Minireview

Co-translational protein targeting by the signal recognition particle

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Received 2 November 2004; revised 2 November 2004; accepted 22 November 2004

Available online 28 November 2004

Edited by Gunnar von Heijne and Anders Liljas

Abstract The signal recognition particle (SRP) mediates the co-translational targeting of nascent proteins to the eukaryotic endoplasmic reticulum membrane, or the bacterial plasma membrane. During this process, two GTPases, one in the SRP and one in the SRP receptor (SR), form a complex in which both proteins reciprocally activate the GTPase reaction of one another. The recent crystal structures of the *T. aquaticus* SRP · SR complex show that the two GTPases associate via an unusually extensive and highly cooperative interaction surface, and form a composite active site at the interface. GTPase activation proceeds through a unique mechanism, stimulated by both interactions between the twinned GTP molecules across the dimer interface and by conformational rearrangements that position catalytic residues in each active site with respect to the bound substrates. Distinct classes of mutations have been isolated that inhibit specific stages during SRP–SR complex formation and activation, suggesting discrete conformational stages during formation of the active SRP · SR complex. Each stage provides a potential control point in the targeting reaction at which regulation by additional components can be exerted, thus ensuring the binding and release of cargo at the appropriate time.

1. Introduction

All cells are divided into multiple subcellular compartments, each containing a unique set of proteins that carries out distinct structural and functional roles. However, the synthesis of almost all proteins begins on the ribosome in the cytoplasm. Thus, cells have evolved molecular machineries that deliver proteins to various subcellular compartments either during or shortly after their synthesis, and the efficiency and fidelity of the targeting process are crucial for maintaining the remarkable organization that is essential for life.

The signal recognition particle (SRP) and its receptor (SR) constitute a universally conserved molecular machinery that delivers nascent membrane and secretory proteins to the eukaryotic ER membrane or the bacterial plasma membrane [1–5]. Like many other cellular processes, the targeting reaction involves a series of ordered steps that need to be closely coordinated. First, SRP binds to the signal sequence of a nascent polypeptide as it emerges from the ribosome [6]. The ribosome · nascent chain complex (RNC) is then delivered to the membrane via an interaction between SRP and SR [7,8]. Upon arrival at the membrane, SRP releases the RNC to the translocation apparatus (translocon; [2,9]). Once the RNC is released, SRP and SR dissociate from one another, allowing the SRP and SR components to be recycled [10,11]. Thus, the precise coordination of the various steps in the targeting reaction requires the SRP and SR to switch between multiple functional states in response to cargo occupancy, spatial information, and time constraints.

Such coordination is achieved by two homologous GTPases, one in SRP and one in SR, which together comprise a unique subgroup in the GTPase superfamily [5,12]. However, the regulatory mechanism utilized by the SRP-type GTPases provides a notable exception to the 'GTPase switch' paradigm established for classical GTPases such as Ras, EF-Tu, and Ran [13]. In the "classic" model, a GTPase switches between two distinct conformational states: an active, GTP-bound state and an inactive, GDP-bound state. Inter-conversion between these states allows the GTPase to interact with other macromolecules in temporal succession, thus achieving regulation of a biological process. Inter-conversion between the two states is inherently slow and is facilitated by external effectors, such as guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs). In contrast, for the SRP-type GTPases, no external GEFs or GAPs have been identified. Instead, these GTPases bind nucleotides weakly, and nucleotide dissociation and exchange is very fast [14,15]. Thus, there is no requirement for external GEFs to facilitate their conversion from the GDP to the GTP-bound forms. Moreover, SRP and SR reciprocally activate each other's GTPase activity upon formation of the SRP · SR complex [16]. Thus, there is also no requirement for external GAPs to facilitate their conversion from the GTP-bound to the GDP-bound forms.

These unusual properties of SRP-type GTPases raise intriguing mechanistic questions. First, what is the mechanism by which these two GTPases reciprocally activate each other? And second, how do these GTPases, which in essence have only a single nucleotide-bound state and no external regulatory factors, function as molecular switches to regulate the protein targeting reaction? The latter question arises because under cellular conditions, SRP and SR are predominantly bound with GTP as they enter the targeting cycle, without a stable GDP-bound form. Thus, GTP binding per se cannot be the molecular event that switches these GTPases to the active state. Here, we summarize recent structural and biochemical characterizations of the bacterial SRP and SR GTPases (called Ffh and FtsY, respectively; [17–19]), which elucidate an unusual mechanism of reciprocal GTPase activation, and underscore the remarkably dynamic nature of this activation

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0014-5793/$30.00 © 2004 Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies.
doi:10.1016/j.febslet.2004.11.049

process. These results suggest the interesting possibility that the intrinsic conformational plasticity of these GTPases regulates the protein targeting reaction in the absence of external GEFs or GAPs.

2. Crystallographic analyses of the conserved GTPase core

The domain organization of the SRP-type GTPases is shown schematically in Fig. 1A. Both proteins contain a central GTPase 'G'-domain that shares homology with the classical Ras GTPase fold [20,21]. Four characteristic GTPase sequence motifs (Fig. 1A, I–IV), highly conserved throughout the GTPase superfamily, mediate interactions with the bound guanine nucleotide [22]. In addition, the SRP-type GTPases contain an insertion box domain (IBD) between motifs II and III; this β–α–β–α domain is highly conserved in the SRP-type GTPases, but not present in other GTPase subfamilies. A second unique feature of the SRP-type GTPases is an N-terminal four-helix bundle (Fig. 1A, the N domain) that is tightly packed against the G domain, forming a single structural and functional unit with it called the NG domain. In addition to the shared NG domains, SRP and SR GTPases each contain a unique domain that allows them to carry out their specific roles during protein targeting. The NG domain of Ffh is connected, via a flexible linker, to a methionine rich 'M' domain that contains the binding sites for signal sequences and for the SRP RNA [23,24]. FtsY has an additional N-terminal, acidic 'A' domain that is thought to anchor it peripherally to the membrane via an interaction with phospholipids.

Comparison of the crystal structures of the nucleotide-free, GDP- and GMPPNP-bound forms of individual NG domains of Ffh and FtsY ([20–22,25] and Reyes, C. and Stroud, R.M., manuscript in preparation) showed that both proteins have a wide-open GTP-binding pocket, which is stabilized in the empty state by a network of active-site side chain interactions [20,21]. Significant re-positioning of active site residues occurs upon binding the nucleotide [22]. These structural analyses thus explain the low nucleotide affinities and the stable nucleotide-free forms of the SRP-type GTPases. Unlike most other GTPases, which undergo marked rearrangements in response to GDP and GTP binding, there are only subtle differences between the GMPPNP- and GDP-bound forms of free Ffh and FtsY ([22,25] and Reyes, C. and Stroud, R.M., manuscript in preparation). Therefore, it seems unlikely that the exchange between the GDP- and GTP-bound forms could provide a bimodal molecular switch as observed in other GTPases.

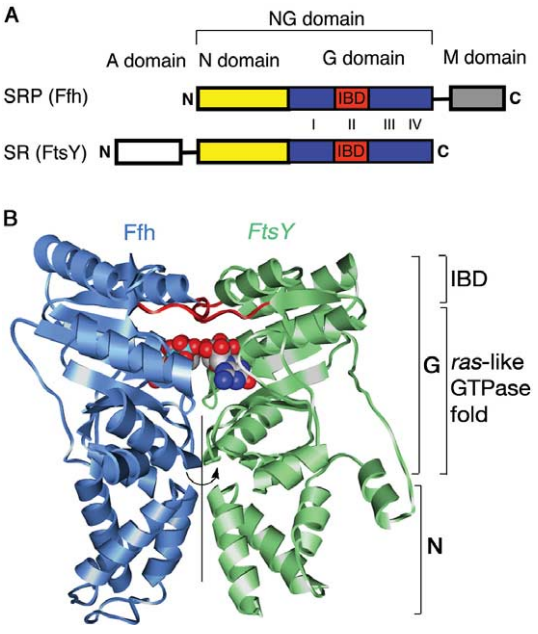

Fig. 1. (A) Domain organization of Ffh and FtsY. I–IV represents the conserved GTPase sequence motifs and IBD represents the insertion box domain unique to the SRP-type GTPases. (B) Crystal structure of the Ffh · FtsY NG domain complex. Ffh and FtsY are shown as blue and green ribbons, respectively, and the two nucleotides are shown as space-filled models. The conserved IBD loop in both proteins is colored in red. Adapted from [17, Figure 1b].

The recent crystal structures of the complex formed between the NG domains of Ffh and FtsY, both bound with the non-hydrolyzable GTP analog GMPPCP, contribute a wealth of new information to our understanding of the mechanism by which complex formation activates both GTPases, and GTP hydrolysis drives dissociation of Ffh from FtsY [17,18]. In particular, the structures reveal several unusual features, such as (i) a remarkably symmetric heterodimer stabilized by an unusually extensive interaction surface; (ii) extensive conformational rearrangements that reposition catalytic residues with respect to the bound substrate; and (iii) a composite active site at the interface, in which the two nucleotides directly interact with each other.

Ffh and FtsY form a quasi-2-fold symmetrical heterodimer through a continuous interaction surface that includes the G and N domains of both proteins (Fig. 1B). The interface is stabilized by 21 hydrogen bonds and 139 van der Waals contacts, burying 3200 $Å^2$ of surface area [17]. In comparison, the surface areas buried in the complexes of Ran and Rap GTPases with their respective GAPs are only ~800–1000 $Å^2$, and the surface areas buried in many antibody–antigen complexes are only ~500–900 $Å^2$, even though these complexes have affinities comparable to, or even higher than that of the Ffh · FtsY complex [26]. Remarkably, biochemical analyses showed that mutation of 25 conserved surface residues at this interface have pronounced deleterious effects on complex formation [17], demonstrating the importance of the extensive interaction surface for the stability of the complex. Presumably, this extensive network of interactions is used to stabilize the conformational changes that occur upon complex formation (see more below).

Two significant conformational rearrangements are observed upon complex formation [17,18]. First, the N- and G-domains of both proteins re-adjust their relative positions, and both N domains bend towards the quasi-2-fold axis to form interface contacts with one another (Fig. 2A). These changes in the N domains are coupled to the G domains through extensive contacts across the N–G domain interface and result in a tighter binding pocket for the guanine base in motif IV of the GTPases. Second, a conserved loop in the IBD of both proteins (Fig. 1B, IBD loop) undergoes major rearrangements as they seal the upper part and lateral entrance of the active sites (Fig. 2B). As a consequence, the two IBD loops move into close proximity with the bound substrates (see Section 3) and contribute additional interactions at the heterodimer interface.

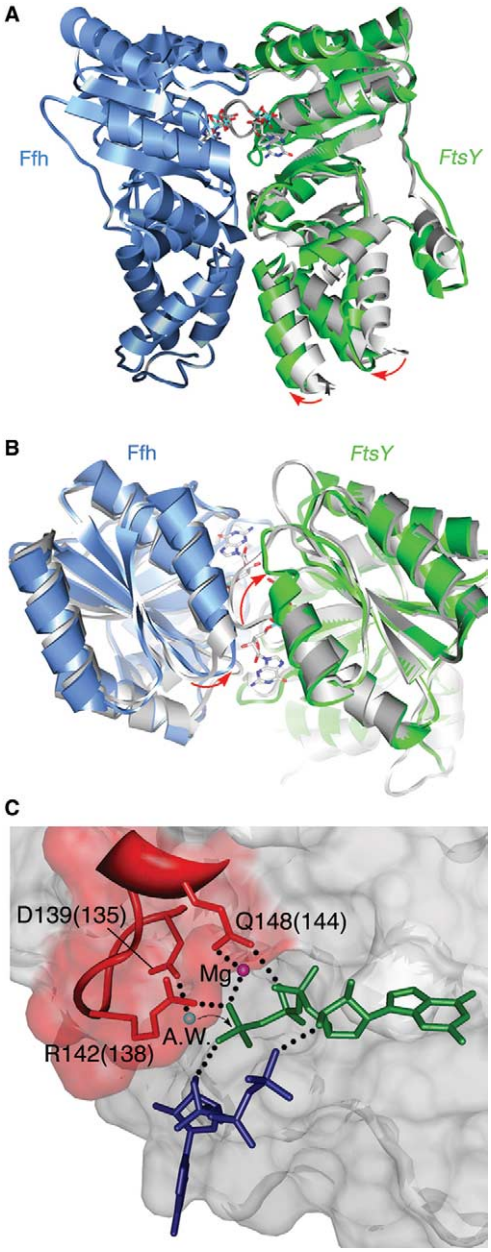

Fig. 2. Conformational rearrangements upon complex formation between Ffh and FtsY. (A) The rearrangement at the N–G domain interface. The structure of the uncomplexed, GMPPNP-bound FtsY (Reyes, C. and Stroud, R.M., in preparation) is shown as a gray ribbon and is superimposed onto the G domain of FtsY in the complex [17]. The red arrows depict the movement of the N domain relative to the G domain. The structure of the individual Ffh protein was not shown for clarity, but a similar N–G domain rearrangement also occurs in Ffh. (B) The rearrangement of the IBD loops. The structures of the individual Ffh and FtsY proteins are depicted as gray ribbons, and superimposed onto the G domains of Ffh and FtsY in the complex, respectively. The red arrows depict the movement of the IBD loops upon complex formation. Adapted from [17, Figure 1c]. (C) Active site interactions involving the IBD loop at the Ffh–FtsY interface. FtsY is in surface representation, the catalytic residues from the IBD loop are depicted as red sticks, the nucleotide bound to FtsY and Ffh are in dark green and dark blue, respectively, and the dotted lines depict hydrogen bonds or van der Waals contacts. The blue ball represents the attacking water molecule (A.W.), the violet red ball represents the active site Mg^{2+}. These catalytic interactions are mirrored in the Ffh active site but are not shown for clarity. Taken from [19, Figure 6b].

At the interface between the two G domains, a composite active site is formed. The two GMPPCP molecules are paired in a head-to-tail manner, forming reciprocal hydrogen bonds between the ribose 3′-OH of one nucleotide and the γ-phosphate oxygen of the other (Fig. 2C). Biochemical analyses, in which each of the 3′-OH groups is replaced by 3′-H, demonstrate that the 3′-OH of each GTP plays a crucial role, contributing 3.0–3.7 kcal mol^{-1} to complex formation and reciprocal GTPase activation [17]. In addition, conformational changes bring several key catalytic residues in the IBD loop into each active site and align them with respect to its bound substrate: Asp135 positions and activates the attacking nucleophilic water molecule, and Arg138 and Gln144 interact with the β- and γ-phosphate groups and the active site Mg to stabilize the transition state (Fig. 2C). Thus, the reciprocal GTPase activation in the Ffh · FtsY complex utilizes a unique mechanism in which each GTPase provides its own catalytic machinery in *cis* and is further stimulated by reciprocal interactions between the twinned GTPs in *trans*. This is in marked contrast to the activation of many other GTPases, whose active site is complemented by insertion of a missing catalytic residue, such as the 'arginine finger', from their respective GAPs ([27] and references therein). Finally, GTP hydrolysis releases the γ-phosphate, and thus severs its extensive connections with both the other substrate and with active site residues. This local perturbation in the highly coordinated interaction network at the complex interface is sufficient to drive dissociation of the complex after GTP hydrolysis.

3. Dynamics of the Ffh–FtsY activation

While structural studies provided us a detailed picture of the catalytic core in the Ffh · FtsY complex, biochemical studies have elucidated the remarkably dynamic nature of this interaction. Through enzymatic analyses of an extensive set of FtsY mutants, we have identified four distinct classes of mutations that map to the interface between the two GTPases and block the Ffh–FtsY interaction at distinct stages (Fig. 3; [19]). Characterization of these mutant proteins, combined with struc-

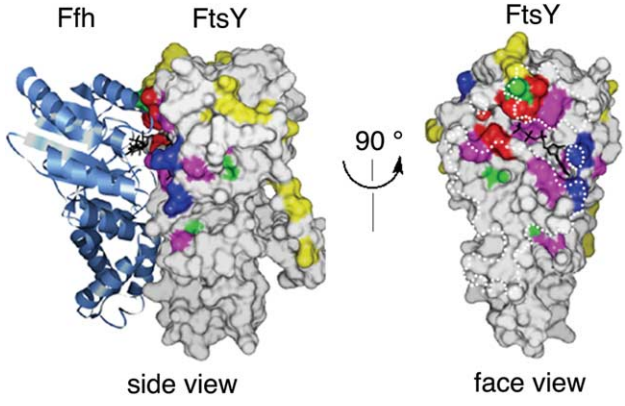

Fig. 3. The mutational effects in *E. coli* FtsY mapped onto the crystal structure of the Ffh · FtsY complex. The bound nucleotides are shown as black sticks and the dotted white lines in the face view outline the contact surface of Ffh with FtsY. The colors denote different classes of mutational effects: blue, mutants defective in complex formation; red, mutants defective in the reciprocal GTPase activation; magenta, mutants defective in both steps; green, mutants exhibiting half-site reactivity; yellow, neutral mutants. Taken from [19, Figure 1].

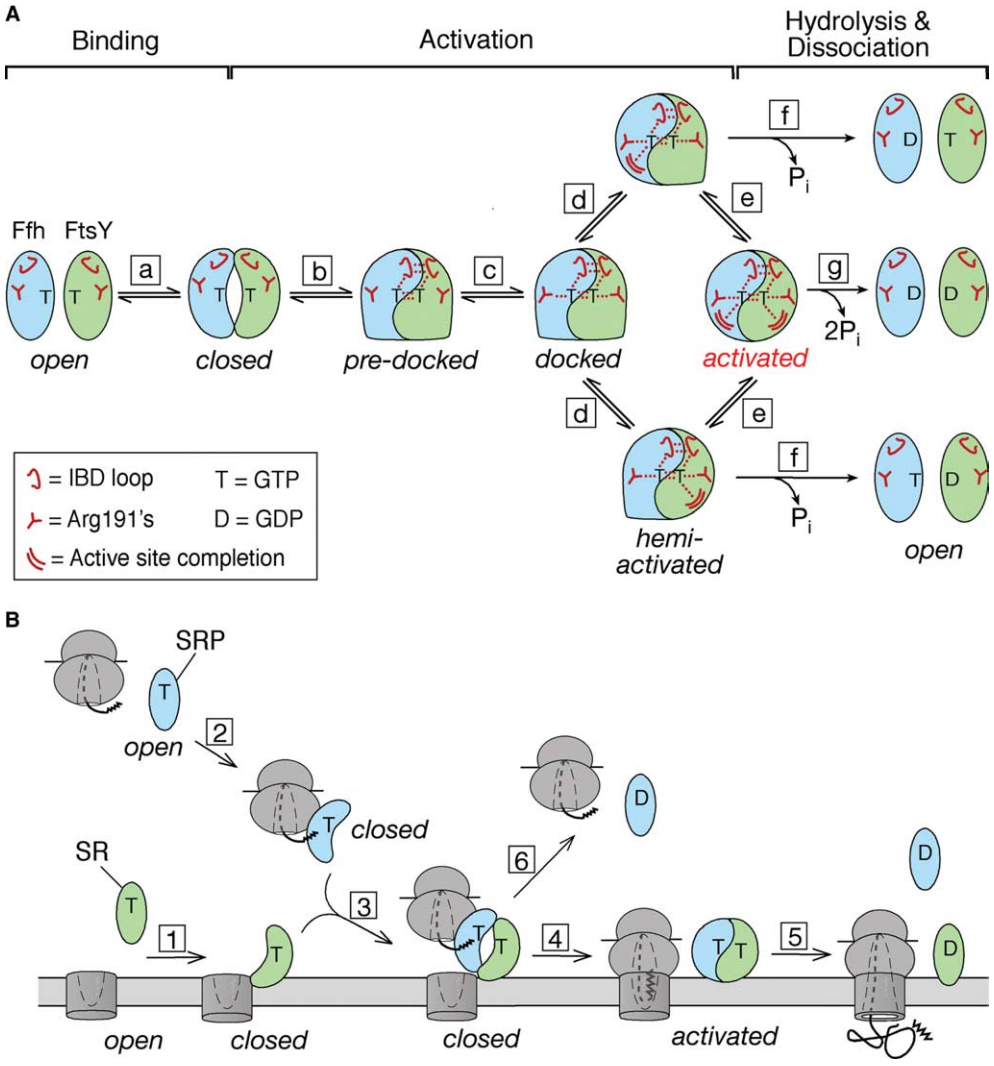

Fig. 4. (A) Model for conformational changes during formation of an activated Ffh–FtsY complex. Step a is the open → closed conformational change during complex formation. Step b is the coordinate docking of the IBD loops into the active sites. Step c is the docking of the Arg191s. Step d is the additional rearrangement of residues that completes one of the GTPase sites. Step e is the rearrangement that completes the other active site. GTP can be hydrolyzed from either the *hemi-activated* complexes (step f) or the *activated* complex (step g) to drive complex dissociation. Taken from [19, Figure 6a]. (B) Conformational changes in the GTPase domains of SRP and SR provide potential regulatory points during the protein targeting reaction. Step 1, SR undergoes an open → closed conformational change upon association with the membrane translocon. Step 2, SRP undergoes an open → closed conformational change upon association with RNC. Step 3, complex formation between SRP and SR delivers the cargo to the membrane. Step 4, cargo release from SRP allows the SRP · SR complex to undergo additional rearrangements to activate GTP hydrolysis. Step 5, SRP dissociates from SR after GTP hydrolysis. Step 6, premature GTP hydrolysis leads to abortive targeting reactions. Adapted from [19, Figure 6e].

tural analyses of the Ffh · FtsY complex, strongly suggest that the reciprocal GTPase activation involves multiple conformational intermediates (Fig. 4A). Here, we summarize the different conformational rearrangements inferred from three classes of mutants.

3.1. Mutation of the nucleotide specificity determinant

A highly conserved Asp residue in motif IV of GTPases forms a hydrogen bonding network with the amino groups on the guanine ring and is known to confer specificity for guanine-based nucleotides. In many GTPases, mutation of this Asp to Asn alters the specificity of the GTPase to that for xanthosine-5′-triphosphate (XTP; e.g., [28–30] and references therein). However, when this mutation was made in FtsY (D449N), we found that free FtsY exhibits little nucleotide discrimination: both the wild type and mutant FtsYs bind and hydrolyze GTP and XTP with efficiencies within 3-fold of each other [31]. In contrast, in the presence of SRP, the mutant FtsY(D449N) exhibits 10^3-fold specificity for XTP relative to wild type FtsY [31]. This mutation primarily affects the complex formation step (Fig. 3, blue). Thus, FtsY acquires the ability to distinguish between cognate and non-cognate nucleotides only when it forms the SRP · FtsY complex.

These observations suggest that upon complex formation, FtsY changes from a floppy, non-specific 'open' state to a more specific, 'closed' state in which the nucleotide is better positioned at the active site and contacts between the guanine ring and Asp449, the nucleotide specificity determinant, are established (Fig. 4A, Step a; 31). Consistent with this notion, crystallographic analysis shows that the rearrangements at the N–G domain interface upon complex formation (see above) allow Asp449 in FtsY to move closer to the guanine

ring and form hydrogen bonds, thus explaining the enhanced nucleotide specificity of FtsY upon complex formation [17]. Therefore, the N–G domain rearrangement is primarily responsible for the 'open' → 'closed' conformational change. The crystal structure also shows that Ffh undergoes a similar N–G domain rearrangement upon complex formation. Thus, both Ffh and FtsY exist predominantly in the 'open' state in their free, uncomplexed form, and complex formation drives the equilibrium towards the 'closed' state (Fig. 4A, step a).

3.2. Activation-defective mutants

A second class of mutants allow stable complex formation but specifically block reciprocal GTPase activation (Fig. 3, red). These mutants suggest that even after a stable, 'closed' complex is formed, activation requires additional conformational changes (the 'docking' process) that align active site residues with respect to the bound nucleotides in both GTPase sites (Fig. 4A, 'closed' →→ 'docked'). Furthermore, as single mutations in FtsY inhibit GTPase activation in *both* active sites, these rearrangements are highly cooperative and bridge the interface between the two GTPases.

Analyses of these mutants in the context of the crystal structure further suggest that the docking event can be broken down into two steps: (i) The concerted rearrangements of the IBD loops (Fig. 4A, step b). This step is inferred from three of the activation-defective mutants that map to the IBD loop. As described in the previous section, this loop can move relatively independently from the rest of the protein and brings multiple catalytic residues into the active site after complex formation. Importantly, as disruption of any of the active site contacts also destroys activation of the other GTPase site, coordinate docking of the IBD loops from both interacting partners into their respective active sites is crucial for reciprocal GTPase activation (Fig. 4A, step b). (ii) The rearrangement of the Arg191s in both GTPases to form the 'docked' complex (Fig. 4A, step c) inferred from another activation-defective mutant, R386(191)A. In contrast to the other mutants in this class, however, the side chains of the Arg191s in both GTPases point away from the γ-phosphate group in the crystal structure. By analogy to the homologous residue Gln61 in the Ras · RasGAP structure, which contacts the γ-phosphate, Focia et al. [18] proposed that the Arg191s are in a 'pending' position, forming a 'latched' structure that requires additional rearrangements to activate the GTPases. The observation that the crystal structure is 'trapped' in a state with the IBD loop docked but with the Arg191s undocked suggests that docking of the IBD loop either precedes that of the Arg191s, as depicted in Fig. 4A, or that these two rearrangements can occur independently of one another.

3.3. Half-site mutants

A third class of mutants specifically block the activation of one, but not the other GTPase site (Fig. 3, green). These mutants break the remarkable coupling between the two GTPase sites in the complex and suggest that, after all the molecular rearrangements required to activate the interacting GTPase have been accomplished, additional rearrangements are required to complete each active site (Fig. 4B, step d). Therefore, these rearrangements occur either late in the docking process, as depicted in the figure, or can occur independently of the various docking steps. In contrast to the docking steps that are tightly coupled between the two active sites, these additional rearrangements can occur independently in one GTPase but not the other, leading to the formation of hemi-activated intermediates (Fig. 4A).

Importantly, all these half-site mutants are less than 2-fold reduced in the rate of multiple turnover GTPase reactions, indicating that multiple cycles of Ffh · FtsY complex formation and dissociation can still occur efficiently. This implies that only one of the two bound GTPs needs to be hydrolyzed in order for the Ffh · FtsY complex to dissociate (Fig. 4A, step f). In the wild type Ffh · FtsY complex, however, both nucleotides are hydrolyzed during each turnover [16]. Thus after a hemi-activated state is formed, rearrangement of the other GTPase site must follow on a time scale faster than the rate of GTP hydrolysis or complex dissociation (Fig. 4A, step e), so that a fully activated complex is formed, and both GTP molecules are hydrolyzed (step g).

4. Model for GTPase regulation during the protein targeting reaction

The conformational intermediates identified above raise the intriguing possibility that instead of distinguishing between the GTP- and -GDP forms or utilizing external regulatory factors, the intrinsic conformational plasticity of the SRP and SR GTPases could provide the molecular switch that regulates the protein targeting reaction. Each of the conformational changes in the GTPase domains of SRP and SR described above could be transmitted to their interaction domains with their cargos – the RNC and the translocon, respectively – thereby coordinating the loading and unloading of cargos at the appropriate stage during the targeting reaction (Fig. 4B).

The switch between the 'open' and 'closed' conformation would provide an attractive regulatory point at which free from cargo-loaded SRP and SR can be distinguished. Interaction with phospholipid membranes and/or the translocon could shift the conformational equilibrium of the SR GTPase from the 'open' to the 'closed' state (step 1), thereby facilitating its interaction with SRP. Analogously, association with the RNC could shift the conformational equilibrium of the SRP GTPase from the 'open' to the 'closed' state (step 2). In this way, the SRP and SR that are pre-bound to their respective cargos are 'primed' to interact with each other, ensuring efficient delivery of cargo proteins to the membrane and avoiding futile cycles of SRP–receptor interactions (step 3).

Once at the membrane, it is crucial that SRP releases its cargo to the translocon before it dissociates from the SRP receptor. As both GTPases reciprocally activate each other, regulation of GTP hydrolysis must involve mechanisms different from regulation by external GAPs as it is the case for classical signaling GTPases. The conformational changes required for GTPase activation (Fig. 4A, steps b–d) provide attractive points to control the relative timing of the cargo release versus the GTP hydrolysis steps. In solution, the SRP · SR complex exists only transiently, with a half-life less than 1 s, because rapid GTP hydrolysis drives complex dissociation [32]. However, binding of RNC could stall the SRP · SR complex in a stable yet inactive 'closed' state, perhaps by inhibiting one of the docking steps (Fig. 4A, steps b–d), thereby ensuring that

GTP hydrolysis is delayed until the cargo is released from SRP (Fig. 4B, step 4). This possibility is supported by the work of Song et al. [33] that suggest that, in the absence of translocon, RNC, SRP, and SR form a stable complex. According to this view, the SRP · SR complex would undergo the additional rearrangements to activate GTP hydrolysis only after release of the cargo (step 5). In this way, cargo delivery would be assured to precede complex dissociation.

The demonstration that hemi-activated complexes can exist and that hydrolysis of a single GTP is sufficient for complex dissociation (Fig. 4A, steps d and f) raises intriguing questions as to the precise role of the individual GTP hydrolysis events during each cycle of the targeting reaction. Asymmetric, half-site hydrolysis could be used to introduce branches into the pathway, leading to abortive targeting reactions (Fig. 4B, step 6). In this way, the GTP hydrolysis events could be used to help enhance the fidelity of the protein targeting reaction.

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