A molecular recognition feature mediates ribosome-induced SRP-receptor assembly during protein targeting

Yu-Hsien Hwang Fu, Sowmya Chandrasekar, Jae Ho Lee, and Shu-ou Shan

Molecular recognition features (MoRFs) provide interaction motifs in intrinsically disordered protein regions to mediate diverse cellular functions. Here we report that a MoRF element, located in the disordered linker domain of the mammalian signal recognition particle (SRP) receptor and conserved among eukaryotes, plays an essential role in sensing the ribosome during cotranslational protein targeting to the endoplasmic reticulum. Loss of the MoRF in the SRP receptor (SR) largely abolishes the ability of the ribosome to activate SRP-SR assembly and impairs cotranslational protein targeting. These results demonstrate a novel role for MoRF elements and provide a mechanism for the ribosome-induced activation of the mammalian SRP pathway. Kinetic analyses and comparison with the bacterial SRP further suggest that the SR MoRF functionally replaces the essential GNRA tetraloop in the bacterial SRP RNA, providing an example for the replacement of RNA function by proteins during the evolution of ancient ribonucleoprotein particles.

Introduction

Signal recognition particle (SRP) is a universally conserved targeting machine that cotranslationally delivers the majority of membrane and secretory proteins, which compose nearly 30% of the proteome, to the eukaryotic ER or the bacterial plasma membrane (Akopian et al., 2013; Zhang and Shan, 2014). Targeting initiates when SRP recognizes an N-terminal signal sequence or the first transmembrane domain of a nascent polypeptide emerging from the ribosome exit tunnel. The interaction of SRP with the SRP receptor (SR) recruits the ribosome-nascent chain complex (RNC) to the target membrane, where the RNC is unloaded onto the Sec61p (or SecYEG in bacteria) translocation machinery, and the nascent protein is integrated into or translocated across the membrane. The most conserved components of SRP and SR can be found in bacteria, where SRP is composed of a 4.5S RNA tightly bound to the SRP54 protein (named Ffh in bacteria). SRP54 contains a methionine-rich M-domain that binds signal sequences on the nascent polypeptide and a special GTPase domain, termed NG, that dimerizes with a homologous NG-domain in SR (named FtsY in bacteria). The GTP-dependent interaction of SRP with FtsY is extensively regulated by the signal sequence and 4.5S RNA in the bacterial SRP pathway to enable efficient and selective cotranslational protein targeting (Zhang and Shan, 2014; Shan, 2016). Specifically, RNCs bearing a functional signal sequence pre-organize SRP into a conformation in which the conserved GNRA (N is A, C, G, or U; R is A or G) tetraloop of the 4.5S RNA is positioned to contact a basic surface on the NG-domain of FtsY; this contributes a key interaction that enables the rapid recruitment of FtsY in response to recognition of the correct cargo (Zhang et al., 2008; Shen and Shan, 2010; Shen et al., 2011).

SRP has undergone an extensive expansion in size and complexity during evolution. The eukaryotic SRP contains a larger 7SL RNA on which five additional protein subunits (SRP19, SRP68/72, and SRP9/14) are assembled. Recent work showed that the interaction between mammalian SRP and SR is accelerated ~100-fold by the 80S ribosome and 20-fold by the signal sequence (Bacher et al., 1996; Mandon et al., 2003; Lee et al., 2018). The ribosome-induced stimulation is eukaryote-specific, and its underlying molecular mechanism remains incompletely understood. Single-molecule measurements showed that the ribosome unlocks SRP from an autoinhibited state and allows SRP to sample an active conformation that is conducive to SR binding (Lee et al., 2018). On the other hand, multiple studies have implicated the eukaryotic SR in interaction with and sensing the ribosome (Bacher et al., 1999; Fulga et al., 2001; Legate and Andrews, 2003; Mandon et al., 2003; Jadhav et al., 2015).
While bacterial SR is a single protein in which the NG-domain is preceded by two amphipathic lipid-binding helices, eukaryotic SR is a heterodimer of SRα and SRβ subunits. SRβ is a single-pass transmembrane protein anchored at the ER. SRα binds tightly to SRβ via its N-terminal X-domain, which is connected to the NG-domain through an ~200-residue intrinsically disordered linker. Eukaryotic SR cosediments with empty 80S ribosomes, and the SR linker is important in mediating ribosome binding (Mandon et al., 2003). More recently, Jadhav et al. (2015) examined two charged segments in the SR linker, channel binding region (CBR; residues 129–176) and ribosome binding region (RBR; residues 205–250; Fig. 1 A), and suggested that RBR is responsible for ribosome binding.

The importance of the SR linker reflects the expansion of intrinsically disordered protein regions (IDRs) in the proteome during the evolution from bacteria to higher eukaryotes (Ward et al., 2004; Oldfield et al., 2005). In contrast to the canonical structure-function paradigm, IDRs mediate critical cellular processes without assuming a preformed stable structure (Oldfield and Dunker, 2014; Latysheva et al., 2015; Wright and Dyson, 2015). IDRs are characterized by low sequence complexity, low conservation, and biased amino acid compositions that promote disorder (Oldfield et al., 2005; Oldfield and Dunker, 2014). These features often lead to low-affinity, transient interactions of IDRs with their binding partners, allowing IDRs to mediate dynamic cellular processes such as signaling, complex assembly, or lipid-droplet formation (van der Lee et al., 2014). IDRs often exert their functions via molecular recognition features (MoRFs), which provide interaction sites with binding partners (Mohan et al., 2006; Mészáros et al., 2009; Disfani et al., 2012; Cumberworth et al., 2013; Fang et al., 2013; Fung et al., 2018). MoRFs are short (10–70-residue) segments in IDRs that undergo disorder-to-order transitions upon binding and have been proposed to help recruit interaction partners to an IDR-mediated molecular hub (Oldfield et al., 2008). However, the disordered nature of IDRs presents major challenges to the elucidation of their structure, dynamics, and activity, and more work is needed to understand the mechanistic principle by which MoRFs mediate diverse cellular functions.

In this study, we used the recently reconstituted human SRP and SR to examine the mechanism by which the mammalian SR senses and responds to the 80S ribosome during cotranslational protein targeting. We identified a conserved MoRF element in the disordered SR linker and showed that it is responsible for accelerating SRP-SR assembly in response to the ribosome. The role of this MoRF element phenocopies that of the GNRA tetraloop in the bacterial 4.5S RNA, which accelerates SRP-FtsY assembly in response to the RNC, whereas the corresponding tetraloop in the mammalian 7SL RNA has lost this stimulatory role. We propose that the MoRF element in mammalian SR functionally replaces the electrostatic tether provided by the bacterial 4.5S RNA during SRP–SR interaction. This and other observations suggest that many functions of the bacterial SRP RNA have been replaced by protein subunits during the evolution of this ancient ribonucleoprotein particle.

**Results**

A MoRF element in the SR linker is important for SR function

The SR linker contains ~200 residues and is intrinsically disordered. Based on charge distribution and evolutionary conservation, a previous work suggested the presence of two functional segments in the SR linker, CBR (residue 129–176) and RBR (residue 205–250), proposed to regulate the Sec6β–Sec62 interaction and to bind the ribosome, respectively (Jadhav et al., 2015). To identify potential interaction motifs, we analyzed the SR linker sequence using multiple MoRF predictors including ANCHOR, MoRFpred, and MFSPSSMPred (Mészáros et al., 2009; Disfani et al., 2012; Fang et al., 2013). The three algorithms are based on very different approaches. ANCHOR uses a scoring function that estimates the likelihood of sequences to undergo folding upon binding of globular partners. MoRFpred uses a machine-learning algorithm to make predictions based on sequence properties including evolutionary conservation, predicted disorder, and selected physicochemical properties of amino acids such as hydrophobicity and charge. MFSPSSMPred uses an algorithm similar to MoRFpred, but the sequences are prefiltered for conservation. All three programs converged on a predicted MoRF at residues 242–261 (Fig. 1 A). Sequence alignments of SRα from diverse species also showed that the MoRF is the most conserved sequence in the SR linker (Figs. 1 B and S1).

To dissect the functions of the various segments in the SR linker, we generated a set of linker deletion mutants, SRdL, SRdC, SRdR, and SRdM, in which the entire linker, CBR, RBR, and MoRF, respectively, are replaced with (GS), (Fig. 1 C). For in vitro assays, we used a functional soluble SR construct, SRαβΔTM, in which the dispensable N-terminal transmembrane domain of SRβ is removed (Fig. 1 C; Ogg et al., 1998; Lee et al., 2018). We first tested these mutants in a cotranslational protein targeting assay, which examines the ability of recombinant, purified SRP and SR to mediate the targeting and insertion of a model SRP substrate, preprolactin (pPL), into ER microsomes. The microsomes were trypsinized and salt washed (trypsinized, salt washed rough ER microsome [TKRM]) to deplete endogenous SRP and SR (see Materials and methods). Deletion of the SR linker severely disrupted the targeting and translocation of pPL (Fig. 1, C and D). Unexpectedly, despite having the smallest deletion in the SR linker, SRdM displayed a stronger defect in SRP-dependent pPL targeting than SRdC and SRdR (Fig. 1, C and D), indicating that the MoRF element contains residues essential for SR function.

To further dissect the potential interactions of the MoRF, we mutated conserved residues in this element (R246, W248, L259, and Y261) to alanines (Fig. 1 B). Both mutants SR(RW/AA) and SR(LY/AA) exhibited modest defects in pPL targeting, and the combination of all four point mutations, SR(RWL/AA), reproduced the targeting defect of SRdM (Fig. 1, B and E). This result
strongly suggests that the conserved aromatic and charged residues in the SR MoRF mediate key molecular interactions during SRP-dependent protein targeting.

The SR MoRF is important for cotranslational protein targeting in yeast
To test the role of SR MoRF in vivo, we leveraged the fact that this MoRF element, especially its functionally important RW/LΦ residues identified above, is conserved across eukaryotic organisms including diverse yeast strains (Figs. 1 B and S2 A). Using CRISPR-Cas9–based gene editing, we introduced a (GS)₆ linker to replace the MoRF sequence (residues 208–230) of genomic Srp101, the yeast SR homologue, in Saccharomyces cerevisiae strain BY4741 (see Materials and methods). To minimize adaptation of yeast cells bearing mutations in components of the SRP pathway (Ogg et al., 1992; Mutka and Walter, 2001; Jiang et al., 2008), the srp101dM strain was maintained in synthetic minimal medium containing ethanol and glycerol (SCEG). We found that srp101dM cells exhibited a significant growth defect compared with SRP101 cells at both 30°C and 37°C (Figs. 2 A and S2 B),

Figure 1. A conserved MoRF in the SR linker is important for preprotein targeting to ER microsomes. (A) Probability of MoRF elements in the SR linker generated using the sequence analysis software ANCHOR (dashed line), MoRFpred (gray line), and MFSPSSMPred (black line). A schematic representation of the SR linker is shown above the MoRF probability plot and aligned to the residue index. CBR, RBR, and the predicted MoRF (M) are highlighted. Note that the MoRF partially overlaps with RBR. (B) Sequence alignment of the SR MoRF region was generated by T-coffee webserver (Notredame et al., 2000) and plotted using Texshade package (Beitz, 2000). The arrows indicate the four conserved residues R246, W248, L259, and Y261 in the MoRF. (C) Domain structures of WT and mutant SRs used in this study. The transmembrane domain of SRβ was removed to make a soluble SRαβΔTM (Lee et al., 2018), which is denoted as SR for simplicity. In SrDL, SrDC, SrDR, and SrDM, the deleted sequences are replaced by a (GS)₆ linker. (D and E) The effects of SR linker deletions (D) and MoRF point mutations (E) on the cotranslational targeting of pPL to TKRM. Representative SDS-PAGE autoradiography images are shown on the left. pPL and PL denote preprolactin and signal sequence-cleaved prolactin, respectively. Translocation efficiencies were calculated from these autoradiographs and their replicates using Eq. 1 in Materials and methods. All values are reported as mean ± SD, with n ≥ 3.
A MoRF element activates SRP-receptor assembly

Hwang Fu et al., Journal of Cell Biology

A MoRF element activates SRP-receptor assembly

Figure 2. Mutation of the SR MoRF impairs yeast cell growth and protein translocation into the ER in vivo. (A) Representative YPD plates showing the growth of SRP101 and srp101ΔM cells at 30°C and 37°C. (B) Representative SDS-PAGE autoradiography images (left) and quantification (right) of pulse-chase experiments to measure the targeting and translocation efficiencies of the SRP-dependent model substrate DHC-αF in SRP101 and srp101ΔM cells. Successful insertion into the ER results in glycosylation of the substrate (gDHC-αF), which migrates at a higher molecular weight. Translocation efficiencies were calculated from these autoradiographs and their replicates using Eq. 2 in Materials and methods. All values are reported as mean ± SD, with n = 3 biological replicates.

indicating that the SR MoRF is important for supporting yeast cell growth.

To test the effect of the SR MoRF deletion on SRP-dependent protein targeting, we measured the in vivo targeting and translocation of a model substrate, DHC-αF, in which the signal sequence of prepro-α-factor is replaced by the hydrophobic core of the dipeptidyl aminopeptidase B signal sequence to convert it into an SRP-dependent substrate protein (Fig. S2 D; Ng et al., 1996; Cho and Shan, 2018). DHC-αF was efficiently glycosylated upon insertion into the ER (Yabal et al., 2003; Wang et al., 2010; Rao et al., 2016), providing a quantitative readout for its targeting and translocation. To measure the targeting kinetics of newly synthesized proteins, we performed pulse-chase assays coupled to immunoprecipitation of HA-tagged substrate proteins (see Materials and methods; Cho and Shan, 2018). The results showed that, while DHC-αF was rapidly and nearly completely translocated in SRP101 cells, the translocation of DHC-αF was substantially delayed and plateaued at <50% in srp101ΔM cells (Fig. 2 B). Western blot analysis of yeast ER microsomes showed that the observed targeting defect was not due to a lower level of ER-localized SR in srp101ΔM compared with SRP101 cells (Fig. S2 C).

The following observations suggested additional defects in srp101ΔM cells. In control reactions, we tested the insertion of a posttranslationally targeted model protein substrate, BirA-Bos1, into the ER (Cho and Shan, 2018). BirA-Bos1 is a model tail-anchored membrane protein substrate in which the transmembrane domain of the SNARE protein Bos1 is fused to the C-terminus of BirA (Fig. S2 D). Due to their topology, tail-anchored proteins are targeted after translation via SRP-independent pathways. A significant defect of ER targeting and insertion was also observed with BirA-Bos1 in srp101ΔM cells (Fig. S2 E), indicating a general defect in protein insertion into the ER. This is not surprising, as many translocation machineries at the ER are substrates of the SRP pathway; thus, defects of the SRP pathway in srp101ΔM cells would compromise the biogenesis and function of ER in general. In Western blot analysis, we also found that a fraction of Srp101pΔM was proteolyzed to an ~65-kD fragment (Fig. S2 C). Although partial proteolysis of SRα during ER isolation was well characterized (Meyer and Dobberstein, 1980; Hortsch et al., 1985; Lauffer et al., 1985) and the amount of proteolysis (<50%) was insufficient to account for the observed targeting defect of DHC-αF, the higher proteolytic susceptibility of Srp101pΔM suggests a loss of contacts that protect the SR linker. Together, these results show that the SR MoRF is conserved across eukaryotic organisms, and loss of this element leads to large and promiscuous protein translocation defects of the ER in vivo.

SR MoRF and the ribosome synergistically stimulate SRP-SR complex formation

To understand the molecular mechanism by which the SR MoRF impacts the targeting reaction, we asked whether the SR MoRF plays a role in SRP-SR complex formation, the first molecular step that the SR participates in during protein targeting. To this end, we first tested the effect of the SR linker mutations on the reciprocally stimulated GTPase reaction between SRP and SR (Fig. 3 A). The GTPase activity of SRP and SR is stimulated 102-103-fold when they form a complex with one another, providing a convenient readout of their interaction (Peluso et al., 2001; Lee et al., 2018). Pre-steady-state fluorescence measurements of the SRP–SR interaction and comparison with the Michaelis–Menten kinetic constants of their stimulated GTPase reaction showed that, at subsaturating SR concentrations, the GTPase rate constant $k_{cat}/K_m$ is rate-limited by and equal to the rate constant of SRP–SR complex assembly in both the bacterial and mammalian systems (Peluso et al., 2001; Lee et al., 2018). The rate constant at saturating SR concentrations, $k_{cat}$, reports on the rate of GTP hydrolysis from a stably formed SRP–SR complex (Fig. 3 A). As the ribosome and signal sequence are required to activate the SRP–SR interaction (Lee et al., 2018), stimulated GTPase reactions between SRP and SR were measured in the presence of saturating 80S ribosome and engineered SRP(4A10L), in which the M-domain of SRP54 is fused to a 4A10L signal sequence. This generates a ribosome- and signal sequence-bound SRP that fully mimics the effect of the RNC in stimulating SRP-SR assembly (Lee et al., 2018).

To decipher the roles of the individual domains of SR in complex assembly, we further tested two SR domain deletion mutants in addition to the linker mutations described above (Fig. 1 C). SRNG contains only the most conserved NG-domain known to mediate dimerization with the homologous NG-domain in SRP54. In Strdx, the X-domain of SRα is deleted, which also abolishes the SRα–SRβ interaction (Fig. 1 C). As reported recently, mutant Strdx is fully functional in mediating rapid recruitment of SR to ribosome and signal sequence-loaded SRP (Lee et al., 2018; Fig. 3 B). In contrast, deletion of the SR linker severely disrupted the SRP–SR
interaction, reducing the value of $k_{cat}/K_m > 20$-fold (Fig. 3, B and C). The effects of SR linker deletion were similar regardless of whether the SRXβ domain was present (Fig. 3, cf. SRNG vs. SRdL), indicating that the linker sequence functions independently of the Xβ domain complex (Fig. 3, B and C). In contrast, the value of $k_{cat}$ was affected less than twofold by these mutations (Fig. 3, B and D), indicating that the SR linker plays a crucial and specific role in efficient complex formation between SRP and SR but does not substantially affect the GTPase activity of the SRP-SR complex. Importantly, the MoRF deletion led to a similar defect as deletion of RBR or the entire SR linker, reducing the $k_{cat}/K_m$ values $\sim 60$-fold and the $k_{cat}$ value approximately threefold (Fig. 4, A and B, filled circles/bars). In contrast, deletion of CBR led to a modest defect, with an approximately fourfold reduction in $k_{cat}/K_m$ and no effects on $k_{cat}$ (Fig. 4, A and B, filled circles/bars). The similar defects of SRdL, SRdR, and SRdM in this assay strongly suggest that the MoRF element is primarily responsible for the role of SR linker in stimulating efficient SRP-SR assembly.

To test whether the SR MoRF is involved in ribosome-induced stimulation of SRP-SR complex assembly, we measured the stimulated GTPase reactions of signal sequence-bound SRP with WT and mutant SRs in the absence of the 80S ribosome (Fig. 4, A and B, open circles/bars). Notably, while the ribosome strongly stimulated complex formation between SRP and WT SR ($\sim 25$-fold, Fig. 4 C), as reported (Lee et al., 2018), the stimulatory effect of the ribosome was much smaller, approximately threefold, in reactions with mutants SRdL, SRdR, and SRdM (Fig. 4 C). The loss of ribosome-induced stimulation of SRP-SR assembly is similar between these three mutants, indicating that the MoRF...
element is primarily responsible for communication between the SR linker and the ribosome. In contrast, the ribosome still had a 12-fold stimulatory effect in the reaction with SRdC, only approximately twofold reduced from that of the reaction with WT SR (Fig. 4 C, blue vs. black). These results show that the MoRF in SR linker is a key element that mediates the ribosome-induced activation of SRP-SR complex formation.

**MoRF mediates a transient interaction to stabilize the transition state of SRP-SR assembly**

To test whether the ribosome and MoRF also affect the equilibrium and kinetic stability of the SRP-SR complex, we used an established Förster resonance energy transfer (FRET) assay based on a donor dye (Cy3B) labeled at SRP54(K47C) and an acceptor dye (Atto647N) labeled at the C-terminus of SR. To improve the solubility of labeled SR for fluorescence measurements, we used the SRdX construct in which the SR TM, and therefore provides a fully functional mimic of SR

[kon]_f calculated (M⁻¹ s⁻¹) 3.09 ± (0.48) x 10⁶ 1.29 ± (0.04) x 10⁶ 1.68 ± (0.21) x 10⁶ 9.72 ± (0.81) x 10⁶

[koff]_calculated (s⁻¹) 0.220 ± 0.0068 0.010 ± 0.0003 3.7 ± (0.35) x 10⁻³ 4.5 ± (0.35) x 10⁻³

The MoRF does not directly mediate ribosome binding to SR

**The SR MoRF does not directly mediate ribosome binding to SR**

The simplest molecular model to explain the synergistic effects of the MoRF and ribosome act synergistically to stabilize the transition state of SRP-SR assembly (cf. Fig. 5 B vs. Fig. 4 B). Equilibrium titrations using this FRET assay (Fig. S3 C) further revealed that the SRdM mutation modestly weakened the SRP-SR complex, displaying an equilibrium dissociation constant (Kd) 10-fold larger than that of WT SR in the presence of the ribosome (Fig. 5, C and D). In the absence of the ribosome, the mutational effect on Kd is smaller (twofold), reflecting a modest synergy between the MoRF and the ribosome in enhancing the equilibrium stability of the SRP-SR complex (Fig. 5, C and D).

Attempts to measure the SRP-SR association rate constants (kon) using the FRET assay were unsuccessful with SrRdM, because the mutant SR coaggregated with ribosome at concentrations >1-2 µM. We therefore calculated kon from the experimentally determined koff and Kd values (kon = koff/Kd). The calculated kon values are in reasonable agreement with the values of kon/Koff measured from the GTPase reaction and corroborated the conclusions from the enzymatic assay, that is, mutant SrRdM slowed SRP-SR association, specifically in the presence of the ribosome, and lost most of the ribosome-mediated activation during this step (Fig. 5 D).

Collectively, these results show that the SR MoRF strongly accelerates complex assembly between SRP and SR (~200-fold), while exerting more modest effects on the kinetic and equilibrium stability of the SRP-SR complex (10-20 fold). Moreover, all the stimulatory effects of the SR MoRF are largely abolished in the absence of the ribosome. Thus, the MoRF element and 80S ribosome act synergistically to stabilize the transition state during SRP-SR complex formation.

**Figure 5. The SR MoRF accelerates both the assembly and disassembly of the SRP-SR complex in the presence of the ribosome.** (A) Representative time courses for dissociation of the SRP-SR complex with WT SR (gray) and mutant SrRdM (red) in the presence (+80S, closed circles) and absence (-80S, open circles) of the ribosome. The time courses for WT SR in the absence of the ribosome were fitted to a double exponential equation (Eq. 4 in Materials and methods), and dissociation rate constants of the fast phase were reported. All other time courses were fitted to a single exponential equation (Eq. 5 in Materials and methods). (B) Summary of the dissociation rate constants (koff) of the SRP-SR complex formed with WT SR or mutant SrRdM in the presence (solid bars) and absence (open bars) of the ribosome. Values of Kd were derived from the equilibrium titrations in Fig. S2 C. (D) Summary of the rate and equilibrium constants of the SRP–SR interaction for WT SR and mutant SrRdM (dM) in the presence (+80S) and absence (-80S) of the ribosome. All values in B–D are reported as mean ± SD, with n ≥ 3.
Deletion of either the CBR or RBR led to more than twofold reductions in SR-80S binding (Fig. 6, A and C). The folded domains in SR, Xβ, and NG also displayed no detectable 80S binding (Fig. 6, B and C). In contrast, mutant SRdX lacking the Xβ domain complex retained significant ribosome binding. These results suggest that the SR linker is primarily responsible for the interaction of SR with the 80S ribosome, and that both the CBR and RBR in this linker provide important ribosome binding sites. In contrast to the CBR and RBR deletions, deletion of the MoRF led to minimal loss in the 80S binding of SR (Fig. 6, A and C). Thus, the MoRF does not directly recruit the ribosome to SR.

The electrostatic tethering of SRP RNA to SR is lost in the mammalian SRP pathway

Previous work with the bacterial SRP showed that the conserved GNRA tetraloop of the 4.5S RNA forms an electrostatic interaction with a basic surface on the FtsY NG-domain, providing a transient tether that holds SRP and FtsY together to stabilize the transition state of their assembly (Fig. 7 A, left; Zhang et al., 2008; Shen and Shan, 2010; Shen et al., 2011). The kinetic signatures of the SR MoRF are highly reminiscent of those of the 4.5S RNA tetraloop: both elements accelerate the association and dissociation of the SRP-SR complex, with a much smaller impact on the equilibrium stability of the complex (Shen et al., 2011). Moreover, both the SR MoRF and 4.5S RNA tetraloop specifically exert their stimulatory effects in response to the RNC (Shen et al., 2011). The GNRA tetraloop is conserved in the mammalian 7SL RNA. We therefore asked if the electrostatic tethering interaction between this RNA tetraloop and SR is preserved in the mammalian SRP pathway (Fig. 7 A, right).

To address this question, we assembled SRPs carrying mutations in the GNRA tetraloop and tested their effects on SRP-SR assembly using the stimulated GTPase assay between SRP and SR. In bacterial SRP, mutation of the RNA tetraloop from GGAA to UUCG reduces the value of \( k_{cat}/K_m \) ∼ 200-fold (Zhang et al., 2008). Even modest mutations, such as GUAA and GUCG, led to ∼20- and ∼50-fold reductions in \( k_{cat}/K_m \), respectively (Fig. 7 B, Escherichia coli). In contrast, the \( k_{cat}/K_m \) value for the reaction of human SRP with SR was minimally affected by any of these tetraloop mutations (Fig. 7 B, mammalian; and Fig. S5 A). Consistent with the results of the GTPase assays, none of the 7SL tetraloop mutations significantly impaired the targeting of pPL to ER microsomes (Fig. S5 B), in contrast to the deleterious effects of the same mutations in the 4.5S RNA (Zhang et al., 2008).

Comparison of the crystal structures of the bacterial and human SRP-SR NG-domain complex further showed that the cluster of basic residues (K399, R402, and K406) on FtsY that comprise the positively charged surface for interaction with the 4.5S RNA tetraloop is reduced to a single K537 in mammalian SR (Fig. S5 C). Moreover, while mutation of K399 in FtsY reduced the rate of SRP-FtsY complex formation ∼100-fold (Fig. 7 C, E. coli; Shen and Shan, 2010), mutation of the corresponding K537 in mammalian SR had a less than fivefold effect on the rate of SRP-SR assembly (Fig. 7 C, mammalian; and Fig. S5 D). Together, these results show that the mammalian SRP pathway no longer uses the electrostatic tether between the RNA tetraloop and the basic cluster in SR-NG to enable rapid SRP-SR complex formation. Instead, the role of the 4.5S RNA tetraloop is phenocopied by the MoRF element in the SR linker.

Discussion

In this work, we identified and characterized a highly conserved MoRF element in the disordered linker domain of mammalian SR that specifically accelerates SRP-SR complex assembly in
response to the ribosome during cotranslational protein targeting. Deletion or mutations of the SR MoRF led to severe defects in protein targeting and translocation to the ER in vitro and in vivo, and resulted in strong growth defects in yeast. Mechanistic dissections showed that the SR MoRF specifically stimulates the recruitment of SR to cargo-loaded SRP, and that its action is synergistic with that of the 80S ribosome. Intriguingly, the roles of the MoRF element in accelerating SRP-SR assembly phenocopy those of the GNRA tetraloop in the bacterial 4.5S RNA, whereas the corresponding RNA tetraloop in the mammalian SRP has lost this essential role.

The kinetic and equilibrium analyses in this work allowed us to construct a free energy diagram that describes the contributions of the ribosome and MoRF to SRP-SR complex formation in a formal model (Fig. 8 A). In the presence of the ribosome, the MoRF specifically stabilizes the transition state of SRP-SR assembly at ∼3.2 kcal/mol. Both the association and dissociation of the SRP-SR complex are significantly accelerated by the MoRF, whereas the equilibrium of complex formation was affected by only ∼1.4 kcal/mol (Fig. 8 A, left, comparing black and red lines). The effects of the MoRF are largely lost in the absence of the ribosome (Fig. 8 A, right, comparing black and red lines). Reciprocally, the ribosome stabilizes the transition state during complex formation with SRP at ∼3.1 kcal/mol, and has a smaller effect, ∼0.7 kcal/mol, on the equilibrium of complex formation (WT, black line, comparing +80S and −80S); these stimulatory effects of the ribosome are largely abolished upon deletion of the MoRF (dM, red line, comparing +80S and −80S). Thus, the SR MoRF and the 80S ribosome synergistically activate the assembly between SRP and SR, and they exert their effects specifically during the transition state of complex formation.

Stimulation of SRP-SR complex assembly by the 80S ribosome is a eukaryote-specific phenomenon (Bacher et al., 1996; Mandon et al., 2003; Lee et al., 2018), as is the ability of the eukaryotic SR to directly bind the ribosome (Mandon et al., 2003; Jadhav et al., 2015). Based on the direct interaction of eukaryotic SR with the ribosome, it was proposed that the 80S
ribose, by contacting both the SRP and SR, could provide a template on which SRP and SR assemble (Mandon et al., 2003; Jadhav et al., 2015). However, the results here indicate that ribosome binding of free SR is largely uncorrelated with the efficiency of SRP-SR complex assembly or cotranslational protein targeting. While SRdC and SRdR showed similarly low affinities for the ribosome, the stimulated GTPase and targeting activities of SRdC are much higher than those of SRdR. On the other hand, deletion of the SR MoRF had minimal impact on SR-ribose binding but severely disrupted SRP-SR complex assembly and cotranslational protein targeting (cf. Fig. 6 C vs. Figs. 1 D and 4 B). These results ruled out the model that the SR MoRF exerts its stimulatory role by helping to recruit the ribosome. Instead, our results suggest that this element acts at a stage downstream of initial ribosome binding, specifically sensing and transmitting the information from the ribosome to the SRP and SR GTPases to activate their interactions. This could occur by optimizing the positioning of the SR NG-domain with respect to the SRP54-NG near the ribosome exit site to promote their assembly. The enrichment of conserved hydrophobic and aromatic residues in this MoRF also suggests that it participates in key, albeit transient, molecular interactions to exert this positioning effect. The precise interactions mediated by the MoRF remain to be determined.

Intriguingly, mutation of the MoRF in the mammalian SR linker phenocopies the effects of mutations in the 4.5S RNA tetraloop in the bacterial SRP. Both elements (the eukaryotic SR MoRF and the bacterial SRP RNA tetraloop) specifically impact the transition state during SRP-SR assembly, with a much smaller effect on the equilibrium of complex formation (Peluso et al., 2000, 2001; Zhang et al., 2008). The actions of both elements are also strongly synergistic with the cargo (Shen and Shan, 2010; Shen et al., 2011). In bacteria, the RNA tetraloop interacts with the basic surface on the FtsY NG-domain to form a transient electrostatic tether that stabilizes the transition state during complex assembly (Fig. 8 B upper panel; Shen and Shan, 2010; Shen et al., 2011). Mutation of either the charged residues in FtsY or the RNA tetraloop significantly impacts SRP and FtsY interactions (Fig. 7, B and C, E. coli; Zhang et al., 2008; Shen and Shan, 2010). In contrast to the bacterial SRP, the assembly of mammalian SRP and SR is not sensitive to either of these mutations (Fig. 7, B and C, mammalian), indicating that the electrostatic tethering via the RNA tetraloop is no longer employed in the mammalian SRP system. Together with the similarities of the effects of SR MoRF and 4.5S RNA tetraloop, we propose that the transient electrostatic tethering via the RNA tetraloop is no longer employed in the mammalian SRP and SR is not sensitive to either of these mutations (Fig. 7, B and C, mammalian), indicating that the electrostatic tethering via the RNA tetraloop is no longer employed in the mammalian SRP system. Together with the similarities of the effects of SR MoRF and 4.5S RNA tetraloop, we propose that the transient electrostatic tethering via the RNA tetraloop is no longer employed in the mammalian SRP and SR is not sensitive to either of these mutations (Fig. 7, B and C, mammalian), indicating that the electrostatic tethering via the RNA tetraloop is no longer employed in the mammalian SRP and SR is not sensitive to either of these mutations (Fig. 7, B and C, mammalian), indicating that the electrostatic tethering via the RNA tetraloop is no longer employed in the mammalian SRP and SR is not sensitive to either of these mutations (Fig. 7, B and C, mammalian), indicating that the electrostatic tethering via the RNA tetraloop is no longer employed in the mammalian SRP and SR is not sensitive to either of these mutations (Fig. 7, B and C, mammalian), indicating that the electrostatic tethering via the RNA tetraloop is no longer employed in the mammalian SRP and SR is not sensitive to either of these mutations (Fig. 7, B and C, mammalian), indicating that the electrostatic tethering via the RNA tetraloop is no longer employed in the mammalian SRP and SR is not sensitive to either of these mutations (Fig. 7, B and C, mammalian), indicating that the electrostatic tethering via the RNA tetraloop is no longer employed in the mammalian SRP and SR is not sensitive to either of these mutations (Fig. 7, B and C, mammalian), indicating that the electrostatic tethering via the RNA tetraloop is no longer employed in the mammalian SRP and SR is not sensitive to either of these mutations (Fig. 7, B and C, mammalian), indicating that the electrostatic tethering via the RNA tetraloop is no longer employed in the mammalian SRP and SR is not sensitive to either of these mutations (Fig. 7, B and C, mammalian), indicating that the electrostatic tethering via the RNA tetraloop is no longer employed in the mammalian SRP and SR is not sensitive to either of these mutations (Fig. 7, B and C, mammalian), indicating that the electrostatic tethering via the RNA tetraloop is no longer employed in the mammalian SRP and SR is not sensitive to either of these mutations (Fig. 7, B and C, mammalian), indicating that the electrostatic tethering via the RNA tetraloop is no longer employed in the mammalian SRP and SR is not sensitive to either of these mutations (Fig. 7, B and C, mammalian), indicating that the electrostatic tethering via the RNA tetraloop is no longer employed in the mammalian SRP and SR is not sensitive to either of these mutations (Fig. 7, B and C, mammalian), indicating that the electrostatic tethering via the RNA tetraloop is no longer employed in the mammalian SRP and SR is not sensitive to either of these mutations (Fig. 7, B and C, mammalian), indicating that the electrostatic tethering via the RNA tetraloop is no longer employed in the mammalian SRP and SR is not sensitive to either of these mutations (Fig. 7, B and C, mammalian), indicating that the electrostatic tethering via the RNA tetraloop is no longer employed in the mammalian SRP and SR is not sensitive to either of these mutations (Fig. 7, B and C, mammalian), indicating that the electrostatic tethering via the RNA tetraloop is no longer employed in the mammalian SRP and SR is not sensitive to either of these mutations (Fig. 7, B and C, mammalian), indicating that the electrostatic tethering via the RNA tetraloop is no longer employed in the mammalian SRP and SR is not sensitive to either of these mutations (Fig. 7, B and C, mammalian), indicating that the electrostatic tethering via the RNA tetraloop is no longer employed in the mammalian SRP and SR is not sensitive to either of these mutations (Fig. 7, B and C, mammalian), indicating that the electrostatic tethering via the RNA tetraloop is no longer employed in the mammalian SRP and SR is not sensitive to either of these mutations (Fig. 7, B and C, mammalian), indicating that the electrostatic tethering via the RNA tetraloop is no longer employed in the mammalian SRP and SR is not sensitive to either of these mutations (Fig. 7, B and C, mammalian), indicating that the electrostatic tethering via the RNA tetraloop is no longer employed in the mammalian SRP and SR is not sensitive to either of these mutations (Fig. 7, B and C, mammalian), indicating that the electrostatic tethering via the RNA tetraloop is no longer employed in the mammalian SRP and SR is not sensitive to either of these mutations (Fig. 7, B and C, mammalian), indicating that the electrostatic tethering via the RNA tetraloop is no longer employed in the mammalian SRP and SR is not sensitive to either of these mutations (Fig. 7, B and C, mammalian), indicating that the electrostatic tethering via the RNA tetraloop is no longer employed in the mammalian SRP and SR is not sensitive to either of these mutations (Fig. 7, B and C, mammalian), indicating that the electrostatic tethering via the RNA tetraloop is no longer employed in the mammalian SRP and SR is not sensitive to either of these mutations (Fig. 7, B and C, mammalian), indicating that the electrostatic tethering via the RNA tetraloop is no longer employed in the mammalian SRP and SR is not sensitive to either of these mutations (Fig. 7, B and C, mammalian), indicating that the electrostatic tethering via the RNA tetra...
trypsin-digested microsomal membrane (TKRM) to a total volume of <3.5 µl. Reactions were quenched by addition of 2× SDS-loading buffer and boiling after 40 min and analyzed by SDS-PAGE and autoradiography. The efficiency of translocation was quantified from Eq. 1, in which PL and pPL are the integrated intensities for prolactin and preprolactin bands from autoradiography:

\[
\% \text{ translocation} = \frac{PL}{pPL + \frac{I}{6} + pPL} \times 100.
\]  

Construction of SRP101FLAG and srp101dMFLAG strains

Genomic SRP101 in strain BY4741 was replaced with SRP101-FLAG or srp101dM-FLAG (denoted as SRP101 and srp101dM in the text) using CRISPR-Cas9 genome editing (Ryan et al., 2016). First, a DNA fragment containing the SRP101 ORF and the flanking 5′ UTR (524 bp) and 3′ UTR (184 bp) was amplified from yeast genomic DNA (BY4741) and cloned into the pUC19 vector. To insert a C-terminal FLAG tag, pCAS-sgRNAFLAG plasmid encoding Streptococcus pyogenes Cas9 and sgRNA containing a 20-bp reverse guide sequence (5′-TTTGTTGAAT AACATTGTCTG-3′) that targets the sequence 36 bp downstream of the SRP101 ORF was cloned. The guide sequence was designed using Benchling CRISPR analysis tool. A flexible linker sequence (GSGAASG) followed by 1xFLAG sequence (DYKDDK) was inserted at the C-terminus of the SRP101 coding sequence in pUC19, and synonymous codon substitutions were introduced at the sequence targeted by the guide sequence using QuickChange Mutagenesis (Stratagene). The resulting plasmid was used to amplify a DNA repair fragment containing the SRP101-FLAG coding sequence and ~100 bp of the 3′ UTR downstream of the sgRNAFLAG site. pCAS-sgRNAFLAG plasmid and the linear repair fragment were cotransformed into freshly prepared BY4741 competent cells and grown on yeast extract–peptone–dextrose (YPD) + G418 plates at 30°C. Multiple single colonies were cultured and streaked on YPD to ensure the loss of pCAS-sgRNAFLAG plasmid. The SRP101-FLAG strain was verified using PCR and DNA sequencing.

To generate the srp101dM-FLAG strain, pCAS-sgRNAFLAG plasmid encoding a guide RNA sequence (5′-TTTGTTGAAT AACATTGTCTG-3′) was designed to target nucleotide 626 in the SRP101 coding sequence (5 bp into the MoRF region). To prepare the repair DNA fragment, the coding sequence for the MoRF (residues 208–230) in pUC19-SRP101-FLAG was by replaced by that for a (GS)₆ linker, and synonymous codon substitutions were introduced at the sequence targeted by the sgRNAFLAG guide sequence using QuickChange Mutagenesis. The srp101dM-FLAG coding sequence and the flanking 5′ UTR and 3′ UTR were amplified to produce the linear repair fragment. pCAS-sgRNAFLAG plasmid and the linear repair fragment were cotransformed into the SRP101-FLAG strain and grown on YPD + G418 plates. To prevent adaptation of yeast cells to the MoRF deletion (Ogg et al., 1992; Mutka and Walter, 2001), transformed colonies were picked as soon as they reached ~0.5 mm in diameter. All the subsequent culturing of srp101dM cells were performed in synthetic minimal media with ethanol and glycerol (SCEG). The same procedure was used to generate a WT control SRP101-FLAG strain that contains the same synonymous mutation at the sgRNAFLAG region as the srp101dM-FLAG strain. Both SRP101-FLAG and srp101dM-FLAG strains were verified by DNA sequencing and were stored and grown in SCEG unless otherwise specified.

Yeast growth assay

2-ml cultures of SRP101-FLAG and srp101dM-FLAG cells were grown at 30°C in SCEG to OD₆₀₀ ~0.6. The cells were then diluted to OD₆₀₀ ~0.1, and 2 µl aliquots of 10-fold serial dilutions were spotted onto YPD plates and incubated at 30°C or 37°C for 2 d.

In vivo pulse-chase assay

SRP101-FLAG and srp101dM-FLAG cells were transformed with a pRS316 vector expressing either 3xHA-DHCαF or 3xHA-BirA-Bos1-opsin under the GPD promoter. Transformed cells were grown in SCEG without uracil (-Ura) to OD₆₀₀ ~0.4. The cells were then washed and shifted to synthetic defined media (SD) without uracil (-Ura) and grown at 30°C for 3.5 h (Jiang et al., 2008). Yeast cells were harvested, washed in SD(-Ura-Cys-Met), resuspended in 1 ml SD(-Ura-Cys-Met) to a final density of OD₆₀₀ ~12, and incubated at 30°C for 30 min. Cells were pulse labeled with 100 µCi/ml EasyTag EXPRESS35S protein labeling mix (PerkinElmer) for 2 min and chased with 1 ml SD(-Ura) supplemented with 10 mM cold methionine and 0.5 mM cysteine. 400-µl aliquots were flash frozen in liquid nitrogen at indicated chase time points.

HA-tagged substrate proteins were immunoprecipitated as described previously (Cho and Shan, 2018). In brief, individual aliquots of cells were harvested and treated with 0.3 M NaOH for 3 min at room temperature, washed with water, and lysed in lysis buffer (20 mM Tris-HCl, pH 8.0, 150 mM NaCl, and 2% SDS) by incubating at 65°C for 15 min. Clarified lysate was diluted 20-fold in anti-HA immunoprecipitation buffer (20 mM Tris-HCl, pH 8.0, 150 mM NaCl, and 1% Triton X-100) before loading on to preequilibrated anti-HA magnetic beads (Thermo Fisher Scientific). Following incubation at room temperature for 10 min, the beads were washed with W1 (20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% Triton X-100, and 2 M urea), W2 (20 mM Tris-HCl, pH 8.0, 500 mM NaCl, and 1% Triton X-100), W3 (20 mM Tris-HCl, pH 8.0, 150 mM NaCl, and 0.1% SDS), and W4 (20 mM Tris-HCl, pH 8.0, and 150 mM NaCl). Proteins were eluted by adding 10 µl of 1× SDS buffer and boiling for 5 min and analyzed by SDS-PAGE and autoradiography. Translocation efficiency was quantified using Eq. 2, in which I₉₅₀ and I₉₅₀⁺ are the integrated intensities for the protein substrate and glycosylated protein bands, respectively:

\[
\% \text{ translocation} = \frac{I_{\text{protein}}}{I_{\text{protein}} + I_{\text{protein}}^+} \times 100.
\]  

Western blot

Yeast microsomes were isolated from SRP101-FLAG and srp101dM-FLAG strains as described (Rao et al., 2016). In brief, yeast spheroplasts were made by incubating harvested cells with

Hwang Fu et al.
A MoRF element activates SRP-receptor assembly

Journal of Cell Biology

https://doi.org/10.1083/jcb.201901001
KHepes, pH 7.5, 200 mM KOAc, 5 mM Mg(OAc)₂, 10% glycerol. GTPase reactions were performed in SRP assay buffer (50 mM KHepes, pH 7.5, 200 mM KOAc, 5 mM Mg(OAc)₂, 10% glycerol, 0.01 g/ml yeast extract, and 0.1 g/ml peptone). The spheroplast reaction was quenched on ice and cleaned up through a 1.5% Ficoll 400 cushion. The spheroplasts were resuspended in sorbitol lysis buffer (0.02 g/ml sorbitol, 50 mM Hepes, pH 7.4, 2 mM EDTA, 1 mM DTT, and 1× protease inhibitor cocktail) using a homogenizer. ER microsomes were then purified from lysed spheroplasts through a 1-M sucrose cushion. Purified and washed ER microsomes were pelleted, resuspended to a final concentration of 50–100 U/ml, aliquoted, and flash frozen until ready. Microsome aliquots were boiled for 5 min in 2× SDS buffer immediately after thawing. 0.5–1 units of microsomes were analyzed by SDS-PAGE and immunoblotting using anti-FLAG antibody (Genscript). IRDye 800CW goat anti-mouse secondary antibody (LI-COR Biosciences) was used for visualization on an Odyssey imaging system.

GTPase assay
GTPase reactions were performed in SRP assay buffer (50 mM KHepes, pH 7.5, 200 mM KOAc, 5 mM Mg(OAc)₂, 10% glycerol, 2 mM DTT, and 0.02% Nikkol) at 25°C. Reactions were followed and analyzed as described previously (Lee et al., 2018). The reciprocally stimulated GTPase reactions between SRP and SR were measured under multiple turnover conditions using 0.15 µM SRP(4A10L) and 0.2 µM 80S when indicated, varying concentrations of SR, and 100 µM GTP doped with trace γ-32P-GTP (PerkinElmer). The SR concentration dependences of observed rate constants (k_{obsd}) were fitted to Eq. 3, where k_{cat} is the GTPase rate constant at saturating SR concentration and K_m is the SR concentration required to reach half of the maximal observed GTPase rate constant:

\[ k_{obsd} = k_{cat} \times \frac{[SR]}{K_m + [SR]}. \]

Fluorescence FRET measurements
All reactions were measured in SRP buffer supplemented with 0.03% BSA and 0.04% Nikkol at 25°C. All fluorescence measurements used SRs carrying the R458A mutation, which specifically blocks GTP hydrolysis, to enable measurements of the assembly and disassembly of SRP and SR in their GTP-bound state. The values of k_{off} were determined using pulse-chase experiments on a stopped-flow apparatus (Jobin Yvon) with mutant SRs. The SR-SR complexes were preformed using 12.5 nM Labeled SRP(4A10L) and 1 µM WT or mutant SR in the presence of 1 mM GTP, plus 50 nM 80S when indicated. 8 µM unlabeled WT SR was added to initiate complex dissociation. Nonspecific fluorescence change was corrected by subtracting the background signal change measured in a parallel reaction in which the same volume of buffer was added. The time courses of fluorescence change were fitted to Eq. 4 (for WT SR in the presence of ribosome) or Eq. 5 (for all other conditions) to extract the dissociation rate constants (k_{off}). F_{obsd} is the measured donor fluorescence signal, F_{AMP} is the corrected fluorescence change, F_0 is the initial fluorescence value at time zero, and t is time:

\[ F_{obsd} = F_{AMP} \left[ 1 - \exp \left( -k_{off}t \right) \right] + F_0, \]  
\[ F_{obsd} = F_{AMP} \left[ 1 - \exp \left( -k_{off}t \right) \right] + F_0. \]

The equilibrium K_d of SRP-SR complexes were measured on a Fluorolog 3-22 spectrofluorometer. The equilibrium titrations were performed using 12.5 nM Cy3B-labeled SRP, 1 mM GTP, and addition of increasing concentrations of Atto647N-labeled WT or mutant SR. Donor fluorescence was recorded when equilibrium was reached. The signal change was corrected by subtracting the background signal of a control titration with unlabeled SR WT/wtmutants performed in parallel. The fluorescence signal was converted to FRET efficiency (E) according to Eq. 6,

\[ E = 1 - \frac{F_{DA}}{F_{DO}}, \]

in which F_{DA} and F_{DO} are fluorescence signals in the absence and presence of the acceptor, respectively. The SR concentration dependences of E were fitted to Eq. 7 to extract the values of K_d. E_{max} is the value of E at saturating SR concentration:

\[ E = E_{max} \times \frac{[SR]}{K_d + [SR]}. \]

SR-80S cosedimentation assay
Binding reactions were performed in 50 mM KHepes, pH 7.5, 100 mM KOAc, 5 mM Mg(OAc)₂, and 1 mM DTT at 25°C. 400 nM SR was incubated with 150 nM 80S in a 50-µl reaction for 10 min. The mixture was loaded onto a 110 µl 1 M sucrose cushion and ultracentrifuged at 100,000 rpm for 2 h in a TLA100 rotor (Beckman Coulter). The pellet fractions were resuspended in 20 µl of 1× SDS loading buffer. Equal amounts of the total and pellet samples were analyzed on 10% SDS-PAGE gels. The gels were Coomassie stained and scanned on a LI-COR Odyssey imager using a wavelength of 700 nm, and the intensities of the bands of interest were quantified. For SR-X, which do not resolve well from ribosomal proteins, the N-terminally His_6-tagged SRs were detected by Western blot using anti-His_6 mouse antibody (Abcam) and IRDye 800CW goat anti-mouse IgG (H + L; LI-COR), and quantified on the LI-COR Odyssey imager.

Online supplemental material
Fig. S1 shows the sequence alignment of eukaryotic SRa. Fig. S2 shows additional in vivo characterizations of yeast cells harboring the SRdM mutation. Fig. S3 shows characterizations of SR linker mutants in the SRdM construct. Fig. S4 shows replicates of the cosedimentation experiments to measure the binding of SR to the ribosome. Fig. S5 shows the SR concentration dependences of the stimulated GTPase reactions for SRPs harboring mutations in 7SL RNA and for mutant SR-K537.
Acknowledgments

We thank the Shan laboratory members for valuable suggestions and H. Bernstein for sharing canine pancreatic microsomes.
This work was supported by National Institutes of Health grant GM078024 and Gordon and Betty Moore Foundation GBMF2939 to S.-o. Shan.

The authors declare no competing financial interests.

Author contributions: Y.-H. Hwang Fu and S.-o. Shan designed research; Y.-H. Hwang Fu, S. Chandrasekar, and S.-o. Shan performed research; Y.-H. Hwang Fu, S. Chandrasekar, and J.H. Lee contributed new reagents/analytic tools; Y.-H. Hwang Fu, S. Chandrasekar, and S.-o. Shan analyzed data; Y.-H. Hwang Fu and S.-o. Shan wrote the paper; S.-o. Shan supervised the project.

Submitted: 1 January 2019
Revised: 28 June 2019
Accepted: 26 August 2019

References

Akopian, D., K. Shen, X. Zhang, and S.O. Shan. 2013. Signal recognition particle: an essential protein-targeting machine. Annu. Rev. Biochem. 82: 693–721. https://doi.org/10.1146/annurev-biochem-072711-164722
Ataide, S.F., N. Schmitz, K. Shen, A. Ke, S.O. Shan, J.A. Doudna, and N. Ban. 2011. The crystal structure of the signal recognition particle in complex with its receptor. Science. 331:881-886. https://doi.org/10.1126/science.1196473
Bacher, G., H. Lütcke, B. Jungnickel, T.A. Rapoport, and B. Dobberstein. 1996. Regulation by the ribosome of the GTase in the signal-recognition particle during protein targeting. Nature. 381:248–251. https://doi.org/10.1038/381248a0
Bacher, G., M. Pool, and B. Dobberstein. 1999. The ribosome regulates the GTase of the β-subunit of the signal recognition particle receptor. J. Cell Biol. 146:723–730. https://doi.org/10.1083/jcb.146.4.723
Beckert, B., A. Kedrov, D. Sohmen, G. Kempf, K. Wild, I. Sinning, H. Stahlberg, D.N. Wilson, and R. Beckmann. 2015. Translational arrest by a prokaryotic signal recognition particle is mediated by RNA interactions. Nat. Struct. Mol. Biol. 22:767–775. https://doi.org/10.1038/nsmb.3086
Beitz, E. 2000. TExshade: shading and labeling of multiple sequence alignments using LATEX2 epsilon. Bioinformatics. 16:135–139. https://doi.org/10.1093/bioinformatics/16.2.135
Cho, H., and S.O. Shan. 2018. Substrate relay in an Hsp70-cochaperone cascade safeguards tail-anchored protein targeting. EMBO J. 37: e99264. https://doi.org/10.15252/embj.201899264
Cumberworth, A., G. Lamour, M.M. Babu, and J. Gsponer. 2013. Promiscuity and functional domain on one side of the protein. RNA. 19:969–979. https://doi.org/10.1006/jmbi.2000.4042
Dobberstein, B. 1985. Topology of signal recognition particle receptor in endoplasmic reticulum membrane. Nature. 318:334–338. https://doi.org/10.1038/318334a0
Fung, H.Y.J., M. Birol, and E. Rhoades. 2018. IDPs in macromolecular complexes: the roles of multivalent interactions in diverse assemblies. Curr. Opin. Struct. Biol. 49:36–43. https://doi.org/10.1016/j.sbi.2017.12.007
Halic, M., M. Gartmann, O. Schlenker, T. Mielke, M.R. Pool, I. Sinning, and R. Beckmann. 2006. Signal recognition particle receptor exposes the ribosomal translocon binding site. Science. 312:745–747. https://doi.org/10.1126/science.1124864
Hortsch, M., D. Avossa, and D.J. Meyer. 1985. A structural and functional analysis of the docking protein. Characterization of active domains by proteolysis and specific antibodies. J. Biol. Chem. 260:937–945.
Jadhav, B., M. McKenna, N. Johnson, S. High, I. Sinning, and M.R. Pool. 2015. Mammalian SRP receptor switches the Sec61 translocase from Sec62 to SRP-dependent translocation. Nat. Commun. 6:10133. https://doi.org/10.1038/ncomms10133
Jiang, Y., Z. Cheng, E.C. Mandon, and R. Gilmore. 2008. An interaction between the SRP receptor and the translocon is critical during cotranslational protein translocation. J. Cell Biol. 180:1149–1161. https://doi.org/10.1083/jcb.200701196
Kobayashi, K., A. Jomaa, J.H. Lee, S. Chandrasekar, D. Boehringer, S.O. Shan, and N. Ban. 2018. Structure of a pretranslocon mammalian ribosomal SRP-SRP receptor targeting complex. Science. 360:322–327. https://doi.org/10.1126/science.aar7924
Latysheva, N.S., T. Flock, R.J. Weatheritt, S. Chavalil, and M.M. Babu. 2015. How do disordered regions achieve comparable functions to structured domains? Protein Sci. 24:909–922. https://doi.org/10.1002/pro.2674
Lauffer, L., P.D. Garcia, R.N. Hanks, I. Coussens, A. Ulrich, and P. Walter. 1985. Topology of signal recognition particle receptor in endoplasmic reticulum membrane. Nature. 318:334–338. https://doi.org/10.1038/318334a0
Lee, J.H., S. Chandrasekar, S. Chung, Y.H. Hwang Fu, D. Liu, S. Weiss, and S.O. Shan. 2018. Sequential activation of human signal recognition particle by the ribosome and signal sequence drives efficient protein targeting. Proc. Natl. Acad. Sci. USA. 115:ES5487–ES5496. https://doi.org/10.1073/pnas.1802252115
Legate, K.R., and D.W. Andrews. 2003. The β-subunit of the signal recognition particle receptor is a novel GTP-binding protein without intrinsic GTPase activity. J. Biol. Chem. 278:27712–27720. https://doi.org/10.1074/jbc.M302152200
Mandon, E.C., Y. Jiang, and R. Gilmore. 2003. Dual recognition of the ribosome and the signal recognition particle by the SRP receptor during protein targeting to the endoplasmic reticulum. J. Cell Biol. 162:575–585. https://doi.org/10.1083/jcb.200303143
Mary, C., A. Scherrer, L. Huch, A.K. Lakkaraju, Y. Thomas, A.E. Johnson, and K. Strub. 2010. Residues in SRP9/14 essential for elongation arrest activity of the signal recognition particle define a positively charged functional domain on one side of the protein. RNA. 16:969–979. https://doi.org/10.1066/j.nra.2010.03005
Mészáros, B., I. Simon, and Z. Dösztányi. 2009. Prediction of protein binding regions in disordered proteins. PLOS Comput. Biol. 5:e1000376. https://doi.org/10.1371/journal.pcbi.1000376
Meyer, D.J., and B. Dobberstein. 1980. A membrane component essential for vectorial translocation of nascent proteins across the endoplasmic reticulum: requirements for its extraction and reassociation with the membrane. J. Cell Biol. 87:498–502. https://doi.org/10.1083/jcb.87.2.498
Mohar, A., C.J. Oldfield, P. Radijovac, V. Vacic, M.S. Cortese, A.K. Dunker, and V.N. Uversky. 2006. Analysis of molecular recognition features (MoRFs). J. Mol. Biol. 362:1043–1059. https://doi.org/10.1016/j.jmb.2006.07.087
Murka, S.C., and P. Walter. 2001. Multifaceted physiological response allows yeast to adapt to the loss of the signal recognition particle-dependent protein-targeting pathway. Mol. Biol. Cell. 12:577–588. https://doi.org/10.1090/mbc.12.3.577
Ng, D.T.W., J.D. Brown, and P. Walter. 1996. Signal sequences specify the targeting route to the endoplasmic reticulum membrane. J. Cell Biol. 134:269–278. https://doi.org/10.1083/jcb.134.2.269
Notredame, C., D.G. Higgins, and J. Heringa. 2000. T-Coffee: A novel method for fast and accurate multiple sequence alignment. J. Mol. Biol. 302: 205–217. https://doi.org/10.1006/jmbi.2000.4042
Ogg, S.C., M.A. Poritz, and P. Walter. 1992. Signal recognition particle receptor is important for cell growth and protein secretion in Saccharomyces cerevisiae. Mol. Biol. Cell. 3:895–911. https://doi.org/10.1090/mbc.3.8.895
Ogg, S.C., W.P. Barz, and P. Walter. 1998. A functional GTase domain, but not its transmembrane domain, is required for function of the SRP receptor beta-subunit. J. Cell Biol. 142:341–354. https://doi.org/10.1083/jcb.142.2.341

Hwang Fu et al.
A MoRF element activates SRP-receptor assembly

Journal of Cell Biology
3318
https://doi.org/10.1083/jcb.201901001
Oldfield, C.J., and A.K. Dunker. 2014. Intrinsically disordered proteins and intrinsically disordered protein regions. Annu. Rev. Biochem. 83: 553–584. https://doi.org/10.1146/annurev-biochem-072711-164947

Oldfield, C.J., Y. Cheng, M.S. Cortese, C.J. Brown, V.N. Uversky, and A.K. Dunker. 2005. Comparing and combining predictors of mostly disordered proteins. Biochemistry. 44:1989–2000. https://doi.org/10.1021/bi047993o

Oldfield, C.J., J. Meng, J.Y. Yang, M.Q. Yang, V.N. Uversky, and A.K. Dunker. 2008. Flexible nets: disorder and induced fit in the associations of p53 and 14-3-3 with their partners. BMC Genomics. 9(Suppl 1):S1. https://doi.org/10.1186/1471-2164-9-S1-S1

Peluso, P., D. Herschlag, S. Nock, D.M. Freymann, A.E. Johnson, and P. Walter. 2000. Role of 4.5S RNA in assembly of the bacterial signal recognition particle with its receptor. Science. 288:1640–1643. https://doi.org/10.1126/science.288.5471.1640

Peluso, P., S.O. Shan, S. Nock, D. Herschlag, and P. Walter. 2001. Role of SRP RNA in the GTPase cycles of Ffh and FtsY. Biochemistry. 40:15224–15233. https://doi.org/10.1021/bi011639y

Rao, M., V. Okreglak, U.S. Chio, H. Cho, P. Walter, and S.O. Shan. 2016. Multiple selection filters ensure accurate tail-anchored membrane protein targeting. elife. 5:e21301. https://doi.org/10.7554/elif.e21301

Ryan, O.W., S. Poddar, and J.H.D. Cate. 2016. Crispr-Cas9 genome engineering in Saccharomyces cerevisiae. Mol. Cell. 62:525–533. https://doi.org/10.1016/j.molcel.2016.07.004

Wang, F., E.C. Brown, G. Mak, J. Zhuang, and V. Denic. 2010. A chaperone cascade sorts proteins for posttranslational membrane insertion into the endoplasmic reticulum. Mol. Cell. 40:1589–171. https://doi.org/10.1016/j.molcel.2010.08.038

Ward, J.J., J.S. Sodhi, L.J. McGuffin, B.F. Buxton, and D.T. Jones. 2004. Prediction and functional analysis of native disorder in proteins from the three kingdoms of life. J. Mol. Biol. 337:635–645. https://doi.org/10.1016/j.jmb.2004.02.002

Wright, P.E., and H.J. Dyson. 2015. Intrinsically disordered proteins in cellular signalling and regulation. Nat. Rev. Mol. Cell Biol. 16:18–29. https://doi.org/10.1038/nrm3920

Yabal, M., S. Brambillasca, P. Soffientini, E. Pedrazzini, N. Borgese, and M. Makarow. 2003. Translocation of the C terminus of a tail-anchored protein across the endoplasmic reticulum membrane in yeast mutants defective in signal peptide-driven translocation. J. Biol. Chem. 278:3489–3496. https://doi.org/10.1074/jbc.M210253200

Zhang, X., and S.O. Shan. 2014. Fidelity of cotranslational protein targeting by the signal recognition particle. Annu. Rev. Biophys. 43:381–408. https://doi.org/10.1146/annurev-biophys-051313-022653

Zhang, X., S. Kung, and S.O. Shan. 2008. Demonstration of a multistep mechanism for assembly of the SRP x SRP receptor complex: implications for the catalytic role of SRP RNA. J. Mol. Biol. 381:581–593. https://doi.org/10.1016/j.jmb.2008.05.049