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Signal recognition particle (SRP) and SRP receptor: A new paradigm for multi-state regulatory GTPases

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Abstract

The GTP-binding proteins or GTPases comprise a superfamily of proteins that provide molecular switches in numerous cellular processes. The 'GTPase switch' paradigm, in which a GTPase acts as a bimodal switch that is turned 'on' and 'off' by external regulatory factors, has been used to interpret the regulatory mechanism of many GTPases for over two decades. Nevertheless, recent work has unveiled an emerging class of 'multi-state' regulatory GTPases that do not adhere to this classical paradigm. Instead of relying on external nucleotide exchange factors or GTPase activating proteins to switch between the 'on' and 'off' states, these GTPases have the intrinsic ability to exchange nucleotides and to sense and respond to upstream and downstream factors. In contrast to the bimodal nature of the 'GTPase switch', these GTPases undergo multiple conformational rearrangements, allowing multiple regulatory points to be built into a complex biological process to ensure the efficiency and fidelity of the pathway. We suggest that these multi-state regulatory GTPases are uniquely suited to provide spatial and temporal control over complex cellular pathways that require multiple molecular events to occur in a highly coordinated fashion.

The GTPase superfamily of proteins provide molecular switches that regulate numerous cellular pathways, including signal transduction, cell growth and differentiation, ribosome assembly and protein synthesis, cytoskeletal organization, nuclear transport and spindle assembly, and intracellular protein transport (1–3). Pioneering work on small GTPases, such as Ras and EF-Tu, established a 'GTPase switch' paradigm to account for their mode of regulation (Fig. 1A). In this mechanism, a GTPase acts as a bimodal switch that alternates between two distinct conformations: a GDP-bound, inactive conformation and a GTP-bound, active conformation that can interact with one or more effector molecules to trigger a cellular response (1). A key to this regulatory mechanism is the extremely slow rate at which a GTPase inter-converts between the active and inactive conformations due to their intrinsically slow rate of nucleotide exchange and GTP hydrolysis (Table 1). Thus, the 'on' and 'off' conformations of a GTPase are temporally separated from one another, and are, in turn, controlled by external regulatory factors such as guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs; Fig. 1A). The recruitment of these external factors allows a GTPase to switch between 'on' and 'off' conformations in temporal succession in response to cellular signaling cues.

This paradigm provided an invaluable framework and has been used to interpret the regulatory mechanism of many GTPases for over two decades. Nevertheless, recent studies have unveiled

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a growing number of GTPases that do not conform to this classical model. The best-studied examples include elongation factor G, the dynamin family of GTPases, and the two GTPases in the signal recognition particle (SRP) and the SRP receptor (SR). In this review, we summarize recent biochemical and biophysical analyses of the bacterial SRP pathway that elucidate a novel mode of regulation by the SRP family of GTPases. We then discuss analogies between SRP/SR and the EF-G and dynamin GTPases, and suggest that they define a new type of multi-state GTPases that can use their intrinsic conformational flexibility to regulate complex biochemical pathways.

The SRP GTPase family: Exception to the GTPase switch paradigm

SRP and SR together comprise the major cellular machinery that mediates the co-translational transport of roughly one third of proteins in a cell's genome to membrane compartments (4, 5). As in many complex cellular pathways, the protein transport reaction mediated by the SRP involves a series of highly orchestrated molecular steps (Fig. 2) that begins when a polypeptide destined for the endoplasmic reticulum (ER) or the secretory pathway emerges from a translating ribosome (Fig. 2, step 1). These proteins carry signal sequences that specify their cellular destination and are recognized, together with the ribosome, by the SRP. The ribosome•nascent chain complex (RNC), referred to here as cargo, is then delivered to the membrane via the interaction of the SRP with the SR (Fig. 2, steps 2–3). Once at the membrane, the SRP switches from a cargo-binding mode to a cargo release mode, unloading the cargo to a protein translocation channel, or translocon, embedded in the membrane (Fig. 2, steps 4–5). After the 'cargo' is unloaded, the SRP and SR dissociate from one another, allowing a new round of protein transport (Fig. 2, step 6). Meanwhile, the nascent polypeptide finishes its synthesis at the translocon and is either integrated into the membrane or translocated across the membrane to enter the secretory pathway.

Efficient and faithful protein transport by the SRP requires exquisite spatial and temporal control, which is provided by two highly homologous GTPases in both the SRP and SR (Fig. 1B). Although the size and composition of SRP varies widely through evolution, the functional core of the SRP responsible for protein transport is comprised of the universally conserved SRP54 GTPase in complex with the SRP RNA. The eukaryotic SRP receptor is heterodimeric complex of a soluble SRα subunit that interacts with the SRP and an SRβ subunit, a transmembrane protein that localizes SRα to the membrane. Bacteria has a simpler SR, comprised of a single protein highly homologous to SRα. Both SRP54 and SRα contain a central GTPase or G-domain that shares homology with the Ras GTPase fold and contains the four sequence motifs (GI–GIV) that are conserved in most GTPases (Fig. 1C) (3). GI, also referred to as the P-loop, provides main chain hydrogen bonding interactions with the α- and β-phosphate groups of GTP. The GII and GIII motifs contain residues essential for coordinating the active site Mg^{2+} and the γ-phosphate of GTP; these loops often change conformation in response to effector binding and are hence also termed the switch 1 and switch 2 loops, respectively. The GIV motif, situated at the opposite end of the GTP binding pocket, provide interactions with the guanine base. Unique to the SRP family of GTPases are two insertions. The first is an N-terminal four helix bundle, the N-domain (Fig. 1C, *yellow*), which packs against the G-domain to form a structural and functional unit called the NG domain (6,7). The N-domain of SRP provides part of the ribosome binding site (8,9) and, as discussed below, plays a critical role in modulating the kinetics and stability of the SRP•SR complex. The second is an insertion box domain (IBD), comprised of a β–α–β–α motif sandwiched between the GI and GIII motifs (6,7). This domain contains the GII motif or the IBD loop (Fig. 1C, *red*), which provides multiple catalytic residues critical for mediating GTP hydrolysis (see below). Aside from the NG domains, SRP54 and SR contain unique structural elements that allow them to carry out their functions in protein transport. The NG domain of the core SRP protein, SRP54, is connected via a flexible linker to a methionine rich M-domain (Fig. 1C, *grey*) that provides

binding sites for signal sequences and for the SRP RNA, another essential component of the SRP (10,11). The bacterial SR protein contains an N-terminal A-domain (Fig. 1C, *white*) that allows the receptor to peripherally associate with the target membrane via interactions with phospholipids (12,13) and with the translocon (14).

Unlike classical GTPases such as Ras, Gα, and EF-Tu, the SRP and SR GTPases by themselves do not exhibit significant conformational changes between the apo-, GDP- and GTP-bound states (6,7,15–17). Further, these GTPases exhibit weak nucleotide affinities and rapid GDP release rates that are 10^4–10^6-fold faster than those observed for signaling GTPases (Table 1) (18–21). Structural studies showed that free SRP and SR contain elongated, wide-open nucleotide binding sites (6,7) that explain their weak nucleotide binding affinities and fast nucleotide exchange rates (Fig. 3, top panel). Moreover, the IBD loops, which encode key catalytic residues, are disordered and not correctly aligned with the bound nucleotide (Fig. 3 top panel, *red*), thus free SRP and SR have low basal GTPase activities (21). Considering these properties and the 10-fold higher cellular concentration of GTP over GDP at steady state, ~90% of the SRP and SR GTPases will be in the GTP-bound state within the cell. Thus the recruitment of an external GEF to facilitate the exchange of GDP to GTP cannot be the mechanism to switch these GTPases to the 'on' state.

An additional distinguishing feature of the SRP and SR GTPases is that they form a thermodynamically stable heterodimeric complex when both of them are bound with GTP (Fig. 1B, step 2) (22). In this complex, the two GTPases reciprocally activate the GTP hydrolysis activities of one another 10^2–10^4 fold (Fig. 1B, step 3; Table 1) (21). Following GTP hydrolysis, the GDP-bound SRP•SR complex is much less stable, and quickly disassembles to regenerate free SRP and SR (Fig. 1B, step 4). Thus there is no need to recruit an external GAP to facilitate GTP hydrolysis and turn these GTPases to the 'off' state. Together, these unique features of the SRP and SR GTPases suggest that they employ an intrinsic mode of regulation of their GTPase cycles that is distinct from the extrinsic mode of regulation depicted in the classical GTPase switch paradigm.

Multiple conformational states in the SRP•SR complex govern GTPase function during protein transport

Given that SRP and SR are intrinsically capable of cycles of dimerization and GTP hydrolysis, how can their kinetics of complex assembly and GTPase activation be controlled so that these GTPases function as molecular switches to regulate the complex series of molecular interactions required for protein transport? Recent biochemical, biophysical, and structural analyses (23–27) demonstrated that the function of SRP and SR are governed by a series of discrete conformational changes during their heterodimeric interactions with each other that culminate in their reciprocal GTPase activation (Fig. 2 and 3). Importantly, each of these conformational rearrangements provides a distinct point whereby regulation can be exerted by interactions with the cargo, the SRP RNA, and the target membrane (26–28), allowing these proteins to sense and respond to their biological cues and thus provide exquisite spatial and temporal coordination of co-translational protein transport (Fig. 2 and 3).

SRP-SR complex assembly is regulated by the cargo

If SRP and SR predominantly exist in GTP-bound states that are capable of forming a stable complex, what prevents futile rounds of dimerization and GTP hydrolysis by these proteins? The answer lies in the slow intrinsic rates of their complex assembly. Structural studies showed that the N-domains of the isolated proteins are not correctly positioned to allow for efficient interaction between one another (6,7,23,29). Indeed, recent work (30) has shown that the first α-helix in the N-domains of both proteins (αN1) act as negative regulators that block the SRP-SR interaction (Fig. 3, top panel, αN1 helix of SR is highlighted in *gold*). Thus, free SRP and

SR GTPases are largely in an inactive, *open* conformation suboptimal for interacting with one another, and substantial conformational rearrangements need to occur to assemble a stable and active SRP•SR complex.

Nonetheless, the first complex detected *in vitro* between these two GTPases is an *early* intermediate that can be formed with or without GTP bound at the active site (Fig. 2 and 3, step 2) (27). While this intermediate forms rapidly, it is highly unstable (27). Little is understood about the structure of this complex, but it is likely to involve loose contacts between the G-domains of both proteins. However, because the steric blocks imposed by the αN1 helices are likely not removed in this intermediate and the two N-domains cannot productively interact with one another, the *early* intermediate is highly transient in the absence of other factors.

We speculate that both SRP and SR explore conformational spaces in this intermediate to search for the correct structure conducive to stable binding. A successful conformational search leads to the formation of a much more stable, *closed* complex (Fig. 2 and 3, step 3). Mutational and crystallographic analyses (23,24) strongly suggest that this transition involves a rearrangement at the intra-molecular interface between the G- and N-domains of both proteins, which acts as a hinge to adjust the relative position of the N-domain with respect to the G-domain (Fig. 3, bottom panel). The αN1 helices in both SRP and SR also rearrange to remove the steric hindrance associated with them (30,31) and as a result, the two N-domains move closer to one another and form additional interface contacts, creating a large, continuous interaction surface between the two proteins that spans 3600 $Å^2$ of surface area (Fig. 3, bottom panel). In addition, the two GTP molecules directly interact with one another across the dimer interface, forming a pair of reciprocal hydrogen bonds between the 3′-hydroxyl of each GTP and the γ-phosphate of the other (Fig. 3, left panel). These rearrangements generate a GTP-dependent *closed* complex that is 400-fold more stable than the *early* intermediate in the absence of additional factors.

Remarkably, in the presence of the cargo the kinetics of stable SRP-SR complex assembly is accelerated over 100-fold (28,32). This rate acceleration is due to an ~100-fold stabilization of the *early* intermediate by interactions with the cargo, such that formation of the *early* intermediate is sufficient to give a stable cargo•SRP•SR *early* targeting complex under physiological conditions (28). Interaction with the cargo also gives the *early* intermediate a much longer lifetime and thus facilitates its rearrangement to the subsequent *closed* complex. Both of these effects allow the cargo-loaded SRP to achieve much faster complex assembly kinetics. Thus, only when the cargo is loaded do the SRP and SR efficiently come together to form a stable complex (Fig. 2 and 3, steps 1–2). This ensures rapid delivery of cargo to the membrane (Fig. 2, step 2), and minimizes futile interactions between the free SRP and SR.

The ability of cargo to stabilize the *early* intermediate has another important consequence: the interaction with the cargo is also significantly strengthened in the *early* complex relative to the interaction with free SRP. This arises from the reciprocity of allosteric effects: if the *early* intermediate is stabilized by the cargo, then conversely, the interaction of cargo with SRP would be stabilized to the same extent, ~100-fold, upon formation of the *early* intermediate. Thus in this *early* targeting complex, the cargo makes the strongest and most extensive interactions with the SRP and SR, with an affinity of K_d ~ 10 pM (28). This could allow the SRP to effectively compete with cytosolic chaperones and other targeting factors such as SecB and trigger factor, directing its substrate proteins to the SRP pathway.

Conformational rearrangements in the SRP•SR complex drive cargo transfer to the translocon

The tight binding of cargo in the *early* intermediate, though beneficial in the early stages of targeting, poses a problem for the subsequent steps during which the cargo needs to be released

from the SRP and transferred to the translocon. With an affinity of ~10 pM, the release of cargo would be expected to take >2 hours, whereas SRP-mediated protein transport is usually complete within 3 seconds *in vivo*. Recent results suggest that a series of conformational rearrangements must occur in the SRP•SR complex to drive the unloading of cargo from the SRP to the translocon (28,33,34). These changes include the rearrangement of the *early* intermediate to the *closed* complex, as discussed above, and an additional rearrangement of the highly conserved IBD loops (Fig. 1B and Fig. 3 left panel, *red*) that leads to GTPase activation in the complex. That the conformational changes leading to the formation of the *closed* and *activated* states occur sequentially was inferred from a class of mutant GTPases that map to the IBD loops and allow formation of a stable SRP•SR complex, but blocks reciprocal GTPase activation (26). Structural studies (23,24) showed that upon complex formation, the IBD loops move into close proximity with the two bound GTP molecules and allow a composite active site to be formed at the interface between the two proteins (Fig. 3, left panel). Each loop provides at least three catalytic interactions that position the nucleophilic water, interact with the α- and γ-phosphate oxygens, and coordinate the active site Mg^{2+} ions (Fig. 3, left panel).

Several lines of evidence showed that the rearrangements of the SRP•SR complex from the *early* intermediate to the *closed* and *activated* states switch the SRP from a cargo-binding mode to a cargo-releasing mode and thus help drive cargo unloading. Equilibrium analysis showed that the interaction of the cargo with the SRP is weakened ~400-fold when the *early* targeting complex rearranges to the subsequent *closed* and *activated* conformations (28). Further, mutant GTPases that block the *closed* → *activated* rearrangement allow protein transport to proceed only to an intermediate stage where a stable cargo•SRP•SR complex can be formed, but the cargo fails to engage with and be translocated by the translocon (34). Finally, cryo-EM analyses suggest that in the presence of SR and GTP analogues, the NG domain of SRP becomes mobile and detaches from the ribosome (33). Together, these results demonstrate that forming an SRP•SR complex and thereby bringing the cargo to the membrane is not sufficient to drive the transfer of cargo from the SRP to the translocon; rather, a series of elaborate conformational rearrangements need to occur in the SRP•SR complex that drives the handover of cargo from the SRP to the translocon at late stages of protein transport (Fig. 2, steps 3–5).

The cargo regulates GTP hydrolysis from the SRP•SR complex

The timing of GTP hydrolysis is crucial for ensuring productive protein transport, as the SRP must unload and transfer its cargo to the translocon (Fig. 2 and 3, steps 4–5) before GTP hydrolysis drives the irreversible disassembly of the SRP•SR complex (Fig. 2 and 3, step 6). In the absence of any spatial and temporal cues, a stable SRP•SR complex has a very short lifetime because rapid GTP hydrolysis drives complex disassembly as soon as it is formed (21). Intriguingly, recent work in the bacterial SRP system (28) showed that the cargo stalls a large fraction of the SRP•SR complex in the *early* conformational state and disfavors its rearrangement to the subsequent conformations (Fig. 2 and 3, ⊥). As a consequence, the cargo uncouples complex formation from GTPase activation, and delays GTP hydrolysis in the SRP•SR complex by ~10-fold (28). A similar effect was suggested from studies of the mammalian system in which, prior to the addition of membrane vesicles, a stable cargo•SRP•SR complex persists in the presence of GTP, suggesting that the cargo may also delay GTP hydrolysis in the mammalian SRP•SR complex (35). This effect, termed ‘pausing’, suggests that the timing of GTP hydrolysis is actively regulated to ensure the efficiency of protein transport. Pausing prevents premature GTP hydrolysis, which would lead to abortive reactions (Fig. 2, dashed arrows), and prolongs the lifetime of the cargo•SRP•SR complex from <1 s to ~8 s, creating an important time window during which the targeting complex can search for the membrane and the translocation machinery. We speculate that the interaction of SR with the phospholipid membrane and perhaps with the translocon may overcome the cargo-

induced 'pausing' and trigger the rearrangement of the GTPase complex to the *closed* and *activated* states, thus initiating cargo unloading (Fig. 2 and 3, steps 4–5). Once the cargo is unloaded, the *activated* SRP•SR complex quickly hydrolyzes GTP to drive the disassembly (36) and recycling of the SRP and SR components (Fig. 2 and 3, step 6).

Multiple conformational changes can provide multiple fidelity checkpoints

The presence of extensive molecular crosstalk between the cargo, the GTPases, and the membrane translocon also introduces the possibility that multiple fidelity checkpoints could be built into this pathway to discriminate between authentic vs. non-authentic cargos. SRP binds to authentic cargos carrying strong signal sequences with high affinity ($K_d \leq 1$ nM; (37,38)). However, SRP has appreciable affinity ($K_d \sim 100$ nM) even for empty ribosomes and RNCs containing weak or no signal sequences (38). Hence, given the cellular SRP concentration of ~400 nM, a significant fraction of the 'incorrect' cargo would be associated with the SRP. Could the subsequent steps during protein transport help reject the incorrect cargo? Authentic cargos carrying strong SRP signal sequences accelerate SRP-SR complex assembly over 100-fold, and one could envision that the 'incorrect' cargos with weak or no signal sequences could not provide similar rate accelerations, and are thus rejected kinetically. Further, formation of the *early* SRP•SR complex is stabilized by the cargo ~100-fold (28), thus preventing the premature disassembly of the *early* cargo•SRP•SR targeting complex. One could envision that the incorrect cargos would form less stable *early* targeting complexes and thus would not efficiently move along a productive targeting pathway. Finally, cargo-induced 'pausing' prevents premature GTP hydrolysis and increases the efficiency of cargo transfer to the translocon and therefore, the fraction of cargos that undergo a productive protein transport cycle. One could envision that the incorrect cargo could not delay GTP hydrolysis as effectively and would thus be more likely to be rejected through premature GTP hydrolysis, akin to kinetic proofreading mechanisms that are used during translation. Given that the SRP pathway needs to handle the transport of one third of cellular proteins and that signal sequences vary widely in length, shape, and amino acid composition (39–43), it is conceivable that multiple fidelity checkpoints are built into this pathway to allow small differences in signal sequences to be effectively distinguished.

A new class of multi-state regulatory GTPases

Despite the absence of a classical bi-modal 'GTPase switch' and without recruiting external regulatory factors, the SRP and SR GTPases nevertheless provide exquisite spatial and temporal control of the protein transport reaction. Using their ability to undergo multiple allosteric regulations driven by protein-, lipid- and nucleotide-interactions, these GTPases couple the loading of cargo to its efficient membrane delivery and unloading, ensuring the spatial and temporal fidelity of the molecular interactions required for protein transport. We suggest that the unique design features of the SRP and SR GTPases are best suited for controlling complex cellular processes that require multiple allosteric regulation. The ability of these GTPases to respond to biological cues by themselves may also allow such complex cellular pathways to be regulated with fewer components, in contrast to the classical 'GTPase switch' that requires at least three components (the GTPase, GEF, and GAP) to impose a single point of regulation.

Needless to say, many cellular processes share features similar to the protein transport reaction, requiring highly efficient action and multiple stages of allosteric regulation. GTPases that behave analogously to SRP and SR would be well suited for these processes. Indeed, rather than being an 'exception to the rule', new evidence suggest that these design features are shared by a growing number of proteins, which may define a new class of GTPases that can use their intrinsic conformational flexibility to exert multiple allosteric regulation. These include elongation factor G, the dynamin family of GTPases, and all the GTPases identified thus far

that mediate ribosome assembly (Table 1). Below we briefly summarize the mechanism of elongation factor G and the dynamin GTPase and their analogies with the SRP and SR as multi-state regulators.

Elongation factor G (EF-G)

EF-G promotes a translocation step in the translation elongation cycle, during which the peptidyl-tRNA moves from the A-site of the ribosome to the P-site, and deacylated tRNA moves from the P site into the E site from where it dissociates. Like the SRP and SR GTPases, EF-G binds nucleotides weakly and GDP dissociation from EF-G is rapid (Table 1), thus this GTPase does not require external nucleotide exchange factors to switch from the GDP- to the GTP-bound state. Also analogous to the protein transport reaction mediated by SRP and SR, the translocation of tRNAs catalyzed by EF-G also includes a sequential series of events, including: (i) binding of EF-G to the pre-translocation ribosome; (ii) rapid GTP hydrolysis by EF-G triggered by the ribosome; (iii) movement of the tRNAs on the ribosome; and (iv) dissociation of EF-G from the post-translocation ribosome.

The mechanism of EF-G has been extensively studied through biochemical and kinetic analyses, and these studies indicate that the GTPase binding and hydrolysis cycle of EF-G and its interaction with the ribosome drives a series of conformational changes in this GTPase as well as in the ribosome, thus coordinating sequential events during tRNA translocation. In the beginning of the cycle, GTP binding allows EF-G to assume an active conformation in which it binds favorably to the pre-translocation ribosome (Fig. 4, step 1) (44,45). The interaction of EF-G with the ribosome triggers another conformational change in this GTPase that activates its GTP hydrolysis reaction (Fig. 4, step 2) (44). GTP hydrolysis by EF-G drives a conformational rearrangement of the ribosome, referred to as ‘unlocking’, that precedes and limits the translocation of tRNA relative to the mRNA (Fig. 4, step 3, red arrow) (46). Subsequent to the unlocking step, interaction between the ribosomal protein L7/11 and EF-G controls the release of inorganic phosphate from EF-G (Fig. 4, step 4), returning this GTPase to a low-affinity conformation and thus driving its rapid dissociation from the ribosome (Fig. 4, step 5) (47). Although the details of the molecular interactions and conformational changes differ between EF-G and the SRP and SR GTPases, their fundamental operating principles – the ability to respond to their biological targets without external regulatory factors, and to use their intrinsic conformational flexibility to exert multiple points of allosteric control in a complex biological process – appear to be remarkably similar between these GTPases.

Dynamin

Dynamin is a 100 kD tetramer that shares many features in common with SRP family GTPases. It has a low affinity for GTP, exhibits rapid GDP dissociation (48), and crystal structures of its isolated GTPase domain suggest that it does not undergo large GTP-dependent conformational changes (49,50). Dynamin’s robust basal GTPase activity is further stimulated by assembly, but in this case as a higher order, helical homooligomer (51,52). The mechanism of assembly-stimulated GTPase activity remains unknown, but structural data from distantly related GTPases has suggested that it may involve dimerization and formation of a composite GTPase site, akin to the SRP family GTPases (53).

While dynamin may have many cellular functions, it is best characterized as the master regulator of clathrin-mediated endocytosis (CME). Like those regulated by SRP-family GTPases and EF-G, CME is a multi-step process (Fig. 5). It involves: 1) assembly of coat proteins to form a clathrin-coated pit (CCP), which deforms the underlying plasma membrane, 2) recruitment of transmembrane receptors and their bound ligands (i.e. cargo) into the CCP, 3) progressive development of curvature during coat assembly to form a deeply invaginated CCP, and 4) membrane fission to pinch off a nascent clathrin coated vesicle (CCV) carrying

its cargo into the cell (54). A plethora of endocytic accessory factors, which are also recruited to the growing CCP, are required for cargo selection, curvature generation and membrane fission (55). While the above is the stereotypic progression of events in CME, recent studies using live cell microscopy have revealed that not all initiation events lead to productive CCV formation and that a substantial subpopulation of nascent CCPs disassemble in 'abortive' events (56,57).

Most studies have focused on late stages of CCV formation (58,59) during which dynamin assembles into higher order oligomers that form short helical collars at the necks of deeply invaginated CCPs (Fig. 5). These short dynamin assemblies were recently shown to be sufficient to catalyze membrane fission *in vitro* (60,61). Interestingly, like SRP family GTPases, assembly-stimulated GTP hydrolysis triggers rapid disassembly of the complex (62). The resulting self-limited, short dynamin assemblies are essential to generate the highly localized curvature necessary for membrane fission.

While dynamin self-assembly occurs late in CCV formation, unassembled dynamin is recruited early to CCPs and interacts directly with several SH3 domain-containing endocytic accessory factors through its C-terminal proline/arginine rich domain (PRD). Dynamin's exact function and mechanism of action during early stages of CCP maturation are not well understood; however, recent studies have shown that mutations affecting dynamin's basal GTP binding and hydrolysis activities alter the turnover rates of abortive CCPs and the rate of CCV formation (57). Thus, like the SRP family GTPases, dynamin may govern fidelity checkpoints along the pathway of CCP maturation and productive CCV formation. The commitment to late events in CCV formation is marked by dynamin self-assembly. Importantly, both dynamin's basal GTPase activity and its self-assembly are subject to allosteric regulation by its SH3 domain-containing binding partners (Fig. 5, 'effectors'), with some enhancing these activities and others inhibiting them (62,63). These same binding partners are able to interact with coat proteins, sense membrane curvature and/or recruit cargo molecules (55), thus they are well-positioned to provide input into dynamin's function as a multi-state regulatory GTPase. Crosstalk between these accessory factors and dynamin's self-assembly and GTPase activities may serve to ensure the spatial and temporal hierarchy of molecular events in CCP maturation that precede membrane fission and CCV formation.

Design features of bimodal vs multi-state GTPases

The classical GTPase switch is bi-modal and extrinsically regulated; whereas the paradigm we describe here involves GTPases that are instrinsically regulated and conformationally flexible. What drives the unique design of these multi-state regulatory GTPases? To address this question, we can reflect on two key features of the classical 'GTPase switch'. The first is its bi-modal nature (Fig. 1A), i.e., classical signaling GTPases often have a well-defined 'on' state where they interact with downstream effector molecules. For example, *Ras* and *Rho* GTPases bind and activate a variety of kinases in their GTP-bound state; EF-Tu in its GTP-bound state binds aminoacyl-tRNAs and the ribosome; *Ran* in its GTP-bound state binds importin β to displace the cargo. In contrast, it is difficult to define a single 'on' or 'off' state for multi-state regulatory GTPases such as the SRP (Fig. 1B). The biological events mediated by these GTPases generally involve a complex series of molecular interactions where different functions must be turned 'on' or 'off' at appropriate stages of the pathway. The ability of these GTPases to undergo multiple conformational changes regulated by allosteric interactions with upstream and downstream components is critical for their role in driving cyclic processes where multiple factors must bind and later dissociate in a sequential and highly coordinated manner.

The second key feature of the classical 'GTPase switch' is that the 'on' and 'off' states of small signaling GTPases are temporally and often spatially separated from one another. In the absence of signaling cues, these GTPases are often kept in the 'off' state for prolonged periods

of time, and GTP hydrolysis acts as a timer that allows for a controlled period of action before returning to the 'off' state. This feature is essential for the function of GTPases mediating cellular signaling and other processes that require a high degree of negative regulation, since uncontrolled activation of pathways in the absence of signaling cues are detrimental to the cell. The extrinsic GEFs and GAPs of these GTPases impose this tight regulation. In contrast, the processes mediated by SRP family of GTPases are highly constitutive and must often occur rapidly. For example, co-translational protein transport must compete with ongoing protein translation and when the nascent chain exceeds ~110 amino acids in length, it is no longer competent for transport by the SRP pathway (38,64). Therefore, the SRP and SR must complete each protein transport cycle within less than three seconds. Thus, multi-state GTPases intrinsically regulate their own catalytic activities. The ability of these GTPases to respond to biological cues and undergo conformational transitions by themselves without the need to recruit additional factors may be especially beneficial for vectorial processes that must occur quickly and with high fidelity.

Conclusions

In summary, conformationally flexible GTPases such as SRP and SR, EF-G and dynamin that are both auto- and allosterically regulated are uniquely suited to coordinate largely constitutive, highly efficient biochemical pathways. Auto-regulation gives these GTPases the ability to change conformation without the need to recruit external factors. Allosteric regulation by upstream and downstream components and their ability to undergo multiple conformational rearrangements enables these GTPases to govern complex pathways that require multiple molecular interactions to occur in a highly coordinated fashion. More work will be needed to decipher the precise roles and the molecular mechanisms of these GTPases, and to explore the extent to which multi-state regulatory GTPases are involved in coordinating other important cellular processes.

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Abbreviations

GTP	guanosine 5′-triphosphate
SRP	signal recognition particle
SR	signal recognition particle receptor
GEF	guanine nucleotide exchange factor
GAP	GTPase activating protein
IBD	insertion box domain
CME	clathrin-mediated endocytosis
CCP	clathrin coated pit
CCV	clathrin coated vesicle
PRD	proline/arginine rich domain

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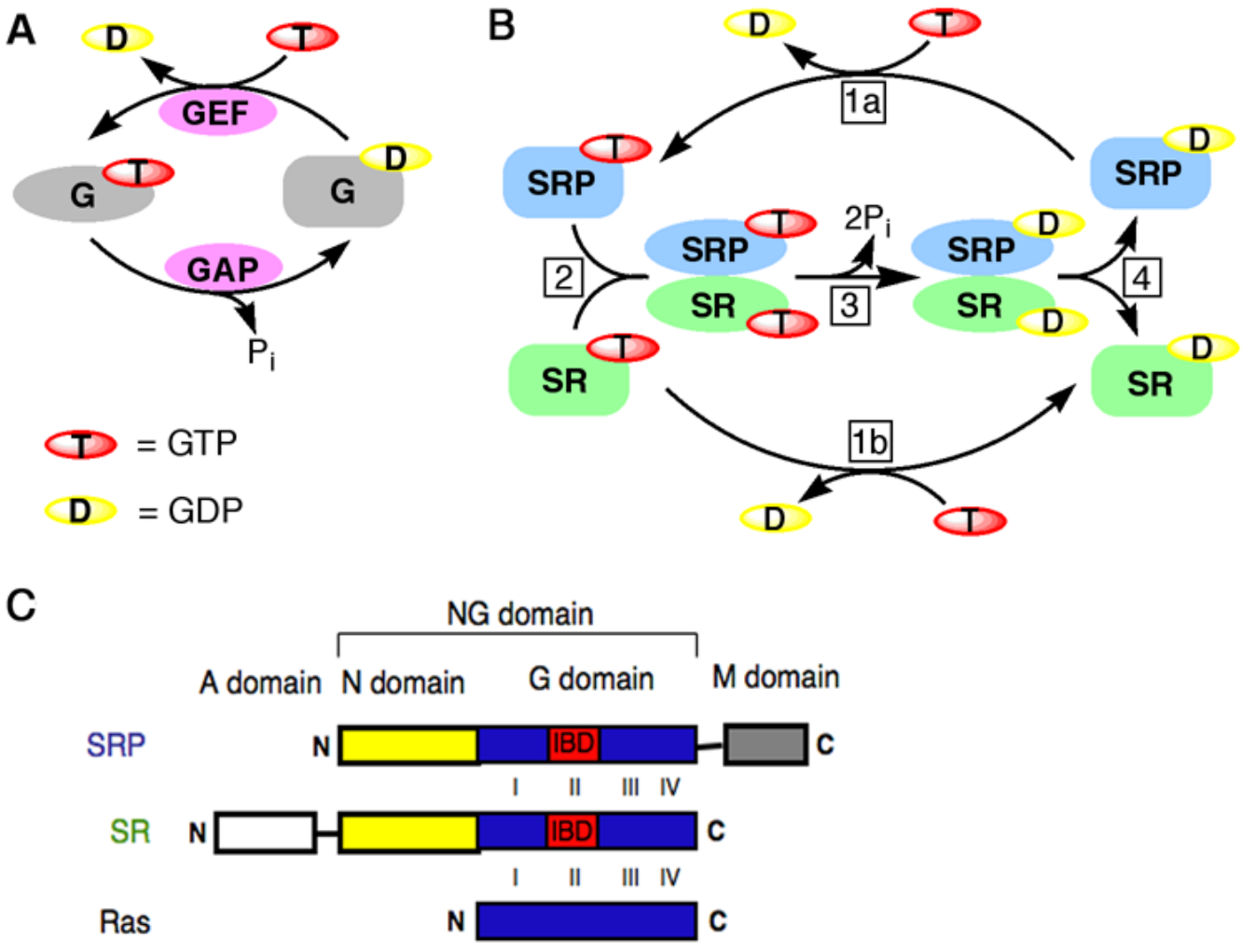

Figure 1. Comparison between the classic GTPase switch and the SRP and SR GTPases

(**A**) The bimodal GTPase cycles of classical signaling GTPases. GEF, guanine nucleotide exchange factor. GAP, GTPase activating protein. (**B**) The multi-state cycle of SRP and SR GTPases. Step 1a and 1b, nucleotide exchange on SRP and SR, respectively. Step 2, complex formation between the SRP and SR GTPases. Step 3, activated GTP hydrolysis from the SRP•SR complex. Step 4, dissociation of SRP and SR after GTP hydrolysis, returning these GTPases to the basal state. (**C**) Domain composition of the core SRP and SR proteins and comparison with Ras. The conserved G-domains are colored in *blue* and the 4 GTP binding elements indicated. The SRP and/or SR specific IBD loops (*red*), N-domains (*yellow*), M-domain (grey) and A-domain (white) are shown.

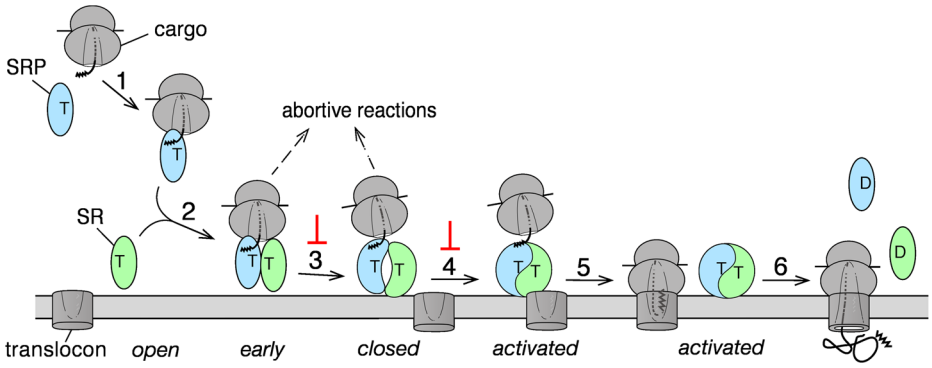

Figure 2. Spatial and temporal regulation of protein targeting by conformational changes in the SRP and SR

Step 1, cargo recognition by the SRP. Step 2, cargo-loaded SRP associates with SR to form a stabilized targeting complex in the *early* conformation. The rearrangements of the GTPase complex to the *closed* and *activated* states are stalled by the cargo (⊥) in the absence of membrane binding. Step 3, association of SR with anionic phospholipids is proposed to drive rearrangement of the *early* intermediate into the *closed* state, during which SRP weakens its affinity for the cargo. Step 4, interaction of SR with the translocation machinery may further relieve the cargo-induced stalling, allowing the SRP•SR complex to rearrange to the *activated* state. Step 5, this rearrangement further weakens the affinity of the cargo for the SRP and drives the handover of cargo from the SRP to the translocon. Step 6, GTP hydrolysis from the SRP•SR complex drives the disassembly and recycling of the SRP and SR.

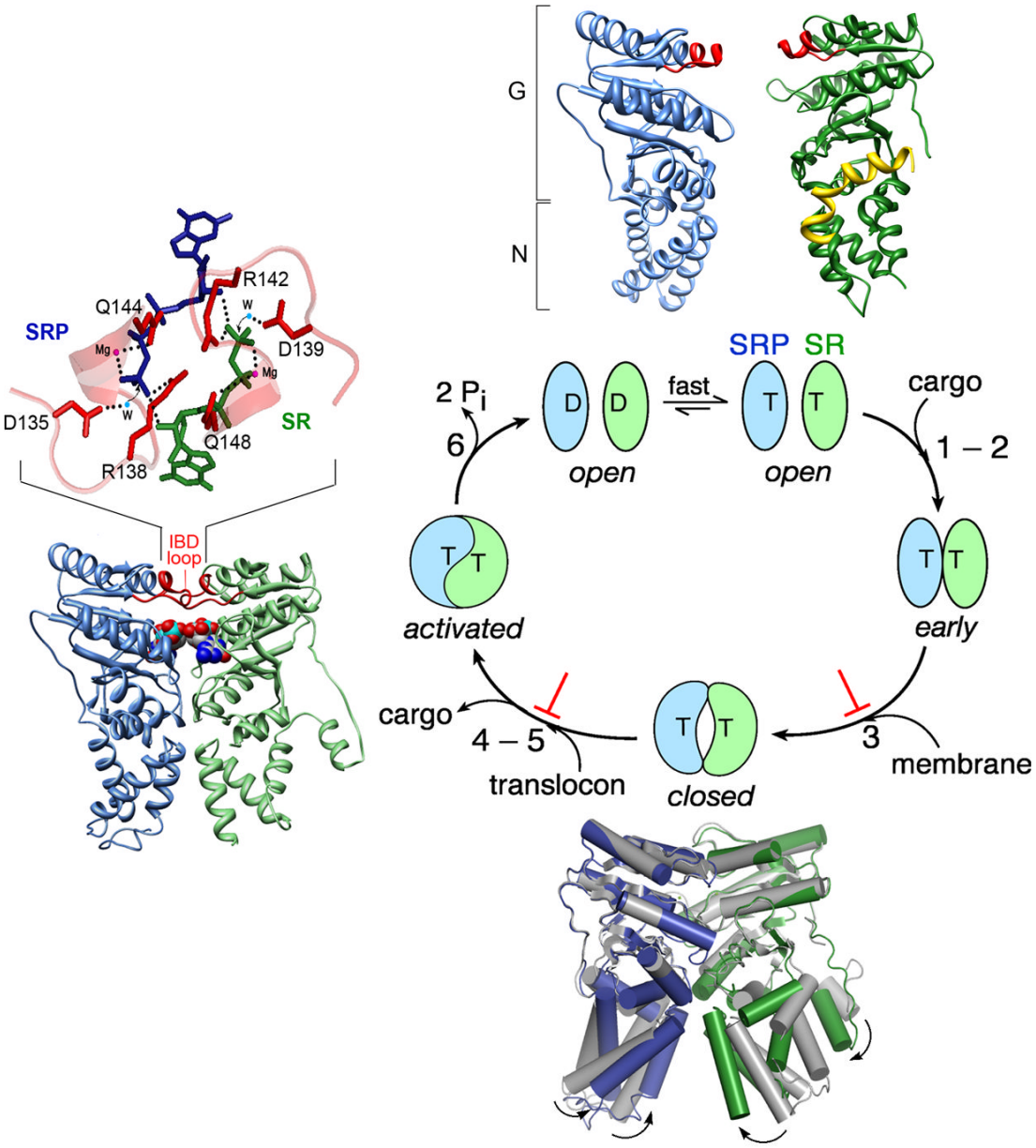

Figure 3. Conformational flexibility and allosteric regulation of the SRP family of GTPases

The steps are numbered to be consistent with those in Figure 2. ⊥ denotes the effect of cargo in preventing the rearrangements of the GTPase complex to the *closed* and *activated* states. Top panel: the crystal structures of free SRP (1ffh) and SR (2iyl) NG domains. The SRP GTPase is in *blue*, the SR GTPase is in *green* and its αN1 helix is highlighted in *gold*, the IBD loops in both proteins are highlighted in *red*. Bottom panel: G-domain superposition of the co-crystal structure of the *T. aquaticus* SRP-SR NG domain complex (1rj9; SRP and SR in *blue* and *green*, respectively) with those of free SRP and SR (*grey*), highlighting the movements in the N-domains of both GTPases. Left panel: Ribbon diagram of the co-crystal structure of the SRP-SR NG domain complex (1rj9) highlighting the catalytic IBD loops (*red*). Catalytic interactions in the composite active site are shown in the zoom-in view, with the GMPPCP molecule from the SRP and SR shown in *blue* and *green*, respectively, the active site Mg^{2+} ions in *magenta*, and the nucleophilic water molecules in *blue*. The backbones of the IBD loops are shaded in

coral and the side chains of the essential catalytic residues from these loops are highlighted in *red*.

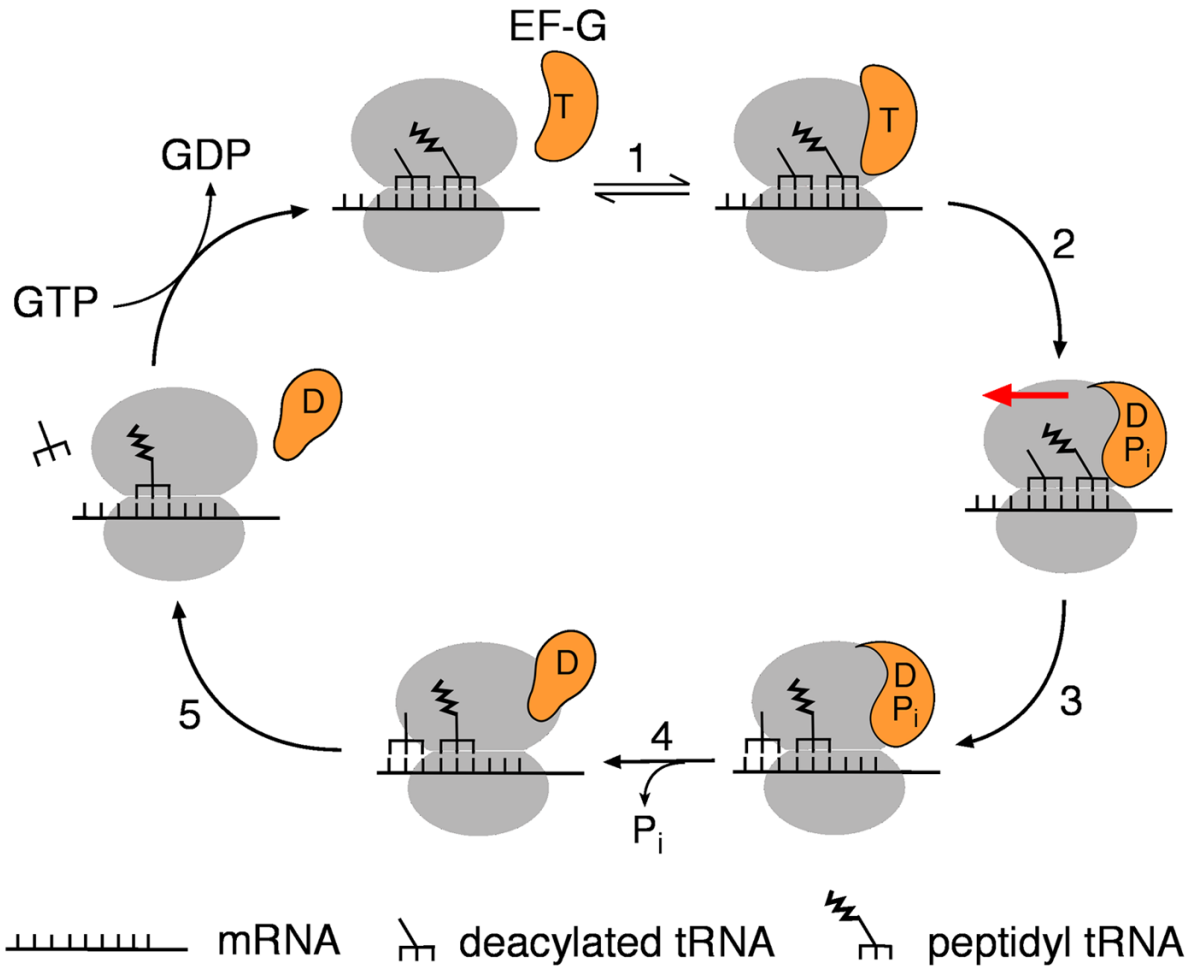

Figure 4. EF-G catalyzed tRNA movement on the ribosome

Step 1, EF-G binds to the pre-translocation ribosome in the GTP-bound form. Step 2, stimulated GTP hydrolysis from EF-G. Step 3, EF-G catalyzed tRNA-mRNA movement on the ribosome. The red arrow depicts the movement of tRNAs relative to the mRNA. Step 4, Release of inorganic phosphate (P_i) from EF-G. Step 5, GDP-bound EF-G dissociates from the post-translocation ribosome.

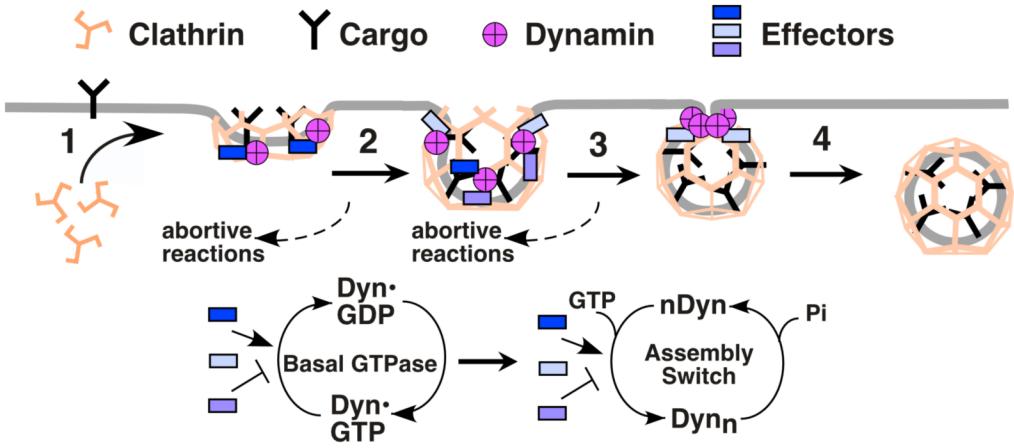

Figure 5. Dynamin is a multi-state regulatory GTPase governing clathrin-mediated endocytosis

Clathrin-mediated endocytosis is a constitutive process involving: 1) coat assembly, 2) cargo recruitment, 3) clathrin coated pit (CCP) invagination, and 4) membrane fission and clathrin coated vesicle formation. Dynamin is recruited to newly assembled coated pits. Its basal GTPase activity governs an endocytosis "restriction/checkpoint" as detected by the turnover of abortive CCPs. Dynamin assembly marks a late event in CCV formation and assembled dynamin-catalyzes membrane fission. Dynamin effectors, which variously recognize cargo, coats and membrane curvature can negatively and positively regulate both dynamin's basal GTPase activity and its ability to self-assemble. Thus, through these activities dynamin can function as a multi-state regulator to monitor the fidelity and progression of CME.

Table 1

Nucleotide binding rate and equilibrium constants.

GTPase	Cellular function	K_d^{GTP} (μM)	K_d^{GDP} (μM)	k_{off}^{GDP} (s^{-1})
Ras[a]	signaling	7.1×10^{-7}	8.3×10^{-6}	4.2×10^{-4}
Cdc42[b]	cytoskeleton organization			2.6×10^{-4}
EF-Tu[c]	translation	6×10^{-2}	1×10^{-3}	2×10^{-3}
Sec4(Rab)[d]	vesicular trafficking	3.5×10^{-3}	7.7×10^{-2}	3.5×10^{-3}
SRP[e]	protein transport	0.39	0.24	14
SR [f]	protein transport	14	26	5
Dynamin [g]	endocytosis	0.5 – 2.5	20	60 – 93
Dnm1[h]	mitochondria fusion	79 – 214	N.D.	N.D.
EF-G [i]	translation elongation	22	40	10 – 300
IF2 [j]	translation initiation	> 20	7	N.D.
Bms1[k]	ribosome assembly	182	22	N.D.
Era [l]	ribosome assembly	3.6	0.6	N.D.
Nug1 [l]	ribosome assembly	200	N.D.	N.D.
Obg [l]	ribosome assembly	1.2	0.5	N.D.
EngA [l]	ribosome assembly	110 – 143	N.D.	N.D.

[a] From reference (65).

[b] From reference (66).

[c] From reference (67).

[d] From reference (68).

[e] From references (18,21).

[f] From references (19,21).

[g] From (48) and references therein and (69).

[h] From reference (70).

[i] From reference (71).

[j] From reference (72).

[k] From reference (73).

[l] From (74) and references therein.