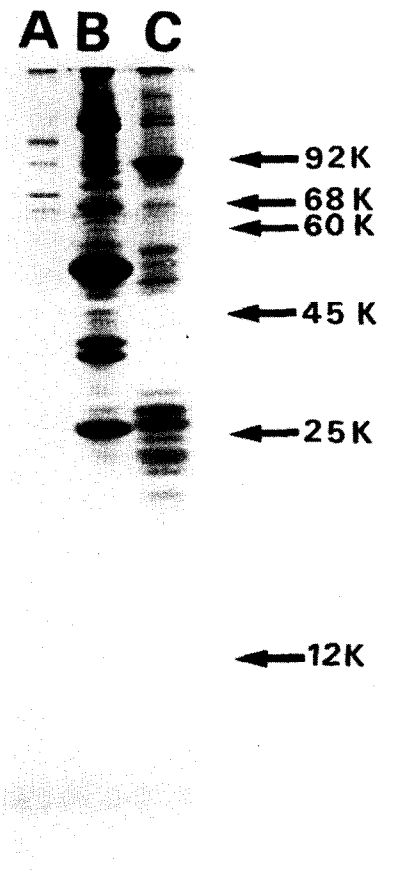

Fig.1. Proteins extracted from nuclease-digested 15 S 'core' mRNP, 13 S and 16 S cytoplasmic free mRNPs were analysed on uniform polyacrylamide gel containing SDS (slots A, B and C, respectively). Arrows indicate the position of protein markers run in parallel.

Furthermore, the fingerprints obtained for 13 S and 16 S mRNPs (fig.2C,D) are more complex than those obtained for the 15 S particle (fig.2A,B) indicating that in the repressed mRNP additional mRNA sequences are protected from nuclease digestion by interaction with proteins. In view of the differences existing between the fingerprints of the repressed mRNP and those obtained for the residual RNA extracted from 15 S mRNP, we may suggest that different proteins bind to different regions of 9 S globin mRNA in polyribosomal and free mRNP particles. The fingerprints obtained for the residual RNAs extracted from nuclease-digested 13 S and 16 S mRNP

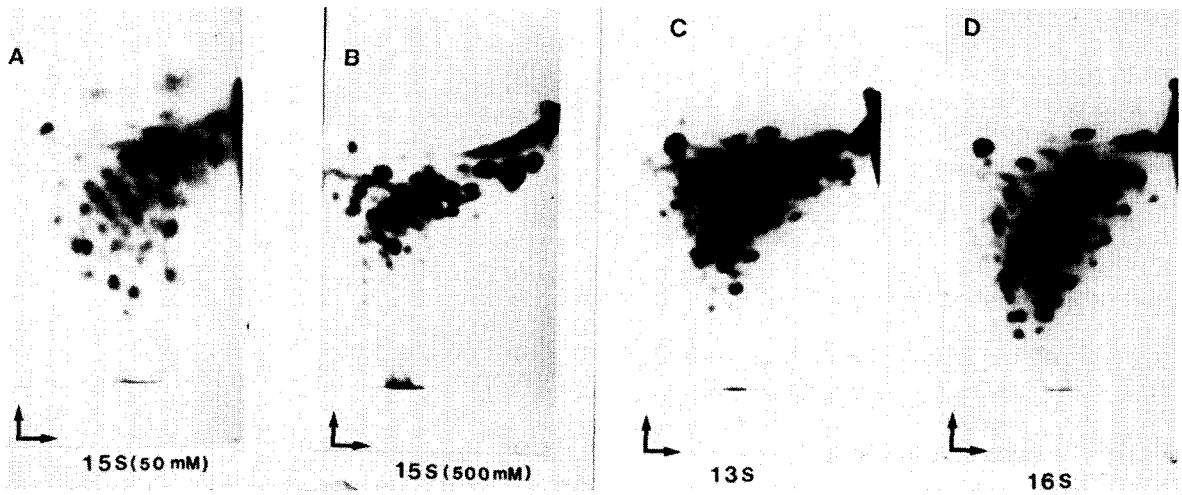

Fig.2. Fingerprint analysis of the staphylococcal nucleaseresistant RNA extracted from polyribosomal 15 S globin mRNP at 50 mM (A) and 500 mM (B) KCl and from 13 S (C) and 16 S (D) cytoplasmic free mRNPs; extracted RNAs were labelled with [γ-^{32}P]-ATP and digested with ribonuclease T1 prior to analysis by fingerprint. First dimension is from the left to the right and second dimension is upwards.

particles (fig.2C,D, respectively), are rather similar with, however some quantitative and minor qualitative differences. Such a result is not surprising since the proteins of the 16 S mRNP are also present in 13 S mRNP, and since proteins do not bind at random but interact with specific mRNA sequence in the mRNP [7]. Furthermore, the qualitative differences between the 16 S and 13 S fingerprints can be related to the presence in the 13 S mRNP only, of the 7 S ScRNA and of 5 major polypeptides [4].

4. Discussion

Summarizing these results, a clear correlation between the protein composition of purified globin mRNPs with sequences protected against nuclease digestion by proteins has been observed; this demonstrates that different proteins interact differently with the same mRNA molecule in translated polyribosomal and repressed free globin mRNPs, possibly inducing changes in secondary and tertiary mRNA structure. In [7,8] mRNP proteins interacted with regions of secondary structure in the mRNA molecule. In [1], the hypothesis of the involvement of a protein factor as a translational repressor associated with globin mRNA in free mRNP was supported. These results show an increased protection of globin mRNA in free mRNP particles compared to that in polyribosomal mRNP, as inferred by fingerprint analysis. One possible explanation of the translational inactivity of these free mRNPs could reside in the induction and fixation by proteins of a particular structure of globin mRNA, so that this mRNA would be unable to bind ribosomes. The importance of the secondary structure of mRNA for the efficiency of in vitro translation has been shown in [13,14].

In [15,16] mRNA was shown refractory to translation by *E. coli* ribosomes unless its secondary structure was destroyed by mild formaldehyde treatment. Different types of globin mRNA show a striking difference of translatability in vitro which can possibly be related to structure: at 37°C rabbit globin mRNA is able to displace the endogenous globin mRNA in duck reticulocyte lysates almost completely [17], whereas at 41°C the ratio of translated duck and rabbit mRNA is 1:1 [18]. In a fully reconstituted in vitro protein-synthetising system, the same type of observation was made in as far as duck globin mRNA required ~5-times higher concentration of ribosome wash factors than rabbit globin mRNA to be able to compete [19]. All these observations demonstrate the importance of the mRNA secondary structure for its translational activity. Correlating these observations on the alteration of mRNA structure in translationally active and repressed globin mRNP and models of

secondary structure of eukaryotic mRNAs derived from sequence data [20], one might suggest that the translational capacity of the globin mRNA is related to its secondary structure which in turn is modulated by the mRNP proteins in the sense of repression in the free globin 20 S mRNP, and of facilitating ribosome binding in case of the translated 15 S mRNP.

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