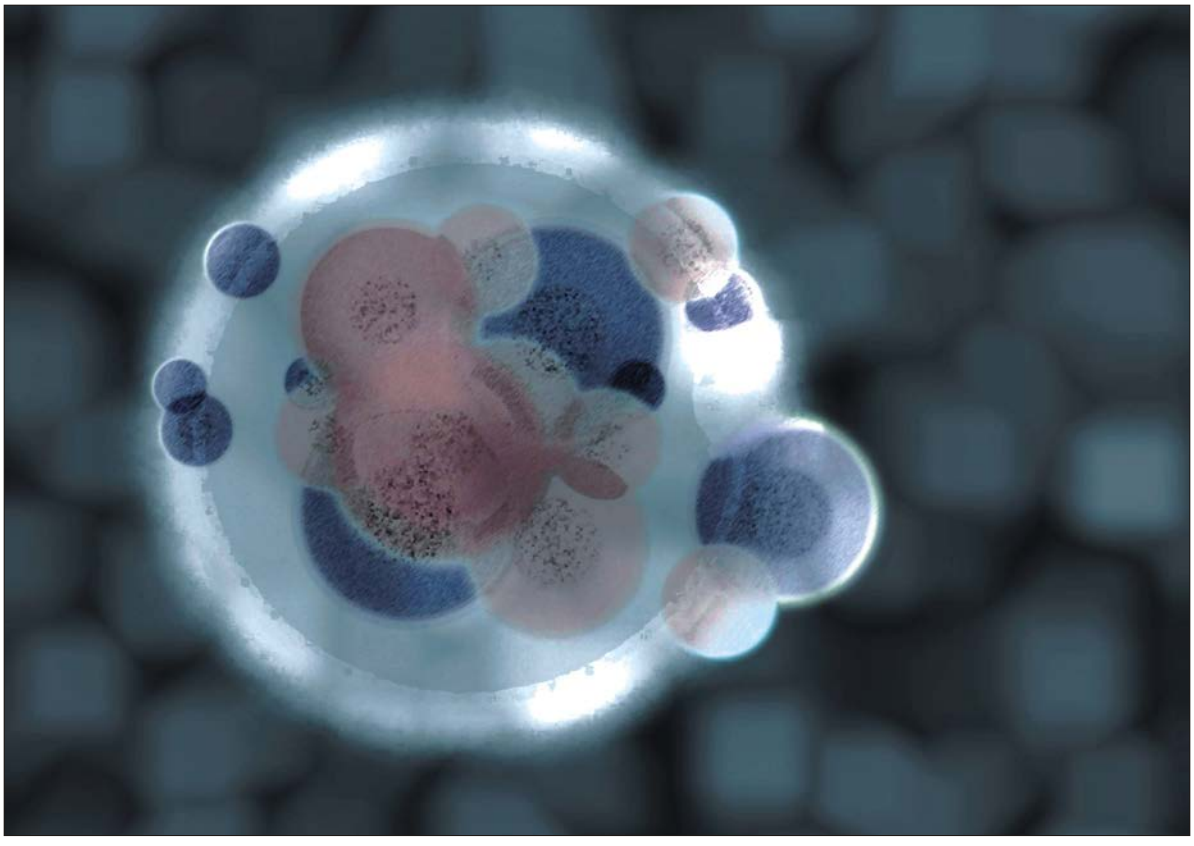

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Signal Recognition Particle Mediated Arrest of Translation Involves Competition with eEF2 on the Ribosome

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Abstract: Signal Recognition Particle (SRP) is a cytosolic particle that is involved in cotranslational transport of presecretory proteins into the endoplasmic reticulum. The *Alu* domain of SRP, comprising the 9 and 14 kDa subunits and part of the 7S RNA, arrests translation. Here we show that SRP14 inhibits translation and employs a highly charged carboxy terminal oligopeptide in doing so. Furthermore, we observed that incubation of SRP14 with ribosomes leads to displacement of elongation factor eEF2. Thus SRP mediated arrest of translation involves competition of SRP14 with elongation factor eEF2 on the ribosome.

Key words: Signal recognition particle, mammalian ER, protein transport, ribosomes, eEF2

INTRODUCTION

Signal Recognition Particle (SRP) is a cytosolic ribonucleoprotein particle that mediates cotranslational transport of presecretory proteins into the Endoplasmic Reticulum (ER) (Wild *et al*., 2004). SRP is a cigar shaped particle and comprises six proteinaceous subunits and a 7S RNA (Wild *et al*., 2004; Siegel and Walter, 1988). Through its 54 kDa subunit (SRP54) SRP recognizes signal peptides of nascent presecretory polypeptides as they emerge from the ribosomal exit tunnel (Kurchalia *et al*., 1986; Krieg *et al*., 1986). Through its 9 plus 14 kDa subunits (SRP9, SRP14) and the *Alu* domain of the 7S RNA SRP arrests elongation until the ribosome/nascent chain-complex has docked to the ER (Siegel and Walter, 1988; Meyer *et al*., 1982). Although the crystal structure of the SRP9/14 heterodimer had been determined the molecular mechanism of translational arrest remained elusive (Birse *et al*., 1997; Weichenrieder *et al*., 2000). A recent structural analysis of SRP-ribosome complexes by cryo-electron microscopy and single particle reconstruction suggested that SRP employs elongation factor mimicry by the *Alu* domain of SRP, i.e., the SRP9/14 heterodimer plus the *Alu* domain of the 7S RNA, as a strategy for translational arrest (Halic *et al*., 2004). However, this analysis did not identify the crucial component within the *Alu* domain.

ERj1p (originally termed Mtj1p) is an ER membrane protein with an ER lumenal J-domain and a cytosolic domain that is in contact with the exit site of translating ribosomes on the ER surface (Dudek *et al*., 2002, 2005; Blau *et al*., 2005). The cytosolic domain of ERj1p as well as a highly charged oligopeptide that is contained in the amino terminus of this cytosolic domain (Fig. 1) have the ability to inhibit translation by an allosteric effect (Dudek *et al*., 2002, 2005). Analysis of the primary structures of SRP9 and SRP14 with the protean option of the DNASTAR sequence analysis software identified an oligopeptide within yeast and mammalian SRP14 that is very similar to the oligopeptide that was characterized as being responsible for the ability of ERj1p to inhibit protein synthesis (Fig. 1). According to the software predictions for the corresponding polypeptides these peptides additionally have in common a high surface probability (data not shown). The crystal structure of the mouse SRP9/14 heterodimer, obtained in the absence of RNA, confirms the surface location of the oligopeptide (Birse *et al*., 1997). Strikingly, it had previously been observed for both yeast and mouse SRP that deletion of the 29 (i.e., amino acid residues 118 through 146) and 20 (i.e., amino acid residues 91 through 110), respectively, carboxy terminal amino acid residues of SRP14 that include the highly charged oligopeptide leads to particles that do not have the ability to arrest translation (Thomas *et al*., 1997; Mason *et al*., 2000). Similarly, deletion of 21 amino terminal amino acid residues of the cytosolic domain of ERj1p led to loss of the ability to inhibit translation ((Dudek *et al*., 2005). Therefore, we proposed that the employment of specifically targeted positively charged oligopeptides that interact with specific domains of rRNA is a unifying theme for a number of ribosomal ligands (Dudek *et al*., 2005). Here, this hypothesis was evaluated with respect to SRP14.

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ERj1p,*M.musculus*	178-192	LLG**RKKR**E**RKKK**TGS
NACβ,*M.musculus*	24- 38	GTA**RRKK**KVV**HR**TAT
Sec62p,*M.musculus*	1- 15	MAE**RRRH**K**KR**IQEVG
Sec62p,*M.musculus*	156-170	PGTP**KKK**ET**KKK**FKL
eIF2β,*M.musculus*	76- 90	FNQ**KKKK**K**K**T**KK**IFD
eIF5B,*M.musculus*	40- 84	SKG**KKKK**E**KKK**QDFD
eIF5B,*M.musculus*	310-324	EGD**KKKK**D**KKKK**KTE
eEF2,*M.musculus*	836-850	AET**RKRK**GL**K**EGIPA
SRP14,*H.sapiens*	91-105	MDGL**KKR**D**KK**N**K**TKK
SRP14,*M.musculus*	91-110	MDGL**KKR**D**KK**N**K**SKKSKPAQ
SRP14,*S.cerevisiae*	118-146	QNLI**KKK**K**KK**S**K**NGTISKTGKKNKVAKKN

Fig. 1: Sequences of highly charged peptides that are present in SRP14 (Birse *et al*., 1997) and ERj1p (Dudek *et al*., 2002), respectively, as well as in various additional ribosomal effectors. The organisms and the positions of the respective oligopeptides within the protein or precursor protein are indicated. In the case of yeast and murine SRP14 the carboxy terminal amino acid residues that were absent from the deletion mutants (Dudek *et al*., 2002; Thomas *et al*., 1997) are presented

RESULTS

Two recombinant hybrid proteins that are related to mouse SRP14, GST-SRP14 and GST-SRP14ΔC (lacking amino acid residues 91 through 110) were analyzed with respect to their ability to inhibit protein synthesis in reticulocyte lysate. Synthesis of preprolactin was sensitive towards GST-SRP14 (Fig. 2a). The inhibitory effect was specific since its extent correlated reciprocally with the ribosome content of the reticulocyte lysate (Fig. 2b) as we had observed for ERj1p (Dudek *et al*., 2002). In comparison, GST-SRP14ΔC and GST did hardly or not at all affect protein synthesis (Fig. 2a), thereby confirming the crucial role of the highly charged carboxy terminal oligopeptide. As we also had observed for ERj1p (Dudek *et al*., 2002), the inhibitory effect of SRP14 was not specific for presecretory proteins, i.e., synthesis of firefly luciferase showed a similar phenotype (data not shown). Thus SRP14 is directly involved in or even responsible for the ability of SRP to arrest translation.

Therefore, we addressed the question of how SRP14 affects translation. When GST-SRP14 was incubated with ribosomes and, subsequently, the ribosomes were reisolated by centrifugation we observed that binding of GST-SRP14 to ribosomes (Fig. 2c) led to the exclusive displacement of a formerly ribosome associated protein (data not shown). In subsequent experiments this protein was characterized as elongation factor eEF2 by amino terminal sequence analysis (data not shown) and by semi-quantitative immunodetection of eEF2 (Fig. 2d). Furthermore, when GST-SRP14ΔC and GST were analyzed in this respect, GST-SRP14ΔC and GST did hardly or not at all bind to ribosomes (Fig. 2c) and there was no displacement of eEF2 (Fig. 2d). Thus the inhibitory effect of SRP14 on translation is due to competition with eEF2 on the ribosome.

DISCUSSION

First of all, our data suggests that SRP14 is responsible for the inhibition of translation by SRP and that SRP inhibits translation by competition of SRP14 with elongation factors for binding to ribosomes. Second, these results support our earlier notion that the employment of specifically targeted positively charged oligopeptides that interact with specific domains of rRNA is a unifying theme for a number of effectors of ribosomes (Dudek *et al*., 2005). With respect to SRP14, this straightforward interpretation of the SRP14 deletion mutants of SRP had originally been dismissed on the basis of two arguments (Thomas *et al*., 1997). Firstly, the deletion affected the tertiary structure of the *Alu* portion of 7S RNA (Thomas *et al*., 1997). Secondly, according to the crystal structure of the mouse SRP9/14 heterodimer, I) Gly93 and Lys95 of SRP14 form hydrogen bonds with amino acid residues of SRP9, and ii) Leu94 of SRP14 is part of the hydrophobic core between the two proteins (Birse *et al*., 1997). We favor the idea that the crystal structure reflects a state of the SRP9/14 heterodimer that would be inactive in translational arrest and that in the case of SRP14 the binding of a signal peptide that is present in a nascent presecretory protein as it emerges from the ribosome to SRP54 affects the structure and/or accessibility of the charged oligopeptide and modulates

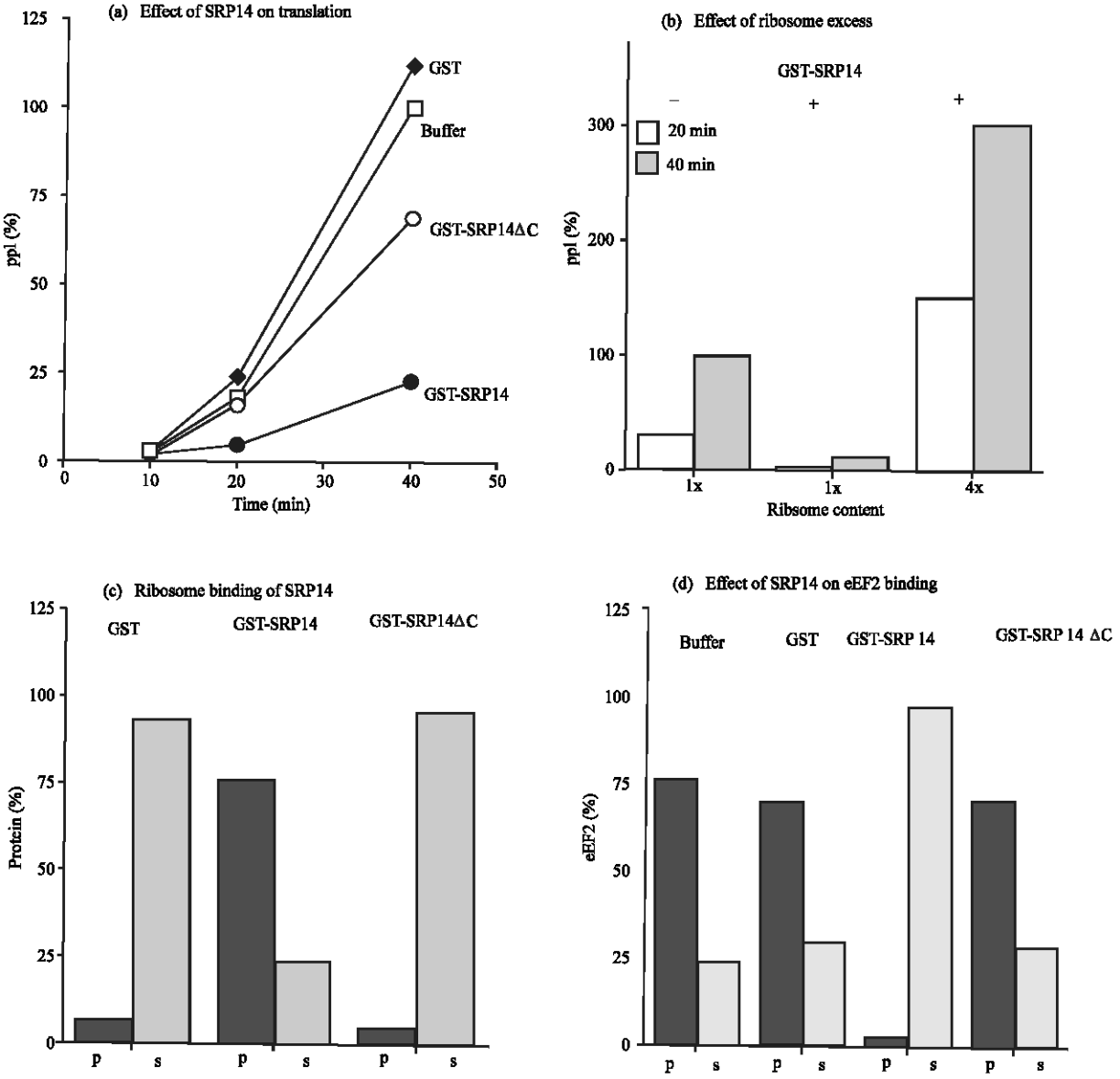

Fig. 2: Effects of SRP14 on ribosomes. GST and the fusion proteins of SRP14 and SRP14ΔC with GST on the amino terminus were produced in *E. coli* and purified as described for ERj1p (Dudek *et al*., 2002). (a,b) Preprolactin was synthesized in rabbit reticulocyte lysate (a) or fractionated lysate (b) in the presence of [^{35}S] methionine as described by Dudek *et al*. (2002). The translation reactions contained either buffer, GST or GST fusion proteins of SRP14 or SRP14ΔC (final concentration: 2 μM). After incubation for various times at 30°C, the translation reactions were subjected to SDS-PAGE. The dried gels were analyzed in a phosphorimager. The amount of full-length radiolabeled preprolactin that was produced at the end of the buffer control reaction was set to 100 percent. We note that the concentration of ribosomes and eEF2 in this reticulocyte based translation reaction is about 150 nM and 300 nM, respectively. (c,d) GST or GST fusion proteins of SRP14 or SRP14ΔC (final concentration: 1 μM) were diluted into buffer and incubated in the presence of ribosomes (final concentration: 50 nM), derived from dog pancreas, for 15 min at 30°C as described Dudek *et al*. (2005). Subsequently the mixture was layered onto a cushion and subjected to centrifugation for 90 min at 100,000 rpm and 2°C (Beckman TLA 120.2 rotor). The ribosomal pellets (p) and postribosomal supernatants (s) were subjected to SDS/PAGE and subsequent protein staining (c) or subsequent electroblotting plus immunodetection (d) and densitometric analysis

the inhibitory activity of SRP14. Accordingly, triggered by binding of a signal peptide to SRP54 the charged oligopeptide within SRP14 could move out of the contact site to SRP9, interact with the ribosome at the eEF2 binding site, and switch off elongation by competing with eEF2. This view is perfectly consistent with the structure of SRP-ribosome complexes (Halic *et al*., 2004). In the SRP14 mutant SRP particles this conformational change, rather than the absence of the carboxy terminal amino acid residues may have affected the conformation of the *Alu* portion of 7S RNA. This view is in fact supported by structural data that were obtained for the human SRP9/14 heterodimer in the presence of parts of the 7S RNA (Weichenrieder *et al*., 2000). Based on these data it was hypothesized that a reversible switch in the folding of the *Alu* domain of SRP, i.e., the SRP9/14 heterodimer plus its associated *Alu* portion of the 7S RNA, may permit alternating onset or release of elongation arrest.

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