Endoplasmic Reticulum Targeting and Insertion of Tail-Anchored Membrane Proteins by the GET Pathway

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Hundreds of eukaryotic membrane proteins are anchored to membranes by a single transmembrane domain at their carboxyl terminus. Many of these tail-anchored (TA) proteins are posttranslationally targeted to the endoplasmic reticulum (ER) membrane for insertion by the guided-entry of TA protein insertion (GET) pathway. In recent years, most of the components of this conserved pathway have been biochemically and structurally characterized. Get3 is the pathway-targeting factor that uses nucleotide-linked conformational changes to mediate the delivery of TA proteins between the GET pretargeting machinery in the cytosol and the transmembrane pathway components in the ER. Here we focus on the mechanism of the yeast GET pathway and make a speculative analogy between its membrane insertion step and the ATPase-driven cycle of ABC transporters.

The mechanism of membrane protein insertion into the endoplasmic reticulum (ER) has been extensively studied for many years (Shao and Hegde 2011). From this work, the signal recognition particle (SRP)/Sec61 pathway has emerged as a textbook example of a co-translational membrane insertion mechanism (Grudnik et al. 2009). The SRP binds a hydrophobic segment (either a cleavable amino-terminal signal sequence or a transmembrane domain) immediately after it emerges from the ribosomal exit tunnel. This results in a translational pause that persists until SRP engages its

receptor in the ER and delivers the ribosome-nascent chain complex to the Sec61 channel. Last, the Sec61 channel enables protein translocation into the ER lumen along with partitioning of hydrophobic transmembrane domains into the lipid bilayer through the Sec61 lateral gate (Rapoport 2007).

Approximately 5% of all eukaryotic membrane proteins have an ER targeting signal in a single carboxy-terminal transmembrane domain that emerges from the ribosome exit tunnel following completion of protein synthesis and is not recognized by the SRP (Stefanovic

Editors: Susan Ferro-Novick, Tom A. Rapoport, and Randy Schekman

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Cite this article as *Cold Spring Harb Perspect Biol* 2013;5:a013334

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and Hegde 2007). Nonetheless, because hydrophobic peptides in the cytoplasm are prone to aggregation and subject to degradation by quality control systems (Hessa et al. 2011), these tail-anchored (TA) proteins still have to be specifically recognized, shielded from the aqueous environment, and guided to the ER membrane for insertion. In the past five years, the guided-entry of TA proteins (GET) pathway has come to prominence as the major machinery for performing these tasks and the enabler of many key cellular processes mediated by TA proteins including vesicle fusion, membrane protein insertion, and apoptosis. This research has rapidly yielded biochemical and structural insights (Tables 1 and 2) into many of the GET pathway components (Hegde and Keenan 2011; Chartron et al. 2012a; Denic 2012). In particular, Get3 is an ATPase that uses metabolic energy to bridge recognition of TA proteins by upstream pathway components with TA protein recruitment to the ER for membrane insertion. However, the precise mechanisms of nucleotide-dependent TA protein binding to Get3 and how the GET pathway inserts tail anchors into the membrane are still poorly understood. Here, we provide an overview of the budding yeast GET pathway with emphasis on mechanistic insights that have come from structural studies of its membrane-associated steps and make a speculative juxtaposition with the ABC transporter mechanism.

TA PROTEIN CAPTURE BY THE GET PATHWAY

The first step in all membrane protein insertion pathways is the selective capture of substrates. The carboxy-terminal hydrophobic anchors of TA proteins do not interact efficiently with SRP after they emerge from the ribosome exit tunnel following completion of protein synthesis (Stefanovic and Hegde 2007). Instead, they are shielded from the aqueous environment by the pretargeting complex of the GET pathway, which comprises Sgt2 (a small glutamine-rich tetratricopeptide repeat [TPR]-containing protein), Get4, and Get5 (Fig. 1). Sgt2 binds directly to tail anchors via its carboxy-terminal

Table 1. A catalog of GET pathway component structures

Component	Role in the pathway	PDB ID
Sgt2	Component of the pretargeting complex that delivers TA proteins to Get3; dimer interacts with Get4/Get5, contains TPR repeats that interact with Hsps	3SZ7
Get5	Component of the pretargeting complex that delivers TA proteins to Get3; dimer interacts with Get4 via amino-terminal domain and with Sgt2 via its ubiquitin-like domain	2LNZ 3VEJ 2LO0
Get4	Component of the pretargeting complex that delivers TA proteins to Get3; interacts with Get3 via amino-terminal domain and with Get4 via carboxy-terminal domain	3LPZ 3LKU 3WPV
Get3	ATPase that binds the TA protein; dimer interacts with the pretargeting complex in the cytosol, and with Get1/2 at the ER membrane	Table 2
Get1	ER receptor for Get3; integral ER membrane protein, three TMDs; forms a complex with Get2	3SJA, 3SJB 3SJC, 3ZS8 3VLC, 3B2E
Get2	ER receptor for Get3; integral ER membrane protein, three TMDs; forms a complex with Get1	3SJD 3ZS9

TA, tail anchored; TPR, tetratricopeptide repeat; TMDs, transmembrane domains.

domain, which is able to discriminate between TA proteins destined for the ER and those destined for the mitochondria (Wang et al. 2010). Defining the structural basis of substrate

Table 2. An itemized list of published Get3 structures with associated nucleotides and conformation nomenclature

Organism	Nucleotide	Conformation	PDB ID	References
Get3				
<i>Schizosaccharomyces pombe</i>	None	Open	2WOO	Mateja et al. 2009
<i>Saccharomyces cerevisiae</i>	None	Open	3H84	Hu et al. 2009
			3A36	Yamagata et al. 2010
<i>Aspergillus fumigatus</i>	ADP	Open	3IBG	Suloway et al. 2009
<i>S. cerevisiae</i>	ADP	Open	3A37	Yamagata et al. 2010
<i>Debaryomyces hansenii</i>	ADP	Closed	3IO3	Hu et al. 2009
<i>Chaetomium thermophilum</i>	AMPPNP-Mg ²⁺	Closed	3IQW	Bozkurt et al. 2009
<i>C. thermophilum</i>	ADP-Mg ²⁺	Closed	3IQX	Bozkurt et al. 2009
<i>S. cerevisiae</i>	ADP•AlF ₄ ⁻ -Mg ²⁺	Fully closed	2WOJ	Mateja et al. 2009
<i>Methanothermobacter</i>	ADP•AlF ₄ ⁻ -Mg ²⁺	Fully closed	3ZQ6	Sherill et al. 2011
<i>thermautotrophicus</i>				
<i>Methanococcus jannaschii</i>	ADP•AlF ₄ ⁻ -Mg ²⁺	Tetrameric	3UG6	Suloway et al. 2012
			3UG7	
Get3/Get2 _{cyto}				
<i>S. cerevisiae</i>	ADP-Mg ²⁺	Closed	3SJD	Stefer et al. 2011
<i>S. cerevisiae</i>	ADP•AlF ₄ ⁻ -Mg ²⁺	Closed	3ZS9	Mariappan et al. 2011
Get3/Get1 _{cyto}				
<i>S. cerevisiae</i>	None	Semiopen	3SJC	Stefer et al. 2011
<i>S. cerevisiae</i>	ADP	Semiopen	3VLC	Kubota et al. 2012
<i>S. cerevisiae</i>	None	Open	3SJA	Stefer et al. 2011
			3SJB	Stefer et al. 2011
			3ZS8	Mariappan et al. 2011
	ADP	Open	3B2E	Kubota et al. 2012

ADP, adenosine diphosphate.

recognition by this Sgt2 domain has been hampered by its poorly folded nature (Chartron et al. 2011) but we have more structural information on the other Sgt2 domains and components of the pretargeting complex. The central TPR domain of Sgt2 has a canonical structure that enables it to associate with a variety of chaperones (Wang et al. 2010; Chartron et al. 2011; Kohl et al. 2011). Sgt2's amino-terminal domain mediates both homodimerization and binding to the central, ubiquitin-like domain of Get5 (Liou and Wang 2005; Chang et al. 2010; Chartron et al. 2011). The carboxy-terminal domain of Get5 assumes a novel, helical bundle fold that mediates homodimerization (Chartron et al. 2012b). Get4 is an elongated, α -helical solenoid (Bozkurt et al. 2010; Chang et al. 2010; Chartron et al. 2010) that forms a tight complex with the amino terminus of Get5 at one end and a more labile complex with Get3 at the other (Chang et al. 2010; Chartron et al.

2010). In addition to this wealth of piecemeal structural information, a key insight into the function of Get4/5 came from the observation that these components of the GET pathway facilitate transfer of TA proteins from Sgt2 to Get3 by a dual mechanism (Wang et al. 2010). They increase the local concentration of Get3 near TA proteins held by Sgt2 and make Get3 more receptive for substrate binding. The precise stoichiometry and mechanistic details of the pretargeting step in the GET pathway remain to be worked out. For example, do Get4/5 enable a TA protein “hand-off” mechanism between Sgt2 and Get3 during which the substrate's hydrophobic anchor is transiently bound to both TA protein chaperones? What mechanism makes Get3 more receptive to bind TA proteins when it becomes transiently recruited to the pretargeting complex? Regardless of these details, the baroque nature of the pretargeting step in the GET pathway suggests that this

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Figure 1. Scheme of the steps in the yeast GET pathway. The cytosolic pretargeting complex (Get4/Get5/Sgt2) captures a newly synthesized tail-anchored (TA) protein released from the ribosome (with differentially shaded large and small subunits) and transfers it to Get3 loaded with ATP (1). The Get3-TA protein complex is subsequently tethered to the endoplasmic reticulum (ER) membrane by the cytosolic domain of Get2 (2) and then docked to the Get1 coiled coil (3). This latter interaction drives TA protein release by forcing open the Get3 dimer (4). Finally, rebinding of ATP dissociates Get3 from the membrane and prepares it for the next round of substrate pretargeting (5). The precise timing of ATP hydrolysis is not known but we have several reasons to favor a model in which it occurs on TA protein binding to Get3. First, the crystal structures of Get3 that have an assembled substrate-binding groove also have an ATP hydrolysis transition-state analog. Second, Get3 ATPase activity is essential for the TA protein release step. Third, the Get1 coiled coil preferentially binds to the apo form of Get3. Also note that for simplicity the higher-order stoichiometry of the pretargeting complex is not shown, whereas the stoichiometry of the Get1/2 transmembrane complex is hypothetically drawn as a 2:2 tetramer.

mechanism is being used to rapidly and selectively channel the appropriate substrates from the ribosome to Get3.

TA PROTEIN RECOGNITION BY THE Get3 ATPase

Get3 is an ATPase of the SIMIBI class of nucleotide triphosphate (NTP)-binding proteins (Leipe et al. 2002). The function of Get3 is to shuttle TA proteins between the pretargeting complex in the cytoplasm and the transmem-

brane components of the GET pathway in the ER (Schuldiner et al. 2008). Numerous crystal structures of fungal Get3 proteins in different nucleotide-bound states have yielded a plausible structural model for substrate recognition by Get3 (Simpson et al. 2010). Specifically, Get3 is a homodimer with subunit interactions stabilized by a coordinated zinc ion. Each monomer comprises a core nucleotide-binding domain (NBD) with interspersed α -helical insertions. Several pieces of evidence argue that these helices comprise the TA protein-binding domain

(TABD). First, the TABD helices are amphipathic and enriched in methionine and hydrophobic residues, a structural feature shared with the M domain of the SRP, which is involved in signal sequence binding (Keenan et al. 1998; Rosendal et al. 2003; Janda et al. 2010; Hainzl et al. 2011). Moreover, hydrophilic mutations at these residues result in reduced TA protein binding to Get3 (Mateja et al. 2009). Second, in certain nucleotide-bound states (see below), the TABD becomes structured into a large, composite hydrophobic groove with contributions from both monomers and the appropriate dimensions to receive an α -helical tail anchor (Mateja et al. 2009). Last, hydrogen–deuterium exchange mass spectrometry experiments have revealed that specific parts of the TABD are protected from exchange by TA protein binding (Bozkurt et al. 2009).

Although the TABD is undoubtedly the site of TA protein binding to Get3, the precise stoichiometry of Get3-TA protein complexes is a matter of debate. Small-angle X-ray scattering and analytical ultracentrifugation studies with fungal Get3 homologs have found that Get3 bound to TA proteins is a tetramer (Bozkurt et al. 2009; Suloway et al. 2012). This view is further supported by the crystal structure of an archaeal Get3 homolog showing a tetramer with a head-to-head arrangement of TABDs (Suloway et al. 2012). It is worth pointing out that tetrameric Get3 species bound to TA proteins come from proteins co-overexpressed in *Escherichia coli* in the absence of the pretargeting pathway components. Future studies should establish the stoichiometry of Get3-TA protein complexes assembled following TA protein delivery by Get4/Get5/Sgt2 (Wang et al. 2010).

The crystal structures of the Get3 dimer have also revealed how nucleotide-dependent conformational changes in the NBD are propagated to the TABD (Fig. 2). Specifically, in the apo and Mg^{2+} -free ADP states, the Get3 dimer is “open,” whereas $\text{ADP} \cdot \text{Mg}^{2+}$ and $\text{AMPPNP} \cdot \text{Mg}^{2+}$ induce a “closed” conformation. Furthermore, in the transition state of ATP hydrolysis (mimicked by $\text{ADP}^- \text{AlF}_4^- \cdot \text{Mg}^{2+}$), Get3 assumes a “fully closed” state in which the hydrophobic groove of the TABD is assembled. A recent free energy

calculation study of the Get3 opening and closing pathway (Wereszczynski and McCammon 2012) corroborates these findings and, in addition, postulates that Get3 can adopt a “wide open” apo state, as well as a “semiopen,” asymmetric nucleotide conformation with one ADP and one ATP. This latter conformation might be reminiscent of the one observed in a crystal structure of Get3 complexed with a piece of its ER receptor (see below). In sum, these experimental and computational studies have revealed a plausible mechanistic framework for how the Get3 nucleotide-linked conformational changes coordinate the pretargeting and membrane-associated steps of the GET pathway.

MEMBRANE RECRUITMENT OF Get3-TA PROTEIN COMPLEXES

Following the pretargeting events in the GET pathway, Get3-TA protein complexes are recruited to the ER membrane by Get1 and Get2 (Fig. 1, steps 2 and 3) (Schuldiner et al. 2008). These two membrane proteins each have three transmembrane domains that mediate formation of a stable transmembrane complex (Mariappan et al. 2011). The intimate nature of this association is well illustrated by the observation that deletion of either Get1 or Get2 leads to a significant reduction in the concentration of the partner protein (Schuldiner et al. 2008; Mariappan et al. 2011; Wang et al. 2011). Two recent structural studies argue that the amino-terminal cytoplasmic domain of Get2 mediates the initial contact between the Get3-TA protein complex and Get1/2 (Mariappan et al. 2011; Stefer et al. 2011). Specifically, even though the isolated cytoplasmic domain of Get2 (~140 amino acids) is for the most part unstructured, it can form a 2:2 complex with Get3. Crystal structures of this complex have revealed a pair of short Get2 helices ($\alpha 1$ and $\alpha 2$) that interact with Get3. Importantly, the Get3 dimer can interact with Get2 in a closed, nucleotide-bound state that is compatible with TA protein binding. This is because the Get2 binding site on Get3 is a monomeric epitope located away from the homodimerization interface. Binding is for the most part mediated by electrostatic

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Figure 2. Get3 conformations during its ATPase cycle. Get3 is a homodimeric ATPase (monomers are in blue and green) that comprises an α -helical TA protein-binding domain (TABD) and a nucleotide-binding domain (NBD). The *upper* panels show cartoon representations of the Get3 dimer, whereas the *middle* and *lower* panels show the corresponding crystal structures from the side or top, respectively (see Table 2 for the PDB entry codes). These structures are presented as a sequence of conformational changes that might accompany the Get3 ATPase cycle. Get3 bound to ATP most likely resembles the closed dimer conformation stabilized by AMPPNP-Mg²⁺ (a nonhydrolyzable ATP analog). In the process of ATP hydrolysis, a state stabilized by the ATP transition-state analog ADP•AlF₄⁻-Mg²⁺, Get3 assumes a full-closed conformation that leads to the assembly of a composite, hydrophobic groove in the TABD. Following release of inorganic phosphate (P_i), Get3 reverts to a more relaxed closed state similar to the one preceding hydrolysis. Last, in the absence of Mg²⁺, Get3-ADP assumes an open conformation, which also resembles the nucleotide-free (apo) state. All Get3 structures have been superimposed by the blue monomer to illustrate that nucleotide-driven dimer opening and closing is not simply a scissor-like motion, but involves substantial rotation of the monomers.

interactions between the conserved RERR sequence on the Get2 α 1 helix and the negatively charged surface patch on Get3's NBD that includes the conserved DELYED motif. Importantly, point mutations in the RERR sequence abolish Get3/Get2 complex formation and TA protein insertion into ER-derived membranes (microsomes).

TA PROTEIN RELEASE FROM Get3

Like Get2, Get1 has a large cytoplasmic domain (~80 amino acids) that in isolation forms a stable 2:2 complex with Get3 (Mariappan et al. 2011; Stefer et al. 2011). Structural studies of this complex support the idea that Get1 induces TA protein release from Get3 (Fig. 1, steps 3 and 4) (Mariappan et al. 2011; Stefer et al.

2011; Kubota et al. 2012). In particular, the cytoplasmic domain of Get1 is a coiled coil of two α helices (α 1b and α 2) stabilized by hydrophobic contacts similar to leucine zippers. In striking contrast to the Get3/Get2 complex, Get3 is bound to the coiled coils in an open conformation that lacks nucleotide (or has ADP; see Fig. 2) and has a disrupted TABD. This is because each coiled coil binds to both Get3 monomers via a composite epitope that is largely occluded in the closed Get3 conformation. Few interactions occur between Get1 α 1b and the TABD helix α 4 on one Get3 monomer, but more extensive contact is established by interactions between Get1 α 2 and NBD helices α 10 and α 11, which include the Get3 DELYED motif recognized by Get2, on the opposing monomer. Notably, Get1 mutations that disrupt interactions

with the DELYED motif abolish Get3/Get1 complex formation and TA protein insertion into microsomes. The overlap between the primary Get3/Get1 interface and the Get2 binding site implied that Get1 and Get2 compete for binding to Get3. This hypothesis was confirmed by an NMR analysis showing that Get1 can displace the Get2 $\alpha 2$ helix from its binding site on Get3 while still allowing the Get2 $\alpha 1$ helix to remain bound in the ternary complex (Stefer et al. 2011). Attempts to crystallize this ternary complex revealed instead the structure of a new Get3/Get1 complex with a novel Get3 conformation: a semiopen state with respect to the closed AMPPNP-Mg²⁺ and ADP-Mg²⁺ bound structures and the open apo form (Fig. 2) (Stefer et al. 2011). Interestingly, the semiopen and open Get3 makes similar contacts with the Get2 $\alpha 2$ helix. Taken together with a related semiopen Get3/Get1 structure (Kubota et al. 2012), these studies suggest that the Get1 $\alpha 2$ helix initiates TA protein release from Get3 tethered to Get2.

The structural model for the membrane-associated steps of the GET pathway is supported by biochemical reconstitution studies (Mariappan et al. 2011; Wang et al. 2011). In particular, TA proteins remain bound to Get3 on Get2 binding, but Get1 binding triggers substrate release from Get3. Importantly, even though high concentrations of the Get1 coiled coil can drive substrate release, under physiological conditions, the Get2 cytoplasmic domain is essential to increase the local concentration of Get3-TA protein complexes near Get1. Moreover, quantitative measurements of Get3 binding to Get1 have shown that in the ATP-bound state Get3 does not interact with Get1, whereas the apo form binds Get1 with high affinity (Mariappan et al. 2011; Stefer et al. 2011; Kubota et al. 2012). Indeed, in the crystal structures of the Get3-Get1 complex, the tip helix of the Get1 coiled coil protrudes into the nucleotide-binding site of Get3 suggesting that Get1 is a nucleotide exchange factor for Get3. Regardless of the details, ATP binding drives dissociation of Get3 from the membrane (Fig. 1, step 5) and prepares it for another round of substrate loading by the pre-targeting complex.

THE INSERTION STEP OF THE GET PATHWAY: A SPECULATIVE ANALOGY TO ABC TRANSPORTERS

The membrane insertion step remains the least well-understood step in the GET pathway. Proteoliposomes with purified Get1/2 afford the minimal membrane machinery necessary for TA protein insertion (Mariappan et al. 2011; Wang et al. 2011), but whether the transmembrane domains of Get1 and Get2 play an active role during this step is not known. A variety of mechanisms for Get1/2-mediated tail-anchor insertion have been hypothesized (Hegde and Keenan 2011; Chartron et al. 2012a; Denic 2012) and we add to this a speculative analogy to the substrate transport mechanism of ATP-binding cassette (ABC) transporters (Fig. 3). ABC transporters couple the energy of ATP hydrolysis to the transport of diverse substrates across the membrane. In general, they consist of two sets of TMDs and two NBDs that bind and hydrolyze ATP. Most ABC transporter NBDs are monomers in the absence of their TMDs but become dimerized in the presence of nonhydrolyzable ATP analogs. At first glance, the mechanistic requirements of ABC transporters and Get1/2/3 membrane machinery might seem disparate. However, closer inspection reveals a striking commonality. ATP binding drives rotation of both types of NBDs toward each other to create an extensive dimer interface. In ABC transporters, changes in the NBD conformation induced by binding and hydrolysis of ATP are transmitted to the neighboring TMDs via coupling helices. The resulting flipping of TMDs from an inward to an outward facing conformation drives substrate movement across the membrane. For TA protein insertion one could envisage a similar scenario whereby Get1 coiled coils act as coupling helices that translate movements associated with Get3 dimer opening into conformational changes in the flanking transmembrane domains (Fig. 4). Future structural and functional analysis of the Get1/2 transmembrane complex should establish if its transmembrane domains carry out the insertion step of the GET pathway.

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Figure 3. ABC transporters and Get1/3 interactions: A structural comparison. The *upper left* panel is a cartoon of a hypothetical 2:2 Get1/2 tetrameric transmembrane complex (Get1 subunits are in yellow and red; Get2 cytoplasmic domains are eliminated for simplicity) bound to a Get3 dimer (monomers are in blue and green; nucleotide and tail-anchor binding domains, NBD and TABD, respectively, are indicated) via the cytosolic coiled-coil domains of Get1. Both Get1 and Get2 are predicted to have three transmembrane domains (TMDs) each. The *upper right* panel is a cartoon of a generic ABC transporter with TMDs (ranging in number from 10 to 20) coupled to nucleotide-binding domains (ABC) by special α helices (cylinders). The *lower panels* are crystal structures of Get1 coiled coils complexed with Get3 and the multidrug ABC transporter Sav1866 (Dawson and Locher 2007) (the dashed line indicates the cytosolic border of the membrane) with structural elements color coded to suggest similarities between the two structures. Note that Sav1866 is a dimer with domain-swapped interactions between the coupling helices and nucleotide-binding domains. Furthermore, the ABC domains are in a closed conformation, whereas the Get3 dimer is open (see Fig. 4 for further details).

A WORKING MODEL FOR THE GET PATHWAY

In summary, in a relatively short period of time, a detailed mechanistic framework for the GET pathway has emerged (Fig. 1). ATP•Mg²⁺-bound Get3 receives a newly synthesized TA protein from the Get4/Get5/Sgt2 pretargeting complex. ATP hydrolysis, which probably occurs on substrate binding to Get3, ensures the formation of a stable, closed Get3-TA protein complex containing the hydrolyzed nucleotide (step 1). This complex is recruited to the ER membrane by the interaction with Get2, which

tethers it into proximity with Get1 (step 2). Binding of Get1 to Get3 displaces Get2 and induces Get3 dimer opening to release the TA protein substrate and hydrolyzed nucleotide (steps 3 and 4). These changes in the Get3 dimer conformation might be directly coupled to the Get1/2 transmembrane domains to facilitate the membrane insertion step. Last, binding of ATP to Get3 weakens the Get3–Get1 interaction to recycle Get3 for a new round of pretargeting (step 5).

Arguably, the most challenging problem that remains in the field is to define the structural and biochemical basis of the insertion step. For

Figure 4. Hypothetical structural model for the insertion step of the GET pathway. Get1 coiled coils might enable coupling between the opening of the Get3 dimer and movement of the transmembrane domains (TMDs). The predocking complex (*left*) is a hypothetical model of the Get3–Get1 interaction based on the fully closed Get3 state (2WOJ). Get1 docking causes the opening of the Get3 dimer via a semiopen state. Also shown are the associated hypothetical movements of Get3 relative to the membrane (from near to distant), as well as the coupled conformational changes in the Get1/2 TMDs (from wide to narrow).

example, does the release of substrate and hydrolyzed nucleotide from Get3 generate a “power stroke” that enables Get1/2 transmembrane domains to catalyze the insertion step? Furthermore, detailed biophysical and modeling studies will also be needed to turn the structural snapshots of the Get3 ATPase cycle into a movie of TA protein insertion. Together with characterization of conserved pathway components in higher eukaryotes and archaea, studies of the yeast GET pathway are well on their way to adding another textbook example of how cells chaperone membrane proteins into lipid bilayers.

ACKNOWLEDGMENTS

The authors thank Yin-Yuin Pang and Gert Bange for discussion, comments on the manuscript, and preparing the figures. I.S. acknowledges support by the DFG (SFB 638). V. Dötsch acknowledges support by the Deutsche Forschungsgemeinschaft (Do545/9), the Centre for Biomolecular Magnetic Resonance (BMRZ), and the Cluster of Excellence Frankfurt (Macromolecular Complexes). V.D. acknowledges support by the National Institutes of Health (RO1GM0999943-01), the

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Ellison Medical Foundation, and Harvard University.

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