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Signal Sequences Activate the Catalytic Switch of SRP RNA

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Abstract

The signal recognition particle (SRP) recognizes polypeptide chains bearing a signal sequence as they emerge from the ribosome, and then binds its membrane-associated receptor (SR), thereby delivering the ribosome–nascent chain complex to the endoplasmic reticulum in eukaryotic cells and the plasma membrane in prokaryotic cells. SRP RNA catalytically accelerates the interaction of SRP and SR, which stimulates their guanosine triphosphatase (GTPase) activities, leading to dissociation of the complex. We found that although the catalytic activity of SRP RNA appeared to be constitutive, SRP RNA accelerated complex formation only when SRP was bound to a signal sequence. This crucial control step was obscured because a detergent commonly included in the reaction buffer acted as a signal peptide mimic. Thus, SRP RNA is a molecular switch that renders the SRP-SR GTPase engine responsive to signal peptide recruitment, coupling GTP hydrolysis to productive protein targeting.

Secretory and transmembrane proteins are delivered to the membrane cotranslationally by the signal recognition particle (SRP) and its membrane-associated receptor (SR) (1). SRP recognizes signal sequences as they emerge from the ribosome (2) and then associates with SR at the membrane where the ribosome is transferred to the translocon. The guanosine triphosphatase (GTPase) domains of SRP and SR mediate this interaction cycle (3). Interaction of SRP with SR leads to the reciprocal stimulation of their GTPase activities, and GTP hydrolysis dissociates the complex (4,5). In *Escherichia coli*, SR is a single protein, FtsY, and SRP consists of 4.5S RNA and a single protein, Ffh (6). 4.5S RNA catalyzes the interaction of Ffh and FtsY, accelerating both on and off rates by a factor of more than 100 (7).

If the energy of GTP hydrolysis is to be harnessed for protein targeting, recruitment of targeting substrates by SRP should be coupled to the SRP-SR interaction cycle. Both signal sequences and 4.5S RNA bind to the M domain of Ffh, which suggests that the catalytic activity of 4.5S RNA could be responsive to signal sequence binding (8). However, under typical assay conditions, 4.5S RNA is constitutively active, negating this role for the RNA (4,7,9,10). A small amount of the nonionic detergent octaethyleneglycol dodecylether (C₁₂E₈) has been used in assays for SRP function, including kinetic characterization of the Ffh-FtsY interaction (4,

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Supporting Online Material

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Materials and Methods

Figs. S1 to S5

Table S1

References

7,9–11). We found that C₁₂E₈ was required for the stimulation of Ffh-FtsY binding rate caused by 4.5S RNA (Fig. 1A and table S1).

Assembly of the Ffh-FtsY complex can be measured by tryptophan fluorescence (7,9). In the presence of 4.5S RNA, C₁₂E₈ stimulated the rate of Ffh-FtsY association by a factor of 70 (Fig. 1A). Likewise, the stimulation of Ffh-FtsY disassembly caused by 4.5S RNA required C₁₂E₈ (faster with C₁₂E₈ than without by a factor of 23; Fig. 1B and table S1). C₁₂E₈ had no effect on the assembly or disassembly reactions in the absence of 4.5S RNA (Fig. 1, A and B). Thus, C₁₂E₈ is not a passive stabilizing additive but “activates” 4.5S RNA to accelerate Ffh-FtsY complex formation. Moreover, as most previous studies characterizing 4.5S RNA catalysis of the Ffh-FtsY interaction were carried out with detergent, they monitored this activated state.

The molecular properties of C₁₂E₈ that are important for activating 4.5S RNA suggested that it acts as a signal peptide mimic. We tested E₈, the nonionic head group of C₁₂E₈, and the detergents cetyltrimethylammonium bromide (CTABr) and sodium dodecyl sulfate (SDS), which share a long carbon chain with C₁₂E₈ but are positively and negatively charged, respectively (Fig. 1, C and D). CTABr stimulated binding similarly to C₁₂E₈, whereas SDS and E₈ did not (Fig. 1D). Thus, the long carbon chain of C₁₂E₈ with a neutral or positively charged head group is sufficient to activate 4.5S RNA. This suggests that C₁₂E₈ acts as a signal peptide mimic, because signal peptides generally have a hydrophobic core and positively but not negatively charged amino acids (12). Additionally, Ffh was crystallized with detergents (13), and density in the signal sequence-binding groove may have been attributable to the detergent. Finally, the Hill coefficient ($n = 5.8$) for C₁₂E₈ stimulation of Ffh–4.5S RNA–FtsY complex formation (fig. S1A) suggested that at least six detergent molecules cooperate to activate each Ffh–4.5S RNA and corresponded well with the size of the putative signal sequence-binding pocket in Ffh (fig. S1B).

We sought to determine whether signal peptides activate 4.5S RNA in the absence of C₁₂E₈. Because most signal peptides are insoluble (14), we chose the ΔEspP signal peptide (15), which is less hydrophobic than most signal peptides. We measured binding of ΔEspP peptide labeled with carboxyfluorescein (ΔEspP-FAM) to Ffh by fluorescence anisotropy. ΔEspP-FAM bound Ffh–4.5S RNA with an equilibrium dissociation constant (K_d) of $1.5 \pm 0.4 \mu\text{M}$ (Fig. 2A). The K_d for Ffh alone was $19.6 \pm 6.4 \mu\text{M}$ (Fig. 2A), which confirms that 4.5S RNA contributes to the binding of signal peptides as predicted (8). The addition of C₁₂E₈ weakened ΔEspP-FAM binding to Ffh–4.5S RNA ($K_d = 5.5 \pm 1.5 \mu\text{M}$) but not to Ffh alone ($21.6 \pm 7.9 \mu\text{M}$) (Fig. 2B), suggesting that ΔEspP and detergent compete for binding to SRP. ΔEspP-FAM did not bind Ffh lacking its signal sequence-binding M domain (Fig. 2A), binding was reversible (fig. S2A), and ΔEspP-FAM did not impair the solubility of Ffh–4.5S RNA (fig. S2, B and C).

To test whether saturating concentrations of ΔEspP stimulate the activity of 4.5S RNA, we used ΔEspP with added lysines at the C terminus to improve its solubility [ΔEspP* (16)]. Like C₁₂E₈, ΔEspP* accelerated Ffh–4.5S RNA–FtsY association (by a factor of >40; Fig. 2C and table S1) and dissociation (by a factor of ~10; table S1) but had no effect in the absence of 4.5S RNA (Fig. 2C and table S1). In the presence of both C₁₂E₈ and ΔEspP*, the rate of Ffh–4.5S RNA–FtsY complex formation was not substantially changed relative to individual additions (table S1). Thus, the ΔEspP peptide and C₁₂E₈ act by the same mechanism.

If ΔEspP* activates 4.5S RNA by associating with SRP, then the rate of Ffh–4.5S RNA–FtsY interaction should correlate with the fraction of ΔEspP*-bound SRP (calculated from the K_d in Fig. 2A). We measured the rate of Ffh–4.5S RNA and FtsY interaction as a function of ΔEspP* concentration (Fig. 2D). When we compared the observed Ffh-FtsY binding rates to

the rate predicted from the fraction of SRP bound to ΔEspP^* (Fig. 2A), the data matched this model exceptionally well (Fig. 2D).

In addition to accelerating Ffh-FtsY association, 4.5S RNA increases the rate of GTP hydrolysis by Ffh^{GTP}-FtsY^{GTP} complexes (4) (fig. S3). However, neither ΔEspP^* nor C₁₂E₈ affected this rate (fig. S3). Thus, signal peptides specifically affect the ability of 4.5S RNA to accelerate Ffh-FtsY complex formation.

To assess the specificity of 4.5S RNA activation, we used a version of ΔEspP^* bearing Phe¹² → Ala and Leu¹⁵ → Thr mutations [$\Delta\text{EspP}(\text{F12A}, \text{L15T})^*$] that reduce SRP-dependent targeting in vivo (15). In the presence of 10 μM $\Delta\text{EspP}(\text{F12A}, \text{L15T})^*$, the 4.5S RNA-stimulated association and dissociation of Ffh and FtsY was slower than that measured with “wild-type” ΔEspP^* by a factor of ~5 (Fig. 3A and table S1). Similar to ΔEspP^* , $\Delta\text{EspP}(\text{F12A}, \text{L15T})^*$ had no effect in the absence of 4.5S RNA (Fig. 3A). To determine whether this was due to reduced binding of $\Delta\text{EspP}(\text{F12A}, \text{L15T})^*$ to SRP, we measured the K_d by fluorescence anisotropy and found that binding was substantially weaker ($K_d = 87 \pm 18 \mu\text{M}$, Fig. 3B). Consistent with this result, increasing concentrations of $\Delta\text{EspP}(\text{F12A}, \text{L15T})^*$ increased the observed rate for SRP-FtsY association (fig. S4).

Thus, SRP RNA acts as a switchable regulatory module at the center of the SRP protein-targeting machine to link recruitment of cargo (a signal peptide) to the next step in the targeting reaction (binding to SR). If free SRP and SR interacted efficiently with each other, they would undergo futile cycles of binding and GTP hydrolysis. Cargo-dependent activation of SRP RNA prevents this, harnessing the energy of GTP hydrolysis for protein targeting.

High-affinity interaction of SRP with ribosomes can occur before SRP interaction with the signal peptide when a short nascent chain is still inside the ribosome, raising the question of how SRP selectively targets signal sequence-containing substrates (17). Our results show that the interaction of the signal peptide with SRP accelerates SRP-SR complex formation, thereby providing a mechanism for selective delivery of appropriate substrates to the membrane. This is conceptually analogous to the kinetic mechanism by which translation achieves fidelity, where cognate codon-anticodon pairing accelerates GTP hydrolysis by elongation factor Tu (EF-Tu) (18,19).

Our results provide an intuitive model for how each step of the targeting process activates the next to achieve productive, directional targeting. Signal peptides bind to SRP's conformationally flexible M domain that forms a continuous surface with SRP RNA (8,13). Binding induces a conformational change that activates SRP RNA (20). Activated SRP RNA facilitates the displacement of the N-terminal helices of SRP and SR that slow their association without SRP RNA (21). This commits the ribosome-nascent chain complex to membrane targeting. The kinetic control described here, where substrate recruitment accelerates downstream interactions, provides a generalizable principle for coordination of multi-step pathways.

Supplementary Material

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Fig. 1.

Detergent activates 4.5S RNA to catalyze the Ffh-FtsY interaction. **(A)** C₁₂E₈ stimulates the binding of Ffh and FtsY only in the presence of 4.5S RNA. Observed binding rates for formation of Ffh-FtsY complexes are plotted as a function of Ffh concentration, [Ffh], in the presence and absence of 4.5S RNA and 185 μM C₁₂E₈. Lines represent fits to the equation $k_{\text{obs}} = k_{\text{off}} + k_{\text{on}}[\text{Ffh}]$. Inset shows the slow reactions on an expanded scale. **(B)** C₁₂E₈ activates 4.5S RNA stimulation of Ffh-FtsY complex dissociation. Dissociation rate constants are plotted in the absence and presence of C₁₂E₈. **(C)** Chemical structures of C₁₂E₈, E₈, CTABr, and SDS. **(D)** Association rate constants for Ffh-4.5S RNA-FtsY complex formation with no detergent, 185 μM C₁₂E₈, 100 μM E₈, 70 μM CTABr, or 100 μM SDS. Error bars in (B) and (D) are SEs of the fits.

Fig. 2.

Δ EspP binds SRP with micromolar affinity and stimulates 4.5S RNA catalysis of Ffh-FtsY interaction. (A) Fluorescence anisotropy of Δ EspP-FAM is plotted as a function of [Ffh]. Lines represent fits to the equation $Anisotropy = Anisotropy_{free} + Anisotropy_{bound}([Ffh]/(K_d + [Ffh]))$. (B) $C_{12}E_8$ increased the K_d of Δ EspP for Ffh–4.5S RNA. K_d values for Δ EspP binding to Ffh from fluorescence anisotropy in the presence and absence of 4.5S RNA are plotted. Dark bars represent K_d in the presence of 185 μ M $C_{12}E_8$. Error bars are SEs of the fits. (C) In the presence of 4.5S RNA, Δ EspP stimulates the association rate for Ffh-FtsY complex formation. Observed rate constants are plotted as a function of [Ffh]. Lines are fits to the equation $k_{obs} = k_{off} + k_{on}[Ffh]$. The dashed line is a reference to the binding rate in the presence of $C_{12}E_8$ from Fig. 1. (D) Δ EspP* activates 4.5S RNA by binding to SRP. Observed rates for 1 μ M Ffh–4.5S RNA binding to 1 μ M FtsY are plotted as a function of Δ EspP* concentration. The dashed line represents the equation $k_{obs} = [(fraction\ bound)(maximum\ stimulated\ rate)] + [(fraction$

unbound)(unstimulated rate)], where the fraction bound was calculated from the K_d measured in (A); $\chi^2 = 5.4 \times 10^{-6}$.

Fig. 3. Mutations in ΔEspP that impair SRP-mediated targeting show decreased binding to SRP and decreased stimulation of 4.5S RNA. **(A)** $\Delta\text{EspP}(\text{F12A}, \text{L15T})^*$ stimulates SRP-FtsY complex formation less than does ΔEspP^* . The dashed line represents the $\Delta\text{EspP}^* + \text{RNA}$ peptide binding rate from Fig. 2C. **(B)** Fluorescence anisotropy of FAM-labeled ΔEspP bearing Phe¹² → Ala and Leu¹⁵ → Thr mutations [$\Delta\text{EspP}(\text{F12A}, \text{L15T})$] is plotted as a function of [Ffh + RNA]. Line is fit as in Fig. 2A.