Construction of the ‘minimal’ SRP that interacts with the translating ribosome but not with specific membrane receptors in Escherichia coli

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Abstract Escherichia coli signal recognition particle (SRP) consists of 4.5S RNA and Ffh protein. In contrast to eukaryotes, it remains unclear whether translation arrest takes place in prokaryotic cells. To study this problem we constructed a fusion of the M domain of Ffh protein with a cleavable affinity tag. This mutant Ffh, in a complex with 4.5S RNA, can bind signal peptide at the translating ribosome but is unable to bind the membrane. This SRP–ribosome complex should accumulate in the cell if translation is arrested. To test this, the complex was purified from the cells by ultracentrifugation and affinity chromatography. The composition of the complex was analyzed and found to consist of ribosomal RNAs and proteins, the Ffh M domain and 4.5S RNA. The accumulation of this complex in the cell in significant amounts indicated that SRP-mediated translation arrest did occur in bacterial cells. © 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: Signal recognition particle; Ribosome; Translation arrest; Ffh; 4.5S RNA

1. Introduction

The signal recognition particle (SRP) mediates protein export and protein insertion into the cellular membrane. In the case of eukaryotes, the SRP interacts with the ribosome and may cause the arrest of translation [1]. However, it is still unclear whether prokaryotic SRP can mediate translation arrest in a similar manner. The prokaryotic SRP has been shown to be unable to cause translation arrest in the eukaryotic extracts [2], but no data relating to the ribosome–SRP interaction in a homologous prokaryotic system are so far available.

In prokaryotes, the SRP consists of only two components, namely 4.5S RNA [3] and Ffh protein [4], whereby the 4.5S RNA is a much shorter homologue of eukaryotic 7S SRP RNA. Both SRP components are essential for cell viability [5,6]. The lethality, caused by the absence of 4.5S RNA, could be suppressed by several mutations in rRNA [7], but it is not known which ribosomal components are involved in the interaction with SRP. Furthermore, the mechanism of elongation arrest is still obscure. However, up to now attempts to investigate translating ribosome–SRP complex have only been made in an eukaryotic in vitro translation system [2]. Here we describe a mutant ‘minimal’ Escherichia coli SRP which contains only 4.5S RNA and the M domain of Ffh protein fused with affinity tag necessary for the purification of ribosome–SRP particles from living E. coli cells. Such ‘minimal’ SRP will bind the ribosome translating signal peptide but not to membrane receptor. If SRP-mediated translation arrest does exist in E. coli, SRP–ribosomal complex would accumulate in the cell and thus can be isolated and characterized. We have shown the accumulation of such a complex in cells, isolated it and confirmed its composition. This finding favors the existence of the translation arrest in prokaryotes.

2. Materials and methods

2.1. Materials

T4 polynucleotide kinase, T4 DNA polymerase and T4 DNA ligase were purchased from Roche, Germany. Pfu DNA polymerase was purchased from Promega, USA. IgG Sepharose 6 fast flow was purchased from Pharmacia Biotech, Sweden. Nitrocellulose membrane Hybond-N+ was purchased from Amersham, UK.

2.2. Expression of Ffh M domain and isolation of the ribosome–Ffh M–4.5S RNA complex

A fragment encoding the TEV protease cleavage site and ZZ domain was excised from the plasmid pYM10 [8] by digestion with EcoRI (followed by T4 DNA polymerase treatment) and Sall, and cloned to the vector pET33b+ (Novagen) linearized with XhoI, T4 DNA polymerase and Sall. The plasmid obtained was named pET-TEV-ZZ. The gene encoding the Ffh M domain was amplified from total E. coli DNA by Pfu polymerase using the primers GCGGCCATGGCTATTCGTCACATCACTGGC and CCAGGCC-TCGAGGCCGACCAGGGAAGCCTGGGGG. The product was cleaved by NcoI and XhoI endonucleases and cloned into pET-TEV-ZZ cleaved by NcoI and Sall. The Ffh M domain expression vector named pET-FfhM-TEV-ZZ was transformed into the E. coli strain BL21(DE3). Freshly transformed cells growing in LB broth were treated with 1 mM IPTG at 4 h, and allowed to grow for another 2 h. The cells were harvested and lysed by sonication. 10 U of RNase-free DNase I was added to the lysate. After incubation for 5 min at 20°C, the solution was centrifuged for 1 h at 14 000×g. The supernatant, which contains the ribosome fraction, was removed and centrifuged for 16 h at 300 000×g to pellet the ribosomes. The pellet was resuspended in 20 ml of buffer A (25 mM Tris–HCl (pH 7.6), 150 mM KCl, 10 mM MgCl2, 1 mM dithiothreitol) and centrifuged for 18 h at 45 000×g in a 10–30% sucrose gradient. The pellet was resuspended in 2 ml of buffer A. This solution was added to 100 µl of IgG Sepharose 6 fast flow equilibrated in buffer B. After 16 h binding at 4°C the resin was washed three times with 300 µl of buffer A. The complex containing ribosome, Ffh M and 4.5S RNA were cleaved
from the resin by treatment with 10 U of recombinant TEV protease in 100 μl of buffer A for 3 h at 4°C.

2.3. Northern blot hybridization
RNA was separated by electrophoresis in a 1.5% agarose gel. After electroblotting, a nitrocellulose membrane Hybond-N+ was hybridized with 5'[^32P]-TGCATGCATGGTGGGGGCCCTGCCAGC oligonucleotide, which is complementary to the 3'-end of 4.5S RNA. An in vitro transcript of the 4.5S RNA served as a control for the Northern blot analysis.

3. Results and discussion
The Ffh protein (abbreviated from 54 homologues) consists of three domains [9], N, G, and M, listed from the N-terminus (Fig. 1A). The G domain is known to interact with the FtsY receptor in the plasma membrane, thus mediating the steps after translation arrest [10]. The M domain alone has been shown to be sufficient to interact with 4.5S RNA and the signal peptide [11], thus coordinating all SRP activities related to the binding of SRP to the translating ribosome. A ribosome-SRP complex exists only transiently in the cell, just until attachment to the receptor on the membrane has taken place. To prevent the interaction of ribosome-SRP complex with the receptor we decided to express a truncated Ffh gene that encodes only the M domain of the Ffh. We assumed that this truncated protein would form a ‘minimal’ SRP particle and interact with the ribosome. However, the complex of Ffh M domain, 4.5S RNA and the translating ribosome should not be able to proceed to the further steps in the protein export pathway, and should thus accumulate in the cell.

In order to facilitate the purification of the complex by affinity chromatography we introduced a *Staphylococcus aureus* protein A ZZ domain at the C-terminus of the recombi-
nant protein. In addition to the ZZ tag, the recombinant protein carries a His6 sequence C-terminal to the ZZ domain. Further, the affinity tag was separated from the Ffh M domain by a peptide cleavable by TEV protease (Fig. 1B). No additional cleavage sites for TEV protease exist either in the Ffh M domain, or in any of the ribosomal proteins or elongation factors. Thus, the recombinant protein used for in vivo incorporation into the ribosome-SRP complex consists of the Ffh M domain, the TEV cleavage site and the *S. aureus* protein A ZZ domain.

Recombinant protein expression was induced in BL21(DE3) cells by 1 mM IPTG for 2 h. In order to test whether the minimal SRP is formed, can such an SRP interact with translation ribosome and is the complex of minimal SRP with the ribosome really accumulated in the cell, the isolation of SRP-ribosomal complex has been performed. For that purpose firstly the ribosomal fraction was prepared from the Ffh M domain by a peptide cleavable by TEV protease (Fig. 1B). No additional cleavage sites for TEV protease exist either in the Ffh M domain, or in any of the ribosomal proteins or elongation factors. Thus, the recombinant protein used for in vivo incorporation into the ribosome-SRP complex consists of the Ffh M domain, the TEV cleavage site and the *S. aureus* protein A ZZ domain.

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correspondent expression of the full-length Ffh. If there is no translation arrest, no accumulation of the ribosome–Ffh M domain–4.5S RNA should be observed. This experiment indicates for the first time that in prokaryotes the SRP arrests the ribosomal elongation cycle until membrane receptor binding has taken place.

The possibility to isolate the SRP–ribosomal complex with arrested translation offers a unique opportunity to investigate the precise mechanism of elongation arrest by structure probing and cryo-electron microscopy of the complex, both of which are now in progress.

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