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ATPase and GTPase Tangos Drive Intracellular Protein Transport

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Abstract

The GTPase superfamily of proteins provides molecular switches to regulate numerous cellular processes. The 'GTPase switch' paradigm, in which external regulatory factors control the switch of a GTPase between 'on' and 'off' states, has been used to interpret the regulatory mechanism of many GTPases. However, recent work unveiled a class of nucleotide hydrolases that do not adhere to this classical paradigm. Instead, they use nucleotide-dependent dimerization cycles to regulate key cellular processes. Here, we summarize recent studies of dimeric GTPases and ATPases involved in intracellular protein targeting. We suggest that these proteins can use the conformational plasticity at their dimer interface to generate multiple points of regulation, thereby providing the driving force and spatiotemporal coordination of complex cellular pathways.

Keywords

GTPases; ATPases; protein targeting; signal recognition particle; Get3; molecular recognition and regulation

A class of unconventional, dimerization-activated GTPases

Guanosine-triphosphate (GTP) binding proteins, or GTPases, are a superfamily of proteins that regulate numerous cellular pathways[1–4]. Pioneering work on the extended Ras subfamily of GTPases, exemplified by Ras, Rho, Rab, Arf and heterotrimeric GTPases, has established a 'GTPase switch' paradigm to explain their regulatory mechanism[5, 6]. In this paradigm, a GTPase alternates between a GDP-bound inactive conformation and a GTP-bound active conformation in which they interact with effector molecules to trigger cellular responses. A key to this mechanism is the temporal separation of the GTP- and GDP-states in these proteins due to their intrinsically slow rates of nucleotide exchange and GTP hydrolysis. Thus, inter-conversion between these states requires the recruitment of external factors, such as guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs), to turn these GTPases 'on' and 'off', respectively. Additional layers of regulation can be exerted, such as through Guanine nucleotide dissociation inhibitors that further

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stabilize the GDP-state[7]. Nevertheless, these mechanisms are conceptually extensions of the bi-modal regulatory mechanism established by the classic paradigm.

Despite the importance of Ras-type GTPases, analyses of the evolutionary history of the P-loop GTPases and related ATPases suggested a much larger repertoire of proteins that use the energy from nucleotide triphosphates to regulate cellular processes (Figure 1)[8]. As shown by Leipe et al[8], the GTPase superclass underwent seven evolutionary diversifications after the ancestral GTPase (Figure 1, yellow circles) and can be divided into two large classes. The TRAFAC (after translation factors) class includes proteins involved in translation, signal transduction and cell motility. The SIMIBI (after signal recognition particle (SRP), MinD, and BioD) class includes the SRP54 and SRP receptor (SR) GTPases and MinD-like ATPases involved in protein localization, chromosome partitioning and membrane transport. Notably, the extended Ras subfamily is a small subset of GTPases in the TRAFAC class that emerged after the last evolutionary diversification (Figure 1, *blue*). Compared to Ras-type GTPases, the SIMIBI class of nucleotide hydrolases displays distinct biochemical, structural and dynamic properties, and their regulatory mechanisms are far less understood.

A notable feature of the SIMIBI class of GTPases and related ATPases is their ability to form dimers (Fig. 1A, *red* and Figs. 1B–H). As proposed by Gasper et al, many G proteins are activated by nucleotide-dependent dimerization and can be functionally grouped into a class that uses regulatory mechanisms distinct from the ‘GTPase switch’ paradigm[9]. Readers are referred to the reviews by Gasper et al[9] for a more comprehensive summary of G protein systems (within and beyond the SIMIBI family) that might fall into this class, and Bange et al[10] for a detailed description of the structural features of the SIMIBI family GTPases. A major gap in understanding has been the mechanism by which the dimerization-governed nucleotide hydrolase cycles of these proteins are coupled to biological function. Here, we summarize recent findings from two of the best-characterized systems, the SRP/SR GTPase dimer and the Get3 ATPase dimer, which mediate the co- and post-translational targeting of nascent proteins to cellular membranes, respectively. The results show that these proteins not only function as dimers but also generate multiple, functionally and structurally distinct conformational states in the dimer in an ordered sequence (hence the analogy to ‘tango’ as originally coined by Gasper et al). Each state provides a point of regulation and/or fulfills a distinct function in the respective biological pathway. These observations suggest a novel mode of regulation that might be generalized to this class of dimeric nucleotide hydrolases.

The SRP/SR heterodimer: GTPase tangos drive co-translational protein targeting

SRP and SR mediate a universally conserved protein targeting pathway responsible for the delivery of 25–30% of newly synthesized proteome to the eukaryotic endoplasmic reticulum or the bacterial plasma membrane[11–13]. This process begins when a nascent membrane or secretory protein emerges from a translating ribosome. These proteins carry hydrophobic signal sequences or transmembrane domains (TMDs) near the N terminus that are

recognized, together with the ribosome, by SRP. Via the interaction of SRP with SR, the ribosome•nascent chain complex (RNC) is delivered to the membrane. There, the RNC is transferred to the SecYEG (or Sec61p in eukaryotes) protein translocation channel, or translocon, which allows the nascent protein to integrate into or translocate across the membrane.

Efficient and accurate co-translational protein targeting requires spatial and temporal control, which is provided by the two highly homologous GTPases, SRP54 and SR (Figs. 1B and 2A). Both proteins contain a P-loop GTPase domain, termed the G-domain, which contains the GI–GIV sequence motifs conserved in most GTPases[14, 15]. In both proteins, an N-terminal four helix bundle termed the N-domain packs against the G-domain to form a structural and functional unit called the NG domain (Fig. 2A, top structures)[16, 17]. In addition to the NG-domain, SRP54 contains a methionine rich M-domain (Fig. 2A top, dark blue) that provides binding sites for the SRP RNA and for signal sequences or TMDs on the nascent polypeptide[18–20]. Bacterial SR has an acidic A-domain that mediates interactions with phospholipids[21, 22] and possibly with the SecYEG translocon[23]. Eukaryotic SR is anchored on the ER membrane via association of the SRα subunit (containing the NG-domain) with SRβ, an integral membrane protein [22, 24–26].

Unlike the Ras-type GTPases, free SRP and SR do not exhibit significant conformational changes between the apo-, GDP-, and GTP-bound states[16, 17, 27–29]. Further, their NG-domains contain wide-open nucleotide binding sites (Fig. 2, top panel)[16, 17], consistent with biochemical data showing that these GTPases exhibit weak nucleotide affinities and GDP-GTP exchange rates that are 10^4–10^6-fold faster than those of Ras-type GTPases[30–33]. Thus, there is no need to recruit an external GEF to turn these GTPases to the ‘on’ state. Moreover, the GII motif, which contains multiple catalytic residues, is disordered and suboptimally aligned with the bound GTP in free SRP and SR, consistent with their low basal GTPase rates[33]. Notably, GTP hydrolysis is enhanced >10^4-fold when SRP and SR assemble a complex[33, 34]. Thus, there is also no need to recruit an external GAP to turn these GTPases to the ‘off’ state. These features strongly suggest that SRP and SR use a mode of regulation distinct from the classical GTPase switch paradigm.

A combination of molecular genetics, fluorescence spectroscopy and structural analyses, primarily focused on the bacterial SRP and SR, demonstrated that their GTPase cycle is instead driven by multiple conformational changes during SRP-SR dimerization, which culminates in reciprocal GTPase activation (Fig. 2A). Free SRP and SR are in an inactive ‘*open*’ conformation suboptimal for binding one another (Fig. 2A, top)[33, 35, 36]. Their interaction begins with a transient ‘*early*’ intermediate, which is primarily stabilized by electrostatic attractions between their N-domains and by contacts of the GGAA tetraloop of the SRP RNA with a conserved lysine on the SR G-domain (Fig. 2A, right structure)[37–39]. Subsequent rearrangements in both proteins, involving adjustments at the N-G domain interface, generate a stable ‘*closed*’ complex in which the G- and N-domains of SRP and SR together form an extensive interaction surface (Fig. 2A, bottom structure)[38, 40, 41]. The two GTP molecules also hydrogen bond across the dimer interface, further stabilizing the *closed* complex and conferring its specificity for GTP. Finally, cooperative rearrangements in the GII motifs of both proteins bring key catalytic residues into close contact with the two

bound GTP molecules, forming a composite active site at the dimer interface (Fig. 2A, step 4)[40–42]. These movements of the GII motifs are coupled to an 100 Å movement of the SRP•SR NG-domain dimer from the tetraloop to the 5', 3'-distal site of the SRP RNA, where a conserved cyanine base inserts into and further optimizes the GTPase active site to generate the *'activated'* complex (Fig. 2A, left structure)[43–45]. Stimulated GTP hydrolysis then drives the disassembly and recycling of the complex (Fig. 2A, step 5).

Importantly, each conformational state in the SRP•SR dimer provides a distinct point of regulation at which these GTPases can directly sense and respond to different biological cues in the pathway. For example, assembly of a stable '*closed*' complex between SRP and SR is intrinsically very slow (k_{on} ~10^2–10^3 $M^{-1}s^{-1}$)[33, 36, 46], primarily due to the labile nature of the '*early*' intermediate, >98% of which dissociates before it rearranges into the stable '*closed*' complex[38]. However, RNCs bearing SRP substrates stabilize the '*early*' intermediate >100-fold and thus accelerates assembly of the '*closed*' complex up to 10^3-fold (Fig. 2A, steps 1–2)[47–49]. Rearrangement to the '*closed*' state is further driven by the interaction of SR with anionic phospholipids (Fig. 2A, step 3)[50], allowing a stable RNC•SRP•SR '*closed*' targeting complex to accumulate at the membrane. Finally, the last rearrangement that leads to GTPase activation is strongly inhibited by the RNC[48, 51]; this effect, termed 'pausing', is reversed when the targeting complex contacts the SecYEG translocon (Fig. 2A, step 4)[51, 52]. 'Pausing' serves two roles: (i) as a 'timer' that gives the targeting complex an extended time window to search for the SecYEG complex, minimizing premature GTP hydrolysis which would lead to abortive pathways; and (ii) as a spatial sensor that couples GTP hydrolysis to the successful unloading of cargo at the membrane translocon.

Collectively, these findings provide a high-resolution model for how the GTPase cycle in the SRP•SR dimer provides spatiotemporal coordination of co-translational protein targeting (Fig. 2B). SRP-SR interaction is minimized in the absence of cargo and is initiated only when SRP binds RNCs bearing SRP substrates (steps 1–2). Before engaging the membrane translocon, the RNC•SRP•SR complex is primarily in the '*early*' conformational state in which the RNC is tightly bound to SRP, and GTP hydrolysis is delayed. Interactions of SR with phospholipids and with the SecYEG translocon induce GTPase rearrangements into the '*closed*' and '*activated*' states, in which the SRP•SR NG-domain complex detaches from the ribosome exit site and moves to the distal site of the SRP RNA (steps 3–4). These rearrangements reduces the affinity of the SRP•SR complex for RNC over 30-fold and free up the ribosome exit site for subsequent docking onto the SecYEG translocon, initiating a sequential and coordinated cargo handover event[53] (step 5). The same rearrangement also activates GTP hydrolysis in the SRP•SR complex, driving their disassembly and recycling (step 5). Thus, each conformational change in the SRP•SR GTPase dimer allows it to communicate with the cargo, membrane, and translocon; these allosteric communications provide the driving force for the targeting pathway and have also been shown to generate fidelity checkpoints to enhance the accuracy of substrate selection by SRP[37, 49, 54].

The Get3 homodimer: ATPase tangos drive post-translational protein targeting

Get3 (or TRC40 in mammalian cells) belongs to the ArsA subfamily of ATPases, represented by the arsenic-translocating ATPase ArsA (Fig. 1A). Recent biochemical and genetic analyses showed that in eukaryotic cells, Get3 (and TRC40) mediates the delivery of an essential class of membrane proteins, termed tail-anchored (TA) proteins as their sole TMD resides near the C terminus, to the ER [55–58]. This process, termed the Guided-Entry-of-Tail-Anchored proteins (GET) pathway, begins with the co-chaperone Sgt2 that captures TA proteins released from the ribosome[57]. TA proteins are then transferred from Sgt2 to Get3, bridged by a scaffolding complex consisted of Get4 and Get5 subunits[57]. A receptor complex on the ER membrane, comprising Get1 and Get2 subunits, captures the Get3•TA complex and drives the dissociation of TA proteins from Get3 and its insertion into the membrane[58–61].

Initial structural and biochemical work showed that Get3 forms an obligate dimer whose conformation is regulated by the bound nucleotide. Analogous to SRP and SR, Get3 contains a P-loop nucleotide hydrolase domain in which the bound ATPs face one another at the dimer interface (Fig. 3A, top right structure)[62–64]. Also analogous to the SRP and SR NG-domains, the ATPase domain of Get3 is structurally and functionally coupled to a helical domain (Fig. 3A, top structures). Nucleotide binding adjusts the Get3 dimer interface, which is amplified into larger movements of its helical domains. This leads to various conformations, from more '*open*' states in apo-Get3 in which the helical domains are separated, to more '*closed*' states in Get3 bound to non-hydrolyzable ATP analogues, such as AMPPNP- and ADP•AlF_4^-, in which the helical domains are close together (Fig. 3A, step 1)[62–64]. Importantly, multiple hydrophobic residues in the helical domains are brought into a contiguous groove in the '*closed*' Get3 structure, and this site has been shown to mediate TMD binding[62, 65]. In contrast to ATP analogues, the Get1 cytosolic domain preferentially binds apo-Get3 in a wide-*open* conformation (Fig. 3A, top left structure) and promotes the release of TA substrate from Get3[59, 66].

Kinetic analyses of the Get3 ATPase cycle uncovered additional conformational states. The Get4/5 complex specifically stabilizes ATP binding to Get3[67]. Consistent with this, crystallographic analyses showed that Get4/5 bridges the dimer interface of ATP-bound Get3 and selectively stabilizes the latter into one of the most closed conformations observed[68, 69]. Nevertheless, Get4/5 inhibits ATP hydrolysis by Get3, indicating that Get4/5 induces a distinct '*occluded*' state of Get3 in which tight and specific ATP binding is uncoupled from ATPase activation (Fig. 3A, step 3)[67]. In contrast to Get4/5, the TA substrate strongly activates the ATPase activity of Get3, indicating that TA-loaded Get3 adopts an '*activated*' conformation in which its active site is optimized (Fig. 3A, step 4)[67]. Compared to free Get3, TA-loaded Get3 also exhibits significantly weakened affinity for Get4/5[70], further supporting the notion that Get3 adopts a conformation distinct from the '*closed*' or '*occluded*' states upon substrate loading. Finally, while nucleotide-bound Get3•TA complexes exhibit higher affinity for the Get2 than Get1 subunit (Fig. 3A, step 5), Get1 gains significantly higher affinity for the Get3•TA complex upon ADP release[70].

This suggests another '*strained*' conformation of the Get3•TA complex (although this could also be explained by a shift in conformational equilibrium of the Get3•TA complex towards the *open* conformation, rather than a distinct conformational statea), at which stage Get1 can initiate interaction with and remodeling of the targeting complex (Fig. 3A, step 6). Finally, Get1 exhibits the highest affinity for free, apo-Get3, with dissociation rate constants in the low nanomolar range[63, 70]; these results, combined with the crystal structures, strongly suggest that a wide-*open* Get3 bound to Get1 accumulates at the end of the targeting cycle.

Together, these results showed that the GET pathway is driven by an ordered series of nucleotide-, substrate-, and effector-driven conformational changes in the Get3 ATPase dimer (Fig. 3B). Prior to TA binding, the majority of Get3 in the cytosol is loaded with ATP and tightly bound to Get4/5 (steps 1–2). Get4/5 brings Get3 into the vicinity of Sgt2 (step 0) and induces Get3 into the optimal conformation and nucleotide state to capture the TA substrate. TA loading drives the dissociation of Get3 from Get4/5 and activates a rapid round of ATP hydrolysis (step 3). The Get3•TA complex is likely initially captured by the Get2 subunit at the ER membrane (step 4). ADP release allows the Get3•TA complex to explore additional conformations for which Get1 has higher affinity, and thus initiates the remodeling of the targeting complex (step 5). The strong preference of Get1 for wide-open Get3 drives the disassembly of the Get3•TA complex (step 5), followed by insertion of the TA substrate into the ER membrane that may be facilitated by the TMDs of the Get1/2 receptor[61]. At the end of the pathway, Get3 is tightly bound to Get1/2, requiring both ATP and Get4/5 to release it from ER and re-initiate the targeting cycle (steps 1–2)[70].

Unlike the bacterial SRP pathway for which the energetics, kinetics, and structure of almost every intermediate have been characterized, multiple questions remain for the GET pathway (Fig. 3, '?'). First, the targeting pathway demands distinct activities of Get3 before and after substrate loading: prior to TA binding, Get3 must be ATP-bound and tightly bound to Get4/5; after TA loading, Get3 must hydrolyze ATP and detach from Get4/5 so that it can instead interact with the Get1/2 receptor at the ER. Although a structure of Get3•TA peptide complex is now available, the conformation of Get3 in this structure is similar to that in the Get3•Get4/5 complex; it is unclear how the TA substrate drives the transition of Get3 from Get4/5 to the Get1/2 receptors. In addition, the structures of important intermediates in the pathway, such as Get2 and/or Get1 bound to the Get3•TA complex, are still unavailable, and the mechanisms by which Get1/2 remodels the Get3•TA complex and inserts the TA into the membrane remain unclear. Further, the structural basis for regulation of Get3's ATPase activity by Get4/5 and the TA substrate remain to be elucidated. Finally, it is unclear whether additional upstream components are required to help load newly synthesized TA proteins onto Sgt2, and if so, how these substrate relay events are accomplished.

Comparison of SRP•SR with Get3: common regulatory principles?

Although the details of the SRP and GET pathways differ significantly, many similarities between the Get3 ATPase dimer and the SRP•SR GTPase dimer emerge from available data. In contrast to the classical 'GTPase switch' paradigm, both SRP and GET systems forego the use of nucleotide exchange and the recruitment of external GEFs and GAPs as major regulatory elements. In support of the initial proposal by Gasper et al, both systems use

dimers as the functional unit[9]. Further, multiple ‘on’ states can be generated within the dimer. Both the SRP•SR GTPase dimer and Get3 ATPase dimer undergo an ordered series of conformational changes on the global (*open* → *closed* transitions) and local (catalytic loop adjustments) scale to generate multiple, discrete functional states during their NTPase cycle. Each rearrangement provides a distinct regulatory point at which SRP•SR or Get3 can directly communicate with upstream and downstream effector molecules in the pathway. I suggest that these dimeric nucleotide hydrolases provide ‘multi-state navigator’ systems that use their conformational plasticity to ensure the spatiotemporal accuracy of diverse molecular actions required for cellular pathways.

What drove the evolution of these regulatory proteins? To answer this question, it is useful to reflect on the bi-modal nature of the classic ‘GTPase switch’, Ras-type GTPases often have a well-defined ‘on’ state in which they interact with effector molecules. In contrast, it is difficult to define a single ‘on’ or ‘off’ state for SRP•SR and Get3, as the pathways mediated by these proteins require a complex series of molecular events for which different functions must be turned ‘on’ or ‘off’ at distinct stages. For example, SRP and Get3 must effectively capture cargo proteins in the cytosol but promptly release them at the target membrane. SRP and SR must efficiently assemble a complex at early stages of targeting but promptly dissociate at the end of the targeting cycle. Effector interactions for Get3 is particularly complex, as this ATPase must bind three effector proteins, Get4, Get2 and Get1, in an ordered cascade[70]. The ability of SRP•SR and Get3 to adopt multiple conformations is necessary to drive these cyclic processes during which the substrate and various effector molecules must bind and later release in a sequential and controlled manner. Probably, modulation of the dimer interface provides a facile mechanism to increase conformational diversity, which might be particularly suitable for generating a multitude of conformational states required by these processes.

A second feature of the Ras-type GTPases is that their ‘on’ and ‘off’ states are temporally and often spatially separated from one another. This feature is essential for GTPases mediating signaling and other processes that need to be kept ‘off’ until the arrival of signaling cues. The need to recruit extrinsic GEFs and GAPs – which involve transduction of environmental signals, negative or positive feedback control, or translocation of proteins to a different cellular compartment[71] – further impose this tight regulation. In contrast, the processes mediated by the SIMIBI family of GTPases and ATPases are often constitutive and must occur rapidly. For example, co-translational protein targeting must occur before the nascent polypeptide reaches a critical length, which imposes a time window of 3–6 seconds for SRP and SR to complete each targeting cycle[72, 73]. The ability of SRP•SR and Get3 to directly respond to effector molecules in their respective pathways without the need to recruit extrinsic regulatory factors may be especially beneficial for vectorial processes that must occur efficiently.

In conclusion, multiple members of the SIMIBI family GTPases and related ATPases couple nucleotide-dependent dimerization to their biological function. Recent work on the SRP•SR and Get3 systems suggest that these proteins can use the conformational plasticity at their dimer interface to directly communicate with upstream and downstream effectors, thus providing spatiotemporal control of complex cellular pathways. More work is needed to

decipher the molecular mechanisms of this family of nucleotide hydrolases and understand the chemical and biological logic for utilizing this class of regulatory GTPases.

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References

1. Gilman AG. G proteins: transducers of receptor-generated signals. Annu. Rev. Biochem. 1987; 56:615–649. [PubMed: 3113327]
2. Hall, A. GTPases. Oxford: Oxford University Press; 2000.
3. Pfeffer SR. Rab GTPase localization and Rab cascades in Golgi transport. Biochem. Soc. Trans. 2012; 40:1373–1377. [PubMed: 23176483]
4. Trahey MaM F. A cytoplasmic protein stimulates normal N-ras p21 GTPase, but does not affect oncogenic mutants. Science. 1987; 238:542–545. [PubMed: 2821624]
5. Bourne HR, Sanders DA, McCormick F. The GTPase superfamily: conserved structure and molecular mechanism. Nature. 1991; 349:117–127. [PubMed: 1898771]
6. Bourne HR. The arginine finger strikes again. Nature. 1997; 389:673–674. [PubMed: 9338774]
7. Garcia-Mata R, Boulter E, Burridge K. The 'invisible hand: regulation of RHO GTPases by RHOGDIs. Nat. Rev. Mol. Cell Biol. 2011; 12:493–504. [PubMed: 21779026]
8. Leipe DD, Wolf YI, Koonin EV, Aravid L. Classificaiton and evolution of P-loop GTPases and related ATPases. J. Mol. Biol. 2002; 317:41–72. [PubMed: 11916378]
9. Gasper R, Meyer S, Gotthardt K, Sirajuddin M, Wittinghofer A. It takes two to tango: regulation of G proteins by dimerization. Nature Rev. Mol. Cell. Biol. 2009; 10:423–429. [PubMed: 19424291]
10. Bange G, Sinning I. SIMIBI twins in protein targeting and localization. Nature Struct. Molec. Biol. 2013; 20:776–780. [PubMed: 23823071]
11. Akopian D, Shen K, Zhang X, Shan S. Signal recognition particle: an essential protein-targeting machine. Ann. Rev. Biochem. 2013; 82:693–721. [PubMed: 23414305]
12. Pool MR. Signal recognition particles in chloroplasts, bacteria, yeast and mammals. Mole. Membr. Biol. 2005; 22:3–15.
13. Walter P, Johnson AE. Signal sequence recognition and protein targeting to the endoplasmic reticulum membrane. Ann. Rev. Cell Biol. 1994; 10:87–119. [PubMed: 7888184]
14. Mishra AK, Lambright DG. Small GTPases and their GAPs. Biopolymers. 2016; 105:431–448. [PubMed: 26972107]
15. Sprang SR. Activation of G proteins by GTP and the mechanism of Gα-catalyzed GTP hydrolysis. Biopolymers. 2016; 105:449–462. [PubMed: 26996924]
16. Freymann DM, Keenan RJ, Stroud RM, Walter P. Structure of the conserved GTPase domain of the signal recognition particle. Nature. 1997; 385:361–364. [PubMed: 9002524]
17. Montoya G, Svensson C, Luirink J, Sinning I. Crystal structure of the NG domain from the signal recognition particle receptor FtsY. Nature. 1997; 385:365–368. [PubMed: 9002525]
18. Batey RT, Rambo RP, Lucast L, Rha B, Doudna JA. Crystal structure of the ribonucleoprotein core of the signal recognition particle. Science. 2000; 287:1232–1239. [PubMed: 10678824]
19. Hainzl T, Huang S, Merilainen G, Brannstrom K, Sauer-Eriksson AE. Structural basis of signal sequence recognition by the signal recognition particle. Nature Struct. Molec. Biol. 2011; 18:389–391. [PubMed: 21336278]
20. Janda CY, Li J, Oubridge C, Hernandez H, Robinson CV, Nagai K. Recognition of a signal peptide by the signal recognition particle. Nature. 2010; 465:507–510. [PubMed: 20364120]
21. de Leeuw E, te Kaat K, Moser C, Memestrina G, Demel R, de Kruijff B, Oudegam B, Luirink J, Sinning I. Anionic phospholipids are involved in membrane association of FtsY and stimulate its GTPase activity. EMBO J. 2000; 19:531–541. [PubMed: 10675322]

22. Parlitz R, Eitan A, Stjepanovic G, Bahari L, Bange G, Bibi E, Sinning I. Escherichia coli signal recognition particle receptor FtsY contains an essential and autonomous membrane-binding amphipathic helix. J. Biol. Chem. 2007; 282:32176–32184. [PubMed: 17726012]
23. Kuhn P, et al. Ribosome binding induces repositioning of the signal recognition particle receptor on the translocon. J Cell Biol. 2015; 211:91–104. [PubMed: 26459600]
24. Kuhn P, Draycheva A, Vogt A, Petriman NA, Sturm L, Drepper F, Warscheid B, Wintermeyer W, Koch HG. Ribosome binding induces repositioning of the signal recognition particle receptor on the translocon. J. Cell Biol. 2015; 211:91–104. [PubMed: 26459600]
25. Schlenker O, Henricks A, Irmgard S, Wild K. The structure of the mammalian signal recognition particle (SRP) receptor as prototype for the interaction of small GTPases with Longin domains. J. Biol. Chem. 2006; 281:8898–8906. [PubMed: 16439358]
26. Schwartz T, Blobel G. Structural basis for the function of the b-subunit of the eukaryotic signal recognition particle receptor. Cell. 2003; 112:793–803. [PubMed: 12654246]
27. Freymann DM, Keenan RJ, Stroud RM, Walter P. Functional changes in the structure of the SRP GTPase on binding GDP and Mg2+GDP. Nat. Struct. Biol. 1999; 6:793–801. [PubMed: 10426959]
28. Gawronski-Salerno J, Coon YJS, Focia PJ, Freymann DM. X-ray structure of the T. Aquaticus Ftsy:GDP complex suggests functional roles for the C-terminal helix of the SRP GTPases. Proteins. 2006; 66:984–995.
29. Padmanabhan W, Freymann DM. The conformation of bound GMPPNP suggests a mechanism for gating the active site of the SRP GTPase site. Structure. 2001; 9:859–863. [PubMed: 11566135]
30. Jagath JR, Rodnina MV, Lentzen G, Wintermeyer W. Interaction of guanine nucleotides with the signal recognition particle from *Escherichia coli*. Biochemistry. 1998; 37:15408–15413. [PubMed: 9799502]
31. Jagath JR, Rodnina MV, Wintermeyer W. Conformational changes in the bacterial SRP receptor FtsY upon binding of guanine nucleotides and SRP. J. Mol. Biol. 2000; 295:745–753. [PubMed: 10656787]
32. Jaru-Ampornpan P, Chandrasekar S, Shan S. Efficient interaction between two GTPases allows the chloroplast SRP pathway to bypass the requirement for an SRP RNA. Mol. Biol. Cell. 2007; 18:2636–2645. [PubMed: 17475780]
33. Peluso P, Shan S, Nock S, Herschlag D, Walter P. Role of SRP RNA in the GTPase cycles of Ffh and FtsY. Biochemistry. 2001; 40:15224–15233. [PubMed: 11735405]
34. Powers T, Walter P. Reciprocal stimulation of GTP hydrolysis by two directly interacting GTPases. Science. 1995; 269:1422–1424. [PubMed: 7660124]
35. Rosendal KR, Wild K, Montoya G, Sinning I. Crystal structure of the complete core of archaeal signal recognition paricle and implications for interdomain communication. Proc. Natl. Acad. Sci. 2003; 100:14701–14706. [PubMed: 14657338]
36. Shen K, Zhang X, Shan S. Synergistic action between the SRP RNA and translating ribosome allows efficient delivery of correct cargos during co-translational protein targeting. RNA. 2011; 17:892–902. [PubMed: 21460239]
37. Estrozi LF, Boehringer D, Shan S, Ban N, Schaffitzel C. Cryo-EM structure of the E. coli translating ribosome in complex with SRP and its receptor. Nat. Struct. Mol. Biol. 2011; 18:88–90. [PubMed: 21151118]
38. Zhang X, Kung S, Shan S. Demonstration of a two-step mechanism for assembly of the SRP-SRP receptor complex: implications for the catalytic role of SRP RNA. J. Mol. Biol. 2008; 381:581–593. [PubMed: 18617187]
39. Zhang X, Lam VQ, Mou Y, Kimura T, Chung J, Chandrasekar S, Winkler J, Mayo S, Shan S. Direct visualization reveals dynamics of a transient intermediate during proten assembly. Proc. Natl. Acad. Sci. U. S. A. 2011; 108:6450–6455. [PubMed: 21464281]
40. Egea PF, Shan S, Napetschnig J, Savage DF, Walter P, Stroud RM. Substrate twinning activates the signal recognition particle and its receptor. Nature. 2004; 427:215–221. [PubMed: 14724630]
41. Focia PJ, Shepotinovskaya IV, Seidler JA, Freymann DM. Heterodimeric GTPase Core of the SRP Targeting Complex. Science. 2004; 303:373–377. [PubMed: 14726591]

42. Shan S, Stroud R, Walter P. Mechanism of association and reciprocal activation of two GTPases. Plos Biology. 2004; 2:e320. [PubMed: 15383838]
43. Ataide SF, Schmitz N, Shenk K, Ke A, Shan S, Doudna A, Ban N. The crystal structure of the Signal Recognition Particle in complex with its receptor. Science. 2011; 381:881–886.
44. Shen K, Wang Y, Hwang Fu YH, Zhang Q, Feigon J, Shan S. Molecular mechanism of GTPase activation at the signal recognition particle (SRP) RNA distal end. J. Biol. Chem. 2013; 288:36395–36397.
45. Voigts-Hoffmann F, Schmitz N, Shen K, Shan SO, Ataide SF, Ban N. The structural basis of FtsY recruitment and GTPase activation by SRP RNA. Molecular Cell. 2013; 52:643–654. [PubMed: 24211265]
46. Peluso P, Herschlag D, Nock S, Freymann DM, Johnson AE, Walter P. Role of 4.5S RNA in assembly of the bacterial signal recognition particle with its receptor. Science. 2000; 288:1640–1643. [PubMed: 10834842]
47. Bradshaw N, Neher SB, Booth DS, Walter P. Signal sequences activate the catalytic switch of SRP RNA. Science. 2009; 323:127–130. [PubMed: 19119234]
48. Zhang X, Schaffitzel C, Ban N, Shan S. Multiple conformational changes in a GTPase complex regulate protein targeting. Proc. Natl. Acad. Sci. 2009; 106:1754–1759. [PubMed: 19174514]
49. Zhang X, Rashid R, Wang K, Shan S. Sequential checkpoints govern fidelity during co-translational protein targeting. Science. 2010; 328:757–760. [PubMed: 20448185]
50. Lam VQ, Akopian D, Rome M, Shen Y, Henningsen D, Shan S. Lipid activation of the SRP receptor provides spatial coordination of protein targeting. J. Cell Biol. 2010; 190:623–635. [PubMed: 20733058]
51. Shen K, Arslan S, Akopian D, Ha T, Shan S. Activated GTPase movement on an RNA scaffold drives cotranslational protein targeting. Nature. 2012; 492:271–275. [PubMed: 23235881]
52. Akopian D, Dalai K, Shen K, Duong F, Shan S. SecYEG activates GTPases to drive the completion of cotranslational protein targeting. J. Biol. Chem. 2013 in press.
53. Saraogi I, Akopian D, Shan S. Regulation of cargo recognition, commitment, and unloading drives cotranslational protein targeting. J. Cell Biol. 2014; 205:693–706. [PubMed: 24914238]
54. Zhang XaS S. Fidelity of cotranslational protein targeting by the signal recognition particle. Annu Rev Biophys. 2014; 43:381–408. [PubMed: 24895856]
55. Hegde RS, Keenan RJ. Tail-anchored membrane protein insertion into the endoplasmic reticulum. Nature Rev. Mol. Cell Biol. 2011; 12:787–798. [PubMed: 22086371]
56. Stefanovic S, Hegde RS. Identification of a targeting factor for post-translational membrane protein insertion into the ER. Cell. 2007; 128:1147–1159. [PubMed: 17382883]
57. Wang F, Brown EC, Mak G, Zhuang J, Denic V. A chaperone cascade sorts proteins for posttranslational membrane insertion into the endoplasmic reticulum. Mol Cell. 2010; 40:1–13. [PubMed: 20932468]
58. Schuldiner M, Metz J, Schmid V, Denic V, Rakwalska M, Schmitt HD, Schwappach B, Weissman JS. The GET complex mediates insertion of tail-anchored proteins into the ER membrane. Cell. 2008; 134:634–645. [PubMed: 18724936]
59. Mariappan M, Mateja A, Dobosz M, Bove E, Hegde RS, Keenan RJ. The mechanism of membrane associated steps in tail-anchored protein insertion. Nature. 2011; 477:61–66. [PubMed: 21866104]
60. Wang F, Whynot A, Tung M, Denic V. The mechanism of Tail-anchored protein insertion into the ER membrane. Molec. Cell. 2011; 43:1–13. [PubMed: 21726803]
61. Wang F, Chan C, Weir NR, Denic V. The Get1/2 transmembrane complex is an endoplasmic-reticulum membrane protein insertase. Nature. 2014; 512:441–444. [PubMed: 25043001]
62. Mateja A, Szlachcic A, Downing ME, Dobosz M, Mariappan M, Hegde RS, Keenan RJ. The structural basis of tail-anchored membrane protein recognition by Get3. Nature. 2009; 461:361–366. [PubMed: 19675567]
63. Bozkurt G, Stjepanovic G, Vilardi F, Amlacher S, Wild K, Gert B, Favaloro V, Rippe K, Hurt E, Dobberstein B, Sinning I. Structural insights into tail-anchored protein binding and membrane protein insertion by Get3. Proc. Natl. Acad. Sci. U. S. A. 2009; 106:21131–21136. [PubMed: 19948960]

64. Suloway CJM, Chartron JW, Zaslaver M, Clemons WM Jr. Model for eukaryotic tail-anchored protein binding based on the structure of Get3. Proc. Natl. Acad. Sci. U. S. A. 2009; 106:14849–14854. [PubMed: 19706470]

65. Mateja A, Paduch M, Chang HY, Szydlowska A, Kossiakoff AA, Hegde RS, Keenan RJ. Protein targeting. Structure of the Get3 targeting factor in complex with its membrane protein cargo. Science. 2015; 347:1152–1155. [PubMed: 25745174]

66. Stefer S, Reitz S, Wang F, Wild K, Pang UY, Schwarz D, Jorg B, Hein C, Lohr F, Bernhard F, Denic V, Dotsch V, Sinning I. Structural basis for tail-anchored membrane protein biogenesis by the Get3-receptor complex. Science. 2011; 333:758–762. [PubMed: 21719644]

67. Rome M, Rao M, Clemons WM, Shan S. Precise timing of ATPase activation drives targeting of tail-anchored proteins. Proc. Natl. Acad. Sci. U. S. A. 2013; 110:7666–7671. [PubMed: 23610396]

68. Gristick HB, et al. Crystal structure of ATP-bound Get3-Get4-Get5 complex reveals regulation of Get3 by Get4. Nat Struct Mol Biol. 2014; 21:437–442. [PubMed: 24727835]

69. Gristick HB, et al. Mechanism of Assembly of a Substrate Transfer Complex during Tail-anchored Protein Targeting. J Biol Chem. 2015; 290:30006–30017. [PubMed: 26451041]

70. Rome M, Chio US, Gristick HB, Rao M, Shan S. A gradient of interaction affinities drives efficient targeting and recycling in the GET pathway. Proc. Natl. Acad. Sci. U. S. A. 2014; 111:E4929–E4935. [PubMed: 25368153]

71. Bos JL, et al. GEFs and GAPs: critical elements in the control of small G proteins. Cell. 2007; 129:865–877. [PubMed: 17540168]

72. Ariosa AR, Lee JH, Wang S, Saraogi I, Shan S. Regulation by a chaperone improves substrate selectivity during cotranslational protein targeting. Proc. Natl. Acad. Sci. U. S. A. 2015; 112:E3169–E3178. [PubMed: 26056263]

73. Zhang D, Shan SO. Translation elongation regulates substrate selection by the signal recognition particle. J. Biol. Chem. 2012; 287:7652–7660. [PubMed: 22228766]

Outstanding Questions

- When, where, and how do nucleotide binding and hydrolysis events occur during the dimerization cycles of SIMIBI family proteins? What structural and dynamic features of these proteins control these molecular events?
- How are the dimerization-governed GTPase and ATPase cycles coupled to the biological pathways mediated by these proteins?
- Are the principles observed for the SRP•SR and Get3 dimers generalizable to the remainder of SIMIBI family proteins?
- What is the chemical and biological logic for employing this class of regulators?
- Did this regulatory strategy arise from convergent or divergent evolution?

Trends Box

- The SIMIBI family of GTPases and related ATPases provides a notable exception to the classic ‘GTPase switch’ paradigm.
- Nucleotide-, cargo-, and membrane-driven conformational changes during the dimerization cycle of SRP and SRP receptor GTPases provide spatiotemporal control of co-translational protein targeting.
- Nucleotide-, substrate-, and effector-driven conformational changes in the Get3 ATPase dimer coordinate post-translational membrane protein targeting.
- Coupling of nucleotide-dependent dimerization to biological function may be a general feature for the SIMIBI family of nucleotide hydrolases.
- Dimerization-activated GTPases and related ATPases can use modulation of dimer interface to generate multiple regulatory points in a biological pathway. They may constitute a novel class of “multi-state” regulatory systems.

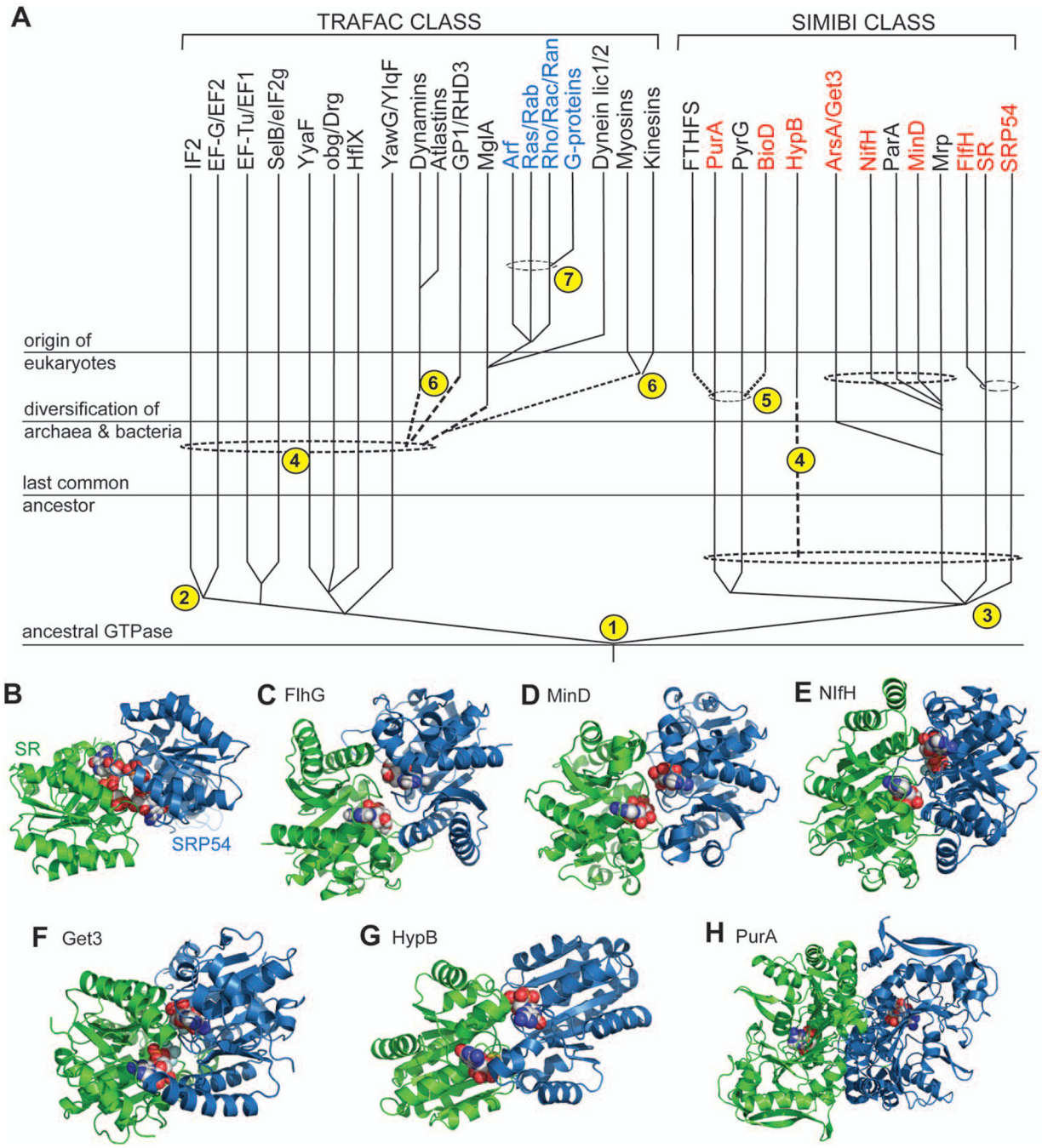

Figure 1.

Multiple members of the SIMIBI GTPase family form dimers. (**A**) Inferred evolutionary history of GTPase families; adapted from Figure 1 in Leipe et al[8]. Numbered circles indicate various evolutionary events associated with the diversification of GTPases. Broken lines denote uncertainty in the exact point of origin of the lineage. Dashed ellipses group the lineages from within which a new lineage potentially could have emerged. Members of the extended Ras subfamily are highlighted in *blue*, members of the SIMIBI family known to form dimers are highlighted in *red*. (**B–H**) Top view of the structures of dimeric GTPases

and ATPases in the SIMIBI family, including SRP54 and SR (B; 1RJ9), FlhG (C; 4RZ3), MinD (D; 3Q9I), NifH (E; 1N2C), Get3 (F; 2WOJ), HypB (G; 2HF8), and PurA (H; 4M9D). The bound nucleotides are in *spacefill*.

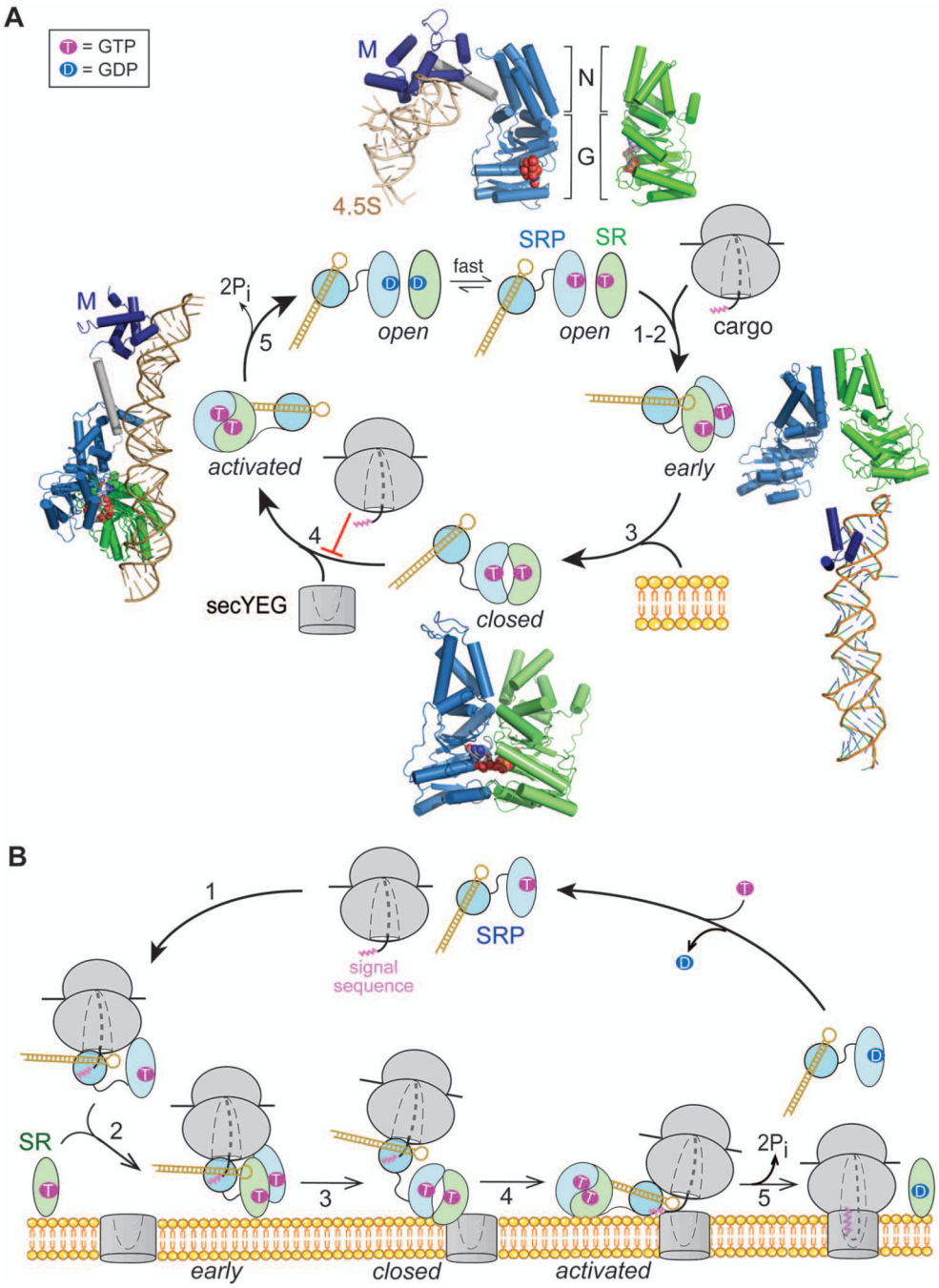

Figure 2.

Conformational changes in the SRP•SR GTPase dimer regulate co-translational protein targeting. SRP54 and SR are in *blue* and *green*, respectively. T denotes GTP; D denotes GDP. (**A**) Cargo, membrane and translocon drive multiple rearrangements during SRP-SR dimerization, as described in the text. '⊥' denotes the effect of RNC in delaying GTPase activation. Top panel: the crystal structures of free SRP54 (left; 1QZW) and the SR NG-domain (right; 2Q9C). The SRP54 NG- and M-domains are in *light* and *dark blue*, respectively, the linker between the two domains is in *grey*, and the SRP RNA is in *tan*. The

bound GTP analogues are in *spacefill*. Right panel: cryo-EM model of the '*early*' SRP•SR complex bound to RNC (2XKV); the RNC is not shown for clarity. Bottom panel: the crystal structure of the '*closed*' SRP-SR NG-domain complex (1RJ9). Left panel: the crystal structure of the '*activated*' complex (2XXA). (**B**) GTPase rearrangements in SRP and SR drive distinct molecular steps during targeting. The steps are numbered to be consistent with part (A). Step 1, RNC with a signal sequence (*magenta*) binds SRP. Step 2, RNC-loaded SRP forms a stabilized '*early*' targeting complex with SR. Step 3, phospholipids drive rearrangement to the '*closed*' state. Step 4, SecYEG drives rearrangement to the '*activated*' state, which frees the ribosome for subsequent unloading. Step 5, the RNC is unloaded from SRP onto the SecYEG complex, and GTP hydrolysis drives the disassembly and recycling of SRP and SR.

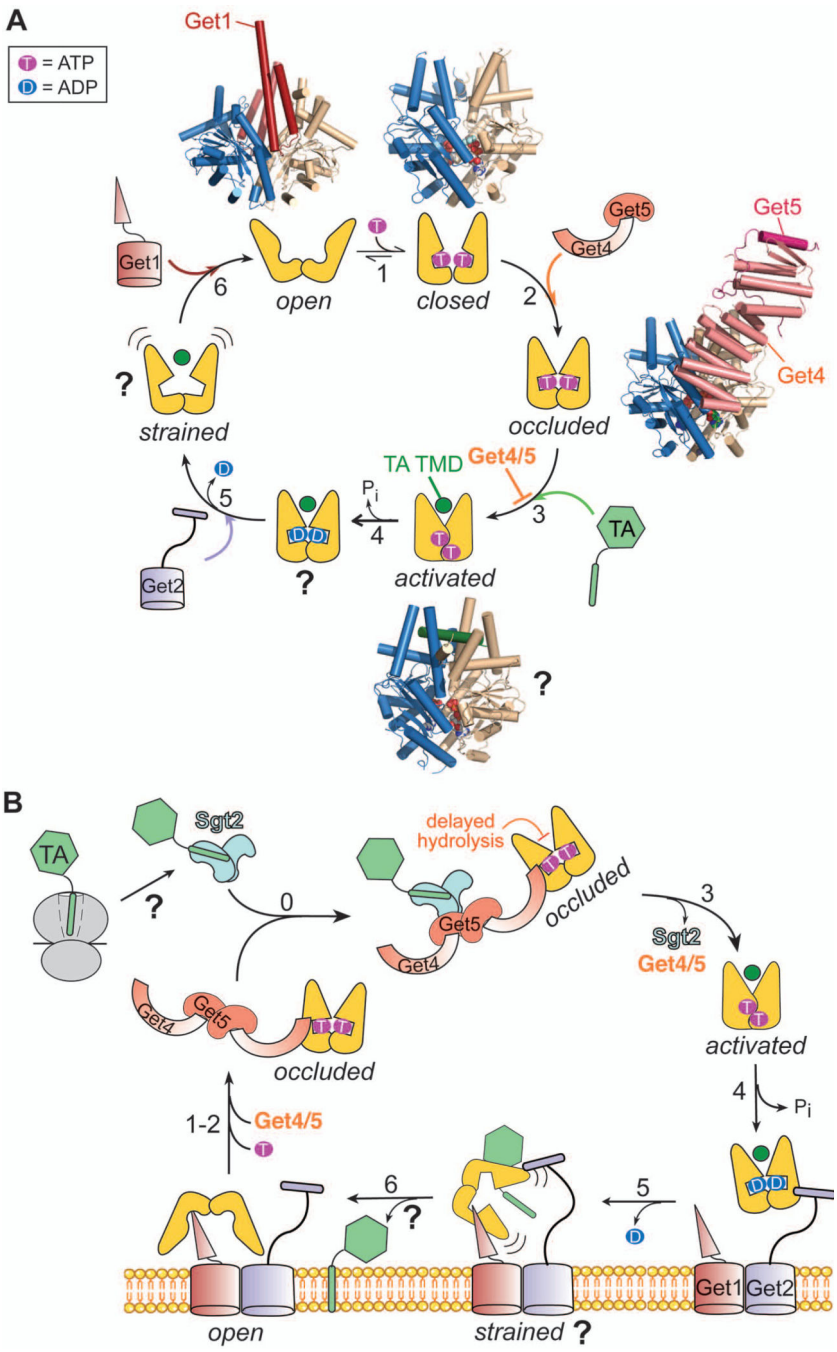

Figure 3.

Conformational changes in the Get3 ATPase dimer drive the post-translational targeting of TA proteins. T denotes ATP; D denotes ADP. (**A**) Nucleotide-, substrate-, and effector-driven conformational changes during the Get3 ATPase cycle, as described in the text. '⊥' denotes the effect of Get4/5 in delaying ATPase activation. Top right panel: crystal structure of *closed* Get3 bound with ADP•AlF_4^- (2WOJ). The two Get3 subunits in the dimer are in *blue* and *tan*, respectively. The bound nucleotides are in *spacefill*. Right panel: crystal structure of Get4/5- and ATP-bound Get3 (4PWX). Bottom panel: crystal structure of Get3

bound with ADP•AlF_4^- and a TA peptide (4XTR). Top left panel: crystal structure of a wide '*open*' Get3 bound to the Get1 cytosolic domain (3SJB). (**B**) ATPase rearrangements in Get3 drive distinct molecular steps during TA protein targeting. The steps are numbered to be consistent with part (A). Get4/5 bridges between Sgt2 and Get3, and primes Get3 into the optimal conformation and nucleotide state for TA loading (step 0). TA binding drives Get3 dissociation from Get4/5 (step 3) and activates ATP hydrolysis (step 4). The Get3•TA complex is probably captured by Get2 at the ER membrane (step 4). ADP release initiates interaction of the Get3•TA complex with Get1 (step 5), which drives disassembly of TA from Get3 (steps 6). Finally, ATP together with Get4/5 displace Get3 from Get1 and reinitiate the cycle (steps 1–2).