Base-pair formation between 18 S ribosomal RNA and globin mRNA during initiation of protein synthesis in vitro

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A stable complex between 18 S rRNA and globin mRNA has been isolated from 40 S initiation complexes in the reconstituted reticulocyte cell free system. This complex is only formed under the conditions which also lead to an initiation complex active in protein synthesis. The mRNA–18 S rRNA interaction has properties compatible with base-pairing. This observation is discussed in the context with other, in part controversial, observations relating to base pairing as a step in initiation of eukaryotic protein synthesis.

Base pairing *Initiation factor* *mRNA–18 S rRNA interaction*
Protein synthesis, eukaryotic *Reconstitution, in vitro* *Ribosome*

1. INTRODUCTION

The correct binding of mRNA to the ribosome is important to ensure proper in-phase translation of the encoded message. In prokaryotes, base pairing between a purine-rich sequence 5′ to the initiator codon of the mRNA and a pyrimidine-rich sequence near the 3′-end of the 16 S rRNA was suggested as the physical basis of mRNA–ribosome interaction during initiation [1]. Indeed, extensive sequence homologies between 5 nucleotides (av.) located about 10 bases from the initiator codon were found in a great number of prokaryotic mRNAs [2]. Hybrids between pieces of mRNA containing these sequences and the 3′-terminal fragment of the 16 S rRNA containing a complementary sequence have been isolated from initiation complexes [3]. In eukaryotes, pyrimidine-rich sequences at the 5′-end of mRNAs complementary to a purine-rich sequence at the 3′-end of 18 S rRNA were discovered in nucleic acid sequencing studies [4,5]. However, the distance between this sequence and the initiator codon varies for different mRNAs; the sequence is absent in some viral mRNAs and the purine-rich sequence of the 18 S rRNA is itself part of a base-paired region and therefore not easily available for base pairing with mRNA [6,7]. The discovery of the capped structure of the 5′-end of eukaryotic mRNA and its functional implications [8] as well as the demonstration of a 40 S subunit scanning the 5′-end of mRNA for the proper initiation codon [9] pointed to a mechanism of mRNA–ribosome interactions entirely different from those in prokaryotes. For all these reasons mRNA–rRNA interaction in eukaryotic initiation still is a controversial topic. Here, we present experimental evidence for physical interaction between globin mRNA and 18 S rRNA which occurs during the initiation factor dependent assembly of functional 40 S initiation complexes in vitro and which is consistent with base pairing.

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Abbreviations: eIF, eukaryotic initiation factor; LDS, lithium dodecylsulfate; Hepes, 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid

2. EXPERIMENTAL

Purification and preparation of all biological components, the general conduct of experiments to form initiation complexes and their analysis by sucrose gradient centrifugation have been described in detail [10,11].

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3. RESULTS

Initiation of protein biosynthesis is an assembly process which includes the binding of Met-tRNA$_f^{Met}$ to the 40 S ribosomal subunit, the binding of mRNA and the subsequent joining of the 40 S initiation complex with the 60 S ribosomal subunit review [12]. In the presence of Met-tRNA$_f^{Met}$, ATP, GTP and the reticulocyte initiation factors eIF-2, eIF-3, eIF-4A and eIF-4B 40 S subunits and mRNA form the 40 S initiation complex [11]. This complex can be separated from excess mRNA by sucrose gradient centrifugation [11] where it is stable between 0.1–0.35 M KCl in the presence of magnesium (fig.1). In the presence of 0.5% LDS and 0.4 M LiCl no 40 S initiation complex is observed, but instead a complex containing globin mRNA which sediments ahead of 18 S rRNA (fig.2a,3a). If a single initiation factor, Met-tRNA or ATP is omitted from the initiation mixture, mRNA does not bind to the 40 S subunit (fig.1b) and no mRNA containing complex appears during centrifugation in the presence of LDS and LiCl (fig.2b–d;3c,d). The latter complex is also not present when LiCl is omitted during centrifugation in the presence of LDS (fig.3a). These observations are compatible with a mechanism of base-pairing between mRNA and 18 S rRNA during initiation. This complex is stable in the presence of LDS and LiCl under conditions where ribosomal proteins

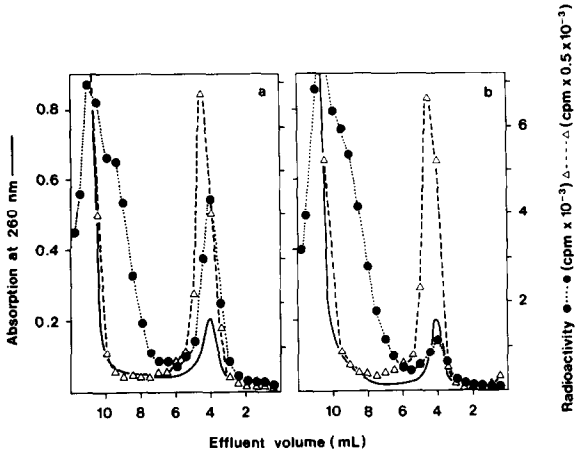

Fig.1. 40 S initiation complexes formed in the presence of all initiation factors (a) and in the absence of eIF-4A (b) analyzed on sucrose density gradients. The reaction mixture (0.15 ml) contained: 15 pmol 40 S subunits, 60 pmol [^{3}H]Met-tRNA (total yeast tRNA charged with methionine; △, 4800 cpm/pmol), 15 pmol globin [^{125}I]-mRNA (•, 3700 cpm/pmol), 0.1 μg eIF-1, 9 μg eIF-2, 18 μg eIF-3, 6 μg eIF-4A, 12 μg eIF-4B, 0.5 μg eIF-4C, 0.5 mM ATP, 0.05 mM β,γ-methylene guanosine 5′-triphosphate, 2.1 mM $MgCl_2$, 0.12 M KCl, 20 mM Hepes buffer (pH 7.3) and 4 mM β-mercaptoethanol. After incubation for 10 min at 37°C the reaction mixtures were chilled on ice and analyzed at 4°C on a 12 ml 5–25% exponential sucrose gradient containing 20 mM Tris–HCl (pH 7.6), 0.1 M KCl and 8 mM MgCl. Centrifugation in a Beckman SW41 Ti rotor was at 40 000 rev./min for 4 h at 4°C. The absorption profile of the gradient (–) was monitored at 260 nm during fractionation and the radioactivity in each fraction was determined.

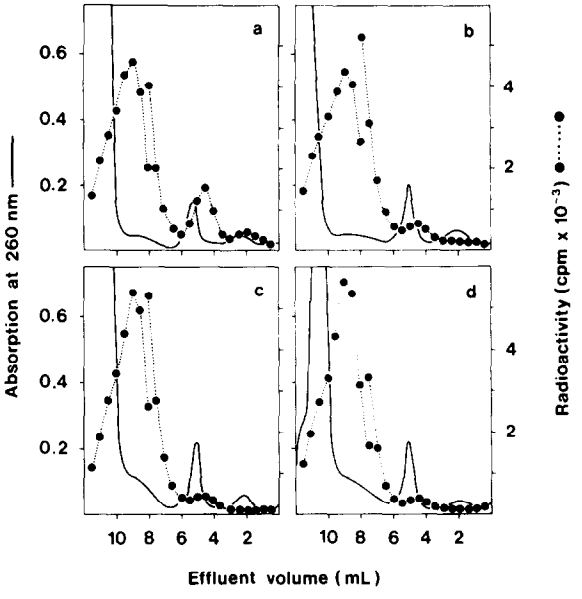

Fig.2. 40 S initiation complexes analyzed on sucrose density gradients in the presence of LDS and LiCl: (a) complete system; (b) minus Met-tRNA; (c) minus eIF-3; (d) minus ATP. Composition and incubation of the mixtures was as indicated in fig.1. To the chilled mixtures 0.15 ml 0.8 M LiCl in 20 mM Tris–HCl (pH 7.6) were added. They were analysed on 12 ml 5–25% exponential sucrose gradients containing 0.4 M LiCl, 20 mM Tris–HCl (pH 7.6) and 0.5% (top) – 0.1% (bottom) LDS with on top a 0.5 ml cushion of 3% sucrose containing Tris–HCl and LiCl but no LDS. Centrifugation in a Beckman SW 41 rotor was at 40 000 rev./min for 7.5 h at 4°C. (•——•) Globin [^{125}I]mRNA (3700 cpm/pmol). At the top of the gradient (left) radioactivity is plotted as measured values/2.

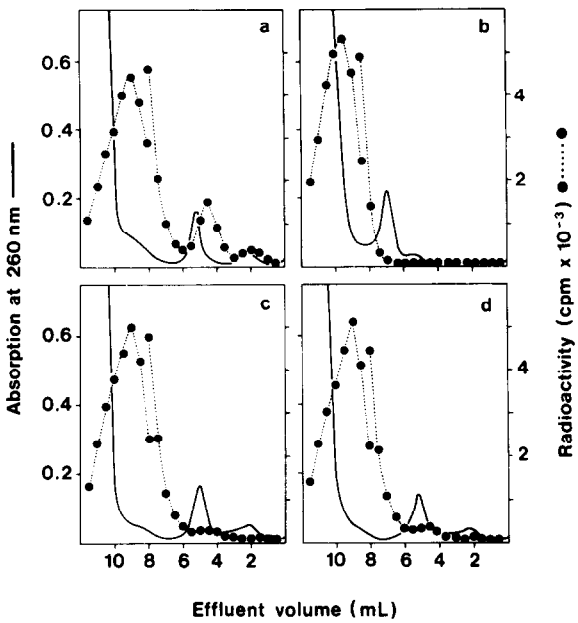

Fig.3. 40 S initiation complexes analyzed on sucrose gradients in the presence of LDS and LiCl (a,c,d): (a,b) complete system; (c) minus eIF-4A; (d) minus eIF-4B. Experiments were performed as in fig.2. Sample (b) was analyzed on a sucrose gradient containing LDS but no LiCl: (•—•) Globin [^{125}I]mRNA (3700 cpm/pmol).

are completely dissociated from rRNA, but it dissociates at low ionic strength as expected of an RNA dimer held together by base pairing (fig.3b). It is obtained only from fully functional 40 S initiation complexes formed in vitro, which upon binding of the 60 S subunit are active in protein synthesis. However, this strong, salt- and detergent-resistant complex can no longer be detected (not shown), when 60 S subunits are added to the 40 S initiation complex, although binding of mRNA is not affected under the (non-denaturing) conditions optimal for in vitro protein synthesis [11].

4. DISCUSSION

Our results indicate that during initiation of protein synthesis in vitro, base-pairing between globin mRNA and 18 S rRNA occurs. Physical proximity between the 5′-region of mRNA and 18 S rRNA has been demonstrated by covalent crosslinking of these two RNAs in initiation complexes [13]. Further, a functional role of base pairing has been inferred from the observed correlation between efficiency of initiation complex formation and extent of sequence complementarity between 18 S rRNA and two viral mRNAs [14]. Such variations in the extent of sequence complementarity of α- and β-globin mRNA with 18 S rRNA could explain, that in the presence of LDS and LiCl only part (40%) of the globin mRNA originally bound to the 40 S subunit (fig.1a) remains associated with the 18 S rRNA (fig.2a) and that α- and β-globin mRNA have different affinities for the ribosome during initiation [15]. Base pairing depends strictly on the presence of initiation factors (fig.2,3) which might explain why no selective binding between 40 S subunits and oligonucleotides from RNase digests of MS2 RNA could be detected [16]. Within the 'scanning model' for initiation of protein synthesis [9], the mRNA sequence complementary to the 18 S rRNA could be the point where the 40 S subunit, migrating along the 5′-end of mRNA, is transiently stalled to allow joining with the 60 S subunit. Subunit joining could reverse the base pairing between mRNA and 18 S rRNA (perhaps by competitive base pairing between rRNAs of the small and large ribosomal subunits, as proposed for prokaryotes, [17,18]) and the 80 S ribosome could continue to move until Met-tRNA$_f^{Met}$ anticodon–AUG codon interaction sets the correct reading frame for polypeptide synthesis. Two observations can be rationalized by such a model of extensive mRNA binding in the 40 S initiation complex, but of weaker binding in the 80 S ribosome:

(1) Regions of mRNA protected against RNase digestion were found to be larger in the 40 S than in the 80 S initiation complex [19–21].

(2) No globin mRNA–18 S rRNA complexes stable in 0.5% LDS and LiCl could be obtained from 80 S initiation complexes although binding of mRNA is obvious under non-denaturing conditions [11].

The functional role of base pairing between mRNA and 18 S rRNA during initiation of protein synthesis in eukaryotes will have to be further confirmed by studies with mRNAs other than globin mRNA and eventually by isolation and characterization of the base-paired complementary sequences.

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