NIH Public Access
Author Manuscript
Structure. Author manuscript; available in PMC 2013 January 28.

Published in final edited form as:
Structure. 2010 August 11; 18(8): 897–902. doi:10.1016/j.str.2010.07.003.

Structures of Get3, Get4, and Get5 Provide New Models for TA Membrane Protein Targeting

Peter J. Simpson[1], **Blanche Schwappach**[2], **Henrik G. Dohlman**[3], and **Rivka L. Isaacson**[1,*]

[1]Division of Molecular Biosciences, Imperial College London, Exhibition Road, South Kensington, London SW7 2AZ, UK

[2]Faculty of Life Sciences, University of Manchester, The Michael Smith Building, Oxford Road, Manchester M13 9PT, UK

[3]Department of Biochemistry and Biophysics, University of North Carolina, Chapel Hill, NC 27599-7260, USA

Abstract

The GET pathway, using several proteins (Gets 1–5 and probably Sgt2), posttranslationally conducts tail-anchored (TA) proteins to the endoplasmic reticulum (ER). At the ER, TA proteins are inserted into the lipid bilayer and then sorted and directed to their respective destinations in the secretory pathway. Until last year, there was no structural information on any of the GET components but now there are ten crystal structures of Get3 in a variety of nucleotide-bound states and conformations. The structures of Get4 and a portion of Get5 also emerged in 2010. This minireview provides a detailed comparison of the GET structures and discusses their mechanistic relevance to TA protein insertion. It also addresses the outstanding gaps in detailed molecular information on this system, including the structures of Get5, Sgt2, and the transmembrane complex comprising Get1 and Get2.

Introduction

A large proportion of cellular proteins reside within membrane bilayers and pose a particular challenge to the cell, in that they are synthesized in the aqueous cytoplasm where their hydrophobic transmembrane portions must be protected until delivery to the requisite membrane. For most membrane proteins, this is solved by cotranslational integration into the bilayer. However, around five percent of human membrane proteins, including SNAREs, signaling proteins and endoplasmic reticulum (ER) translocon components, belong to the family of tail-anchored (TA) proteins. TA proteins are each tethered to their respective membrane by a single transmembrane domain (TMD) at the extreme C terminus. This helix, obscured by the ribosome during translation, is inaccessible to the signal recognition particle (SRP), which cotranslationally directs most membrane proteins to the ER translocon for insertion. An alternative method of targeting is thus required and this function was recently ascribed to a complex of proteins known as GET (for guided entry of TA-proteins) (Stefanovic and Hegde, 2007; Favaloro et al., 2008; Schuldiner et al., 2008).

Each of the proteins so far found to participate in the yeast GET pathway is a relatively recent discovery (see Table 1). Get1 and Get2 are transmembrane proteins that bind tightly to each other and recruit Get3 to the ER membrane (Auld et al., 2006; Schuldiner et al.,

©2010 Elsevier Ltd All rights reserved

*Correspondence: rivka.isaacson@imperial.ac.uk.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

2008). Get3 can be isolated from cytosol in complex with Get4 and Get5 (Jonikas et al., 2009; Chang et al., 2010). Theoretically, Get3 is in a position to transiently link these two GET subcomplexes, which occur at the ER membrane and in the cytosol, respectively. Alternatively, Get3 may interact with Get1/2 or Get4/5 in a mutually exclusive fashion. While Get3 itself is always found as a homodimer and Get4/5 has recently been shown to contain two copies of each protein (Chartron et al., 2010), the full stoichiometry of cytosolic and membrane-associated GET complexes remains to be established. Very recent results from Favaloro et al. (2010) in the mammalian system show that once TRC40, also known as Asna1—mammalian Get3 equivalent (Stefanovic and Hegde, 2007, Favaloro et al., 2008), is fully bound to a TA protein, the TMD can be inserted into the ER membrane without any further requirement for cytosolic factors.

Genetics and biochemical data link Get4/5 with a tetratrico peptide repeat (TPR)-containing protein, Sgt2 (Jonikas et al., 2009; Chang et al., 2010; Costanzo et al., 2010; Leznicki et al., 2010; Battle et al., 2010), which is homologous to a vertebrate glutamine-rich TPR protein called SGTA with roles in androgen-receptor signaling (Buchanan et al., 2007) and mitosis (Winnefeld et al., 2006). Sgt2 also binds to the chaperones Ssa1&2 (homologs of Hsc70) and Ydj1 (Hsp40/DnaJ-related). Bat3, which physically interacts with SGTA, is another newly discovered player in GET-dependent TA protein insertion (Leznicki et al., 2010). Rabu et al. (2009) delineated three mechanisms for TA-membrane insertion (Hsc70/Hsp40, SRP, and GET), based on current experimental evidence, and these new data add support to their speculation that these mechanisms might be closely intertwined.

The central component of the *S. cerevisiae* GET pathway, an ATPase formerly known as Arr4 and now called Get3, first entered the literature in 2003 when Shen et al. described its similarity to a bacterial arsenical resistance factor, ArsA, and its roles in metal stress (Shen et al., 2003). It is the only eukaryotic ATPase member of the SIMIBI (for SRP, MinD, and BioD) class of NTPases (see Leipe et al. (2002) for a review) that is dominated by GTPases, including the main player in membrane protein insertion, the SRP. Prokaryotic ATPases in this class include ArsA, dethiobiotin synthetase (BioD), and Nitrogenase Iron Protein (NifH), which all feature the “deviant P loop” with a lysine in the second position (Koonin, 1993) and offer useful structural comparisons for the Get3 nucleotide cycle.

Further work (Schuldiner et al., 2005; Auld et al., 2006; Metz et al., 2006; Kao et al., 2007; Lee and Dohlman, 2008) identified roles for Get3 in Golgi to ER traffic (the original source of the GET acronym), the ubiquitin-proteasome system, metal homeostasis, signal transduction, and insulin secretion. Upregulation of TRC40/Asna1, the human homolog of Get3, has been observed in tuberculosis (Mistry et al., 2007), breast cancers (Kurdi-Haidar et al., 1998), and ovarian cancer (Hemmingsson et al., 2009a). Inhibition of Asna1 decreases tumor cell resistance to platinum-based therapies (Hemmingsson et al., 2009b). Epistatic miniarray profiling first established the functional and physical association between Get3 and Get1/2 (Schuldiner et al., 2005), or Get4/5 (Jonikas et al., 2009) in the context of protein biogenesis in the ER.

Most of these seemingly disparate functions were collectively rationalized as the result of Get3’s role in TA-protein insertion (Stefanovic and Hegde, 2007). TA proteins have diverse functions in membrane fusion, secretion, apoptosis, and quality control of proteins (for a recent review, see Rabu et al. [2009]) and failure to insert them has wide-reaching consequences, particularly under the conditions of cellular stress. These consequences include defects in removal of terminally misfolded proteins from the ER (Schuldiner et al., 2005) and impaired DNA damage repair (Zewail et al., 2003). Get3 is also known to bind other proteins; e.g., Gpa1 (Lee and Dohlman, 2008) and Gef1 (Metz et al., 2006), whose relation to TA-protein insertion is uncertain.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Since they enable protein-protein interactions, the TPR/TPR-like folds of Sgt2 and Get4 suggest that the cytosolic GET complex may serve as a scaffold to integrate TA biogenesis with other cellular pathways. It is likely that such coordination starts upon emergence of the TA-protein substrate from the ribosome (Rabu et al., 2009). The stress-related phenotypes of the GET pathway mutants imply that a putative interplay between this pathway and other chaperones becomes essential when protein biogenesis is jeopardized by unfavorable physicochemical conditions.

Structures of Get3

Until September 2009, there was no structural information available for Get3 or any other GET pathway component. Concurrent publication in *Proceedings of the National Academy of Sciences* (Suloway et al., 2009) and *Nature* (Mateja et al., 2009) of Get3 crystal structures, was swiftly followed by three further papers (Bozkurt et al., 2009; Hu et al., 2009; Yamagata et al., 2010) that featured “open” and “closed”structures from selected species (summarized in Table 2; examples represented in Figure 1), providing a variety of nucleotide-bound states and insights into the mechanism of TA-protein membrane insertion.

Structural Characteristics of Get3

The Get3 structure (see Figure 1) (Suloway et al., 2009; Mateja et al., 2009; Bozkurt et al., 2009; Hu et al., 2009; Yamagata et al., 2010) exists as a dimer held together by a zinc ion that is tetrahedrally coordinated by cysteines 285 and 288 (*S. cerevisiae* numbering in the CXXC motif). The constituent monomer consists of a nucleotide-binding domain (NBD), which includes the dimerization interface and a methionine-rich, helical domain that renders the dimer “open” or “closed.” “Closing” of the Get3 dimer involves the burial of approximately 1,500 Å of additional surface area, accompanied by a ~35° rotation of the subunits toward each other and a concomitant decrease in the distance between Mg^{2+}-binding sites by over 10 Å (Mateja et al., 2009; Bozkurt et al., 2009; Hu et al., 2009). In both states, the helical domain connects via a flexible region to the NBD, whose nucleotide-bound state appears to influence conformation.

In each case, the Get3 unit adopts the following order of secondary structure elements (13 α helices and 7 β strands) starting from the N terminus: α1, β1, α2, β2, α3, β3, α4, α5, α6, β4, α7, α8, α9, β5, α10, β6, α11, β7, α12, α13. The helical domain, whether “open” or “closed,” is formed from α helices α4 to α9 and includes disordered regions in every published structure. This reflects conformational flexibility in the helical domain, which might gain order in the presence of a TMD or other accessory proteins. The NBD is built from all the remaining helices and strands forming a mixed α-β fold.

The Get3 structure encompasses some canonical features (highlighted in Figure 2) of the P loop NTPase superfamily (Koonin, 1993), including an all-parallel 7 strand beta sheet, the “deviant” P loop (or Walker A motif for nucleotide binding with a rare lysine in the second position) at residues 25–33, a uniquely orientated Switch I at residues 57–61, and Switch II at residues 166–173. These dynamic switch regions bind the γ-phosphate of ATP through two main-chain hydrogen bonds, forming a “loaded spring” that is released upon ATP hydrolysis (Gasper et al., 2009). The A-loop for adenosine recognition occurs at residues 316–322. A TRC40 insert, which distinguishes eukaryotic and archeal versions of Get3 from its prokaryotic homologs (Mateja et al., 2009; Borgese and Righi, 2010) and may have an important role in TA binding, is found between α7 and α9 of the helical domain.

TMD-binding

Though we have yet to see an experimentally determined structure of Get3 bound to a TMD, Mateja et al. (2009), Bozkurt et al. (2009), and Yamagata et al. (2010) biochemically

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

analyzed the interaction between Get3 and the TMD from model TA-proteins Sec61β, Ramp4. and Sec22p, respectively. Moreover, Suloway et al. (2009) generated a panel of over seventy Get3 mutants to probe the TMD binding site in vivo.

A thorough mutational analysis was performed by Mateja et al. (2009), in which 24 hydrophobic amino acids punctuating the composite groove (formed by the "closed" structure of Get3) were individually substituted for aspartate residues. Each of these mutants was tested for binding to the TMD of Sec61β and relative ATPase activity in vitro, and was also screened for its ability to functionally complement a *Δget3 S. cerevisiae* strain in vivo. Thus, Mateja et al. (2009) pinpointed a region comprising helices α7 and α8, and overlapping with the "TRC insert," as the crucial site of TMD binding. These authors suggest that the exposed hydrophobic surface of the "open" state is unsuitable for TMD binding, whereas the "closed" form brings together the hydrophobic residues to create the composite groove.

Suloway et al. (2009) screened a large number of Get3 mutant plasmids for their ability to rescue the growth defects of a *Δget3* yeast strain. By identifying a cluster of "loss of function" mutations, they successfully predicted the "closed" form of Get3 shown by Mateja et al. (2009), with a comparable binding groove for TMDs.

Bozkurt et al. (2009) took a more biophysical approach to analyzing the Get3-TMD interaction. They first coexpressed and copurified Get3 and TA-protein Ramp4, both from *C. thermophilum*, and found that the complex did not contain any nucleotide. They then used amide hydrogen exchange mass spectrometry (HX-MS) to compare its dynamics with those of Get3 in the "open" and "closed" states. Ramp4 shielded amide hydrogens from exchange to deuterons in specific regions corresponding to helices α7, α8, and α9, and the opposite effect was seen at the end of helix α6.

Yamagata et al. (2010) coexpressed GST-tagged Get3 with Sec22p and showed that the proteins copurified on a glutathione column. They replaced nineteen residues between α7 and α9 (and hence most of the "TRC insert") of Get3 with a Gly-Ala-Ala-Gly linker and found that binding to Sec22p was radically reduced.

Although data from these three groups are largely consistent, there is still a lot of missing information on the mode of Get3 binding to TMDs. The inherent flexibility of the helical domain and its changing conformations offer scope for a complex mechanism of TMD association that will become clearer as further atomic-level information becomes available.

Nucleotide Hydrolysis Cycle

ADP.AlF_4^- and AMPPNP-Mg^{2+} binding mimic the ATP-hydrolysis transition state and ATP-bound forms of Get3, respectively, and adopt the "closed" conformation (Mateja et al., 2009; Bozkurt et al., 2009). These analogs (and presumably ATP itself) associate with both molecules in the Get3 dimer. Each nucleotide makes several contacts with the P loop and Switch II domain of the same Get3, while simultaneously binding to the "deviant" lysine 26 of the opposing Get3 molecule in the dimer. The Switch II region is responsible for several dimer contacts in the "closed" state, where its C-terminal half adopts a helical structure extending α7 and shortening the preceding loop region. In the ADP.AlF_4^- structure, a water molecule, coordinated by aspartate 57 in the Switch I domain, is poised for nucleophilic attack where the γ-phosphate would be if ATP, rather than its analog, were present (Mateja et al., 2009). However, in the AMPPNP-Mg^{2+} form, switches I and II are ill defined and not within close enough proximity for ATP hydrolysis (Bozkurt et al., 2009).

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

ADP-bound Get3 occurs in both “closed” (Bozkurt et al., 2009; Hu et al., 2009) and “open” (Suloway et al., 2009; Yamagata et al., 2010) versions. In the latter, each nucleotide discretely contacts one molecule of the dimer, and the α and β phosphate groups of ADP still form hydrogen bonds with the side chains of threonines 33 and 32, respectively. The “closed” ADP-bound structure described by Bozkurt et al. (2009) contains a Mg^{2+} ion coordinating the two helical domains and occupies the same crystal form as their AMPPNP-Mg^{2+} version.

All of the reported nucleotide-free Get3 structures exhibit the “open” form (Mateja et al., 2009; Hu et al., 2009; Yamagata et al., 2010). Since the Get3 structures exhibit differences and the helical domains always include poorly resolved areas, the question of “open” and “closed” forms remains an area for debate. There could well be intermediate forms and structural variation relating to which point in the mechanism is captured and which accessory proteins are found bound.

Each of the Get3 papers suggests a putative mechanistic model (summarized in Figure 3) for the correlation of TA-protein insertion with Get3 nucleotide binding and hydrolysis. Suloway et al. (2009), Mateja et al. (2009), and Bozkurt et al. (2009) speculate that ATP-binding first closes the Get3 dimer, increasing its affinity for TA proteins. Hu et al. (2009) propose that TA proteins might first bind to an “open” nucleotide-free Get3 with ATP binding then closing Get3 around the TMD in a protective fashion. Yamagata et al. (2010) suggest Get3 exists in equilibrium between “open” and “closed” states and that binding to TA proteins plus membrane recruitment to Get1/2 is what stabilizes the closed form. All agree that the Get3-TA protein docks onto the membrane-bound receptor complex Get1/2 and that ATP hydrolysis dissociates Get3 from the membrane, probably resuming its “open” form and releasing the TA protein for membrane insertion. Bozkurt et al. (2009) observed some TA-protein membrane insertion without a requirement for ATP hydrolysis and therefore speculate that the docking of Get3/TMD and TA-protein insertion could precede ATP hydrolysis, which may be necessary to trigger release of the “open” form of Get3 from the Get1/2 receptor. Further investigations are required to truly delineate this mechanism.

Structures of Get4 and Get5

January 2010 saw the publication (Chang et al., 2010) of the first structural information on other members of the GET pathway with a crystal structure of *S. cerevisiae* Get4 in complex with the N-terminal domain of Get5. Bozkurt et al. (2010) have now also solved the structure of *C. thermophilum* Get4 in isolation. Chartron et al. (2010) most recently revealed a structure of Get4 with the N-terminal of Get5 in a different crystal form with the added information that the complex exists as a dimer (two copies of Get4 and two copies of Get5 connected via the C-terminal domains of Get5). Moreover, via mutagenesis, they identified the Get4/5 binding interface with Get3, which occurs at the positive N-terminal face of Get4, confirming the prediction of Bozkurt et al. (2010). Get5 consists of an ubiquitin-like domain (Ubl), whose human homolog (Ubl4a/GDX) was structurally solved by NMR (PDBID: 2DZI), surrounded by N- and C-terminal domains.

All three papers agree that Get4 forms an α-2-solenoid fold with resemblance to a TPR. The structures overlap with a low rmsd, indicating that only minor structural changes occur in Get4 upon binding to Get5. The concave face of Get4 displays little conservation but the convex surface includes two potential binding pockets and the charged patch near the N terminus, which binds to Get3 (Bozkurt et al., 2010). Get5N wraps snugly around Get4 so it is curious that mammalian forms of Get5 lack this domain. Chartron et al. (2010) suggest that, in higher eukaryotes, Get5N may be replaced with a separate protein that bridges Ubl4a with the pertinent Get4 equivalent. Both clefts on the convex body of Get4 are occupied by

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Get5 helices in the structure of the complex or by C-terminal helices from Get4 in the structure of Get4 alone, indicating their potential flexibility for mechanistic changes in protecting and passing on TMDs of TA proteins. This arrangement also offers dynamic possibilities for the changing composition of the GET subcomplexes, both with currently identified GET proteins, and those members and accessory proteins that may still elude us.

Perspectives and Outlook

In vitro TA-protein insertion assays into rough microsomal membranes consistently showed that a membrane-bound receptor is required for the insertion of Get3/TRC40-dependent substrates (Stefanovic and Hegde, 2007; Favaloro et al., 2008; Schuldiner et al., 2008; Bozkurt et al., 2009), in addition to the roles played by Get3, 4, and 5. This is likely the unidentified mammalian counterpart of the yeast Get1/2 receptor, which still evades structure determination by any method. Structure prediction algorithms (Kelley and Sternberg, 2009) indicate that both Get1 and Get2 consist of multiple transmembrane helices that present extreme challenges to the structural biologist. Both proteins also exhibit coiled-coil forming, cytoplasmic domains on the ER membrane that are credible sites of binding to Get3. These, along with Bat3 and Sgt2, are an obvious next step for structural studies.

Several other proteins display interactions with Get3 that await structural characterization. In 2008, Lee and Dohlman reported binding of Get3 to Gpa1, the alpha subunit of a pheromone-activated heterotrimeric G protein in *S. cerevisiae*. Their further characterization revealed that Get3 acts as a “nonreceptor guanine nucleotide exchange factor” (nrGEF), stabilizing the nucleotide-free form of the subunit. Metz et al. (2006) described the copper-dependent interaction between Get3 and an intracellular CLC (voltage-gated chloride channel) chloride-transport protein, Gef1. Further study of these binding proteins, including structural work, will clarify whether the interactions reflect independent cellular roles for Get3 or if they link in with TA-protein biogenesis.

All of the currently available GET structures were solved by X-ray crystallography. The evident flexibility and dynamics of the GET proteins also make them a potentially interesting target for NMR spectroscopy studies, in particular the exciting new methods that have been developed for the study of larger proteins (Isaacson et al., 2007; Xu et al., 2009). In both “open” and “closed” crystal structures of Get3, selected helices in the helical domain and at the junction between the two domains are missing or ill defined, probably due to conformational diversity. This region is an obvious site for examination by NMR, which could be used to map the binding to TMDs at residue-level detail as well as probing the nucleotide hydrolysis cycle. Moreover, if it is possible to purify intact combinations of GET proteins from yeast, containing either the cytosolic or membrane-associated components, it could be a viable target for electron microscopy, potentially revealing a three-dimensional view of the complex with information on its composition, arrangement, and stoichiometry.

Acknowledgments

R.L.I. is supported by an MRC New Investigator Research Grant. B.S. is a Wellcome Trust Senior Research Fellow in Basic Biomedical Science. H.G.D. is supported by NIH grant GM080739. The authors thank Stephen High at the University of Manchester, Shoshanna Isaacson at the University of Cambridge, and Beth Sawyer at the University of Melbourne for critical reading of the manuscript and suggestions. In addition, the authors are grateful to Nica Borgese at CNR Institute for Neuroscience, Milan, William Clemons at CalTech, and Ramanujan Hegde at the NIH for sharing work prior to publication.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

REFERENCES

Auld KL, Hitchcock AL, Doherty HK, Frietze S, Huang LS, Silver PA. The conserved ATPase Get3/Arr4 modulates the activity of membrane-associated proteins in *Saccharomyces cerevisiae*. Genetics. 2006; 174:215–227. [PubMed: 16816426]

Battle A, Jonikas MC, Walter P, Weissman JS, Koller D. Automated identification of pathways from quantitative genetic interaction data. Mol. Syst. Biol. 2010; 6:379. [PubMed: 20531408]

Borgese N, Righi M. Remote origins of tail-anchored proteins. Traffic. 2010; 11:877–885. [PubMed: 20406421]

Bozkurt G, Stjepanovic G, Vilardi F, Amlacher S, Wild K, Bange G, Favaloro V, Rippe K, Hurt E, Dobberstein B, Sinning I. Structural insights into tail-anchored protein binding and membrane insertion by Get3. Proc. Natl. Acad. Sci. USA. 2009; 106:21131–21136. [PubMed: 19948960]

Bozkurt G, Wild K, Amlacher S, Hurt E, Dobberstein B, Sinning I. The structure of Get4 reveals an alpha-solenoid fold adapted for multiple interactions in tail-anchored protein biogenesis. FEBS Lett. 2010; 584:1509–1514. [PubMed: 20206626]

Buchanan G, Ricciardelli C, Harris JM, Prescott J, Yu ZC, Jia L, Butler LM, Marshall VR, Scher HI, Gerald WL, et al. Control of androgen receptor signaling in prostate cancer by the cochaperone small glutamine rich tetratricopeptide repeat containing protein alpha. Cancer Res. 2007; 67:10087–10096. [PubMed: 17942943]

Chang YW, Chuang YC, Ho YC, Cheng MY, Sun YJ, Hsiao CD, Wang C. Crystal structure of Get4-Get5 complex and its interactions with Sgt2, Get3, and Ydj1. J. Biol. Chem. 2010; 285:9962–9970. [PubMed: 20106980]

Chartron JW, Suloway CJM, Zaslaver M, Clemons WM Jr. Structural characterization of the Get4/Get5 complex and its interaction with Get3. Proc. Natl. Acad. Sci. USA. 2010; 107:12127–12132. [PubMed: 20554915]

Costanzo M, Baryshnikova A, Bellay J, Kim Y, Spear ED, Sevier CS, Ding H, Koh JL, Toufighi K, Mostafavi S, et al. The genetic landscape of a cell. Science. 2010; 327:425–431. [PubMed: 20093466]

Favaloro V, Spasic M, Schwappach B, Dobberstein B. Distinct targeting pathways for the membrane insertion of tail-anchored (TA) proteins. J. Cell Sci. 2008; 121:1832–1840. [PubMed: 18477612]

Favaloro V, Vilardi F, Schlecht R, Mayer MP, Dobberstein B. Asna1/TRC40-mediated membrane insertion of tail-anchored proteins. J. Cell Sci. 2010; 123:1522–1530. [PubMed: 20375064]

Gasper R, Meyer S, Gotthardt K, Sirajuddin M, Wittinghofer A. It takes two to tango: regulation of G proteins by dimerization. Nat. Rev. Mol. Cell Biol. 2009; 10:423–429. [PubMed: 19424291]

Hemmingsson O, Nöjd M, Kao G, Naredi P. Increased sensitivity to platinating agents and arsenite in human ovarian cancer by down-regulation of ASNA1. Oncol. Rep. 2009a; 22:869–875. [PubMed: 19724867]

Hemmingsson O, Zhang Y, Still M, Naredi P. ASNA1, an ATPase targeting tail-anchored proteins, regulates melanoma cell growth and sensitivity to cisplatin and arsenite. Cancer Chemother. Pharmacol. 2009b; 63:491–499. [PubMed: 18478230]

Hu Z, Potthoff B, Hollenberg CP, Ramezani-Rad M. Mdy2, a ubiquitin-like (UBL)-domain protein, is required for efficient mating in *Saccharomyces cerevisiae*. J. Cell Sci. 2006; 119:326–338. [PubMed: 16390866]

Hu J, Li J, Qian X, Denic V, Sha B. The crystal structures of yeast Get3 suggest a mechanism for tail-anchored protein membrane insertion. PLoS ONE. 2009; 4:e8061. [PubMed: 19956640]

Isaacson RL, Simpson PJ, Liu M, Cota E, Zhang X, Freemont PS, Matthews SJ. A new labeling method for methyl TROSY spectra of alanine residues. J. Am. Chem. Soc. 2007; 129:15428–15429. [PubMed: 18041839]

Jonikas MC, Collins SR, Denic V, Oh E, Quan EM, Schmid V, Weibezahn J, Schwappach B, Walter P, Weissman JS, Schuldiner M. Comprehensive characterization of genes required for protein folding in the endoplasmic reticulum. Science. 2009; 323:1693–1697. [PubMed: 19325107]

Kao G, Nordenson C, Still M, Ronnlund A, Tuck S, Naredi P. ASNA-1 positively regulates insulin secretion in *C. elegans* and mammalian cells. Cell. 2007; 128:577–587. [PubMed: 17289575]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Kelley LA, Sternberg MJE. Protein structure prediction on the Web: a case study using the Phyre server. Nat. Protoc. 2009; 4:363–371. [PubMed: 19247286]

Koonin EV. A superfamily of ATPases with diverse functions containing either classical or deviant ATP-binding motif. J. Mol. Biol. 1993; 229:1165–1174. [PubMed: 8445645]

Kurdi-Haidar B, Heath D, Lennon G, Howell SB. Immunohistochemical analysis of the distribution of the human ATPase (hASNA-I) in normal tissues and its overexpression in breast adenomas and carcinomas. J. Histochem. Cytochem. 1998; 46:1243–1248. [PubMed: 9774623]

Lee MJ, Dohlman HG. Coactivation of G protein signalling by cell-surface receptors and an intracellular exchange factor. Curr. Biol. 2008; 18:211–215. [PubMed: 18261907]

Leipe DD, Wolf YI, Koonin EV, Aravind L. Classification and evolution of P-loop GTPases and related ATPases. J. Mol. Biol. 2002; 317:41–72. [PubMed: 11916378]

Leznicki P, Clancy A, Schwappach B, High S. Bat3 promotes the membrane integration of tail-anchored proteins. J. Cell Sci. 2010; 123:2170–2178. [PubMed: 20516149]

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]

Metz J, Wächter A, Schmidt B, Bujnicki JM, Schwappach B. The yeast Arr4p ATPase binds the chloride transporter Gef1p when copper is available in the cytosol. J. Biol. Chem. 2006; 281:410–417. [PubMed: 16260785]

Mistry R, Cliff JM, Clayton CL, Beyers N, Mohamed YS, Wilson PA, Dockrell HM, Wallace DM, van Helden PD, Duncan K, Lukey PT. Gene-expression patterns in whole blood identify subjects at risk for recurrent tuberculosis. J. Infect. Dis. 2007; 195:357–365. [PubMed: 17205474]

Rabu C, Schmid V, Schwappach B, High S. Biogenesis of tail-anchored proteins: the beginning for the end? J. Cell Sci. 2009; 122:3605–3612. [PubMed: 19812306]

Schuldiner M, Collins SR, Thompson NJ, Denic V, Bhamidipati A, Punna T, Ihmels J, Andrews B, Boone C, Greenblatt JF, et al. Exploration of the function and organization of the yeast early secretory pathway through an epistatic miniarray profile. Cell. 2005; 123:507–519. [PubMed: 16269340]

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]

Shen J, Hsu CM, Kang BK, Rosen BP, Bhattacharjee H. The *Saccharomyces cerevisiae* Arr4p is involved in metal and heat tolerance. Biometals. 2003; 16:369–378. [PubMed: 12680698]

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]

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

Winnefeld M, Grewenig A, Schnölzer M, Spring H, Knoch TA, Gan EC, Rommelaere J, Cziepluch C. Human SGT interacts with Bag-6/Bat-3/Scythe and cells with reduced levels of either protein display persistence of few misaligned chromosomes and mitotic arrest. Exp. Cell Res. 2006; 312:2500–2514. [PubMed: 16777091]

Xu Y, Liu M, Simpson PJ, Isaacson RL, Cota E, Yang D, Zhang X, Freemont PS, Matthews SJ. Automated assignment in selectively methyl-labelled proteins. J. Am. Chem. Soc. 2009; 131:9480–9481. [PubMed: 19534551]

Yamagata A, Mimura H, Sato Y, Yamashita M, Yoshikawa A, Fukai S. Structural insight into the membrane insertion of tail-anchored proteins by Get3. Genes Cells. 2010; 15:29–41. [PubMed: 20015340]

Zewail A, Xie MW, Xing Y, Lin L, Zhang PF, Zou W, Saxe JP, Huang J. Novel functions of the phosphatidylinositol metabolic pathway discovered by a chemical genomics screen with wortmannin. Proc. Natl. Acad. Sci. USA. 2003; 100:3345–3350. [PubMed: 12615994]

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

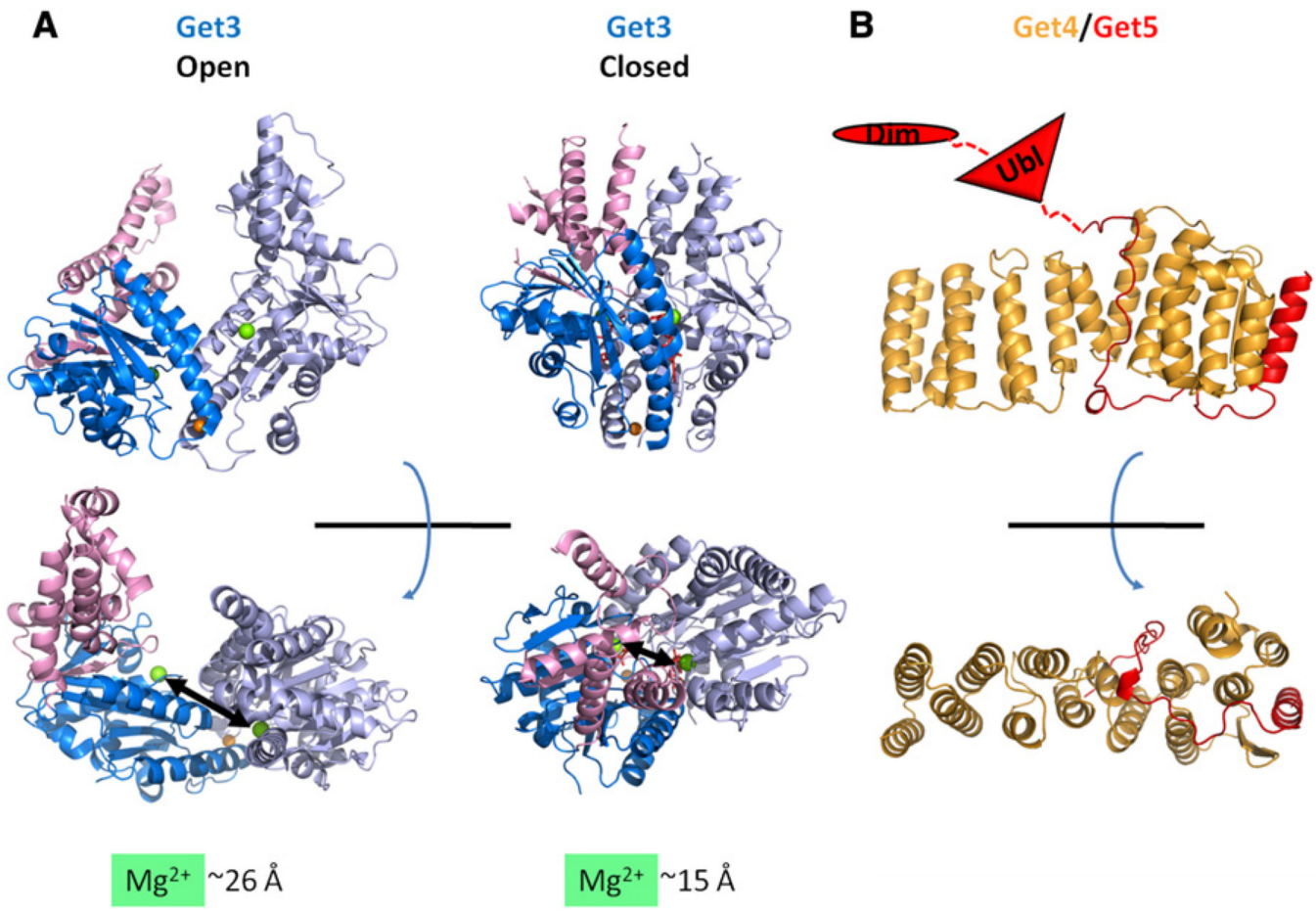

Figure 1. Example GET Structures from *S. cerevisiae*

(A) The highest resolution (1.8 Å) Get3 "open" structure (nucleotide free) (Hu et al., 2009) is compared with the highest resolution (2 Å) "closed" Get3 structure (ADP.AlF_4^- bound) (Mateja et al., 2009). The nucleotide is shown in red, the zinc ion in orange, and the magnesium ions in green. One of the monomers in each dimer is colored lilac and the other is colored blue (NBD) and pink (helical domain).

(B) The structure of Get4 (gold) bound to the N-terminal domain of Get5 (red) from Chang et al. (2010). The Get5 Ubl and C-terminal domain (for dimerization as indicated in Chartron et al. [2010]) are shown as shapes.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

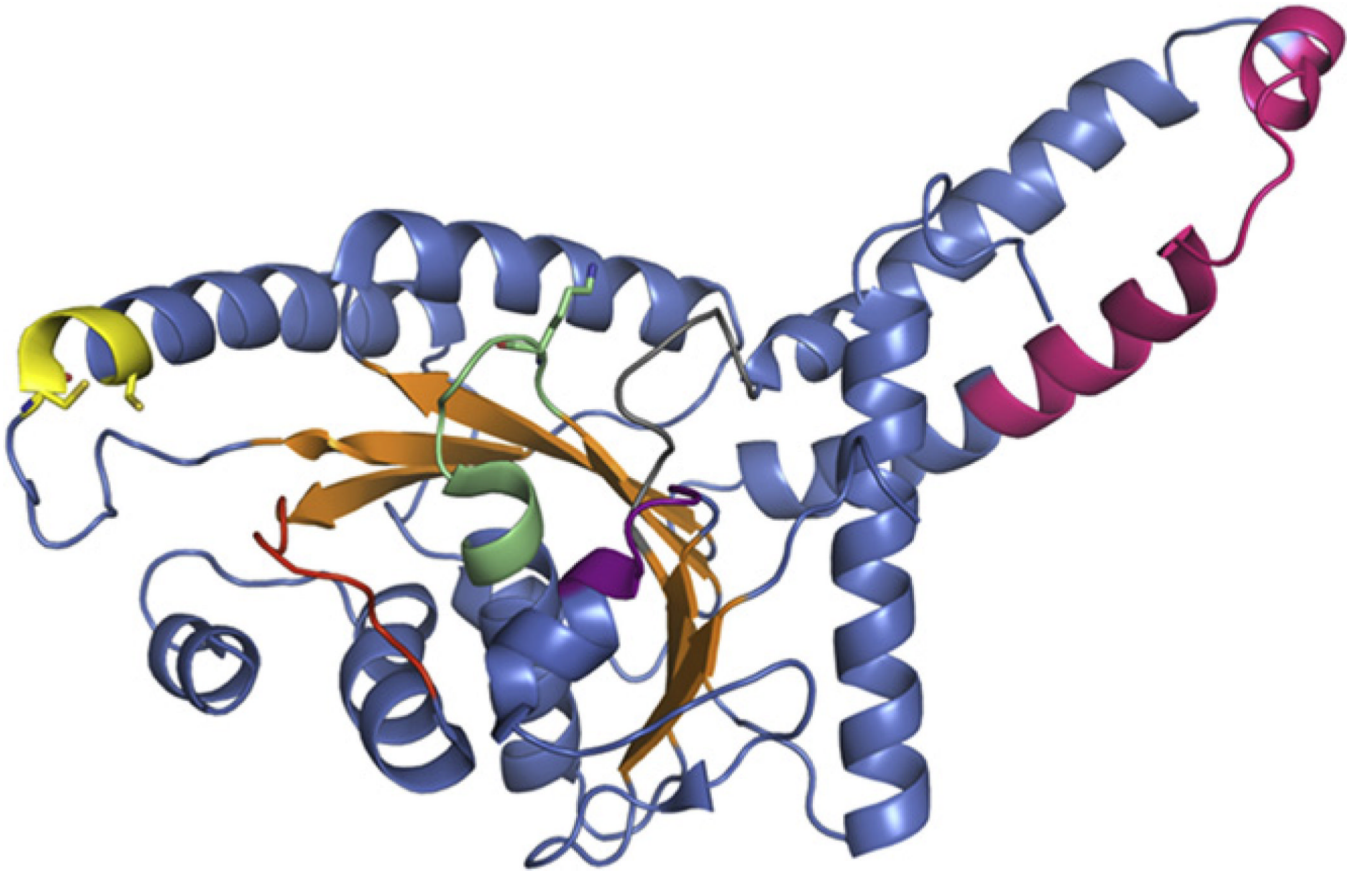

Figure 2. NBD Features

Ribbon diagram of a monomer from an *S. cerevisiae* Get3 dimer (built out of coordinates from Yamagata et al. [2010]), highlighting the nucleotide binding characteristics: the all-parallel 7 strand beta sheet (orange), the “deviant” P loop (green with the rare lysine indicated in stick form), the uniquely orientated Switch I (purple) and Switch II (gray), the A-loop (red), and the CXXC motif (yellow with the cysteines shown in stick form). The TRC40 insert, unique to archeal and eukaryotic versions of this protein, is colored pink.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

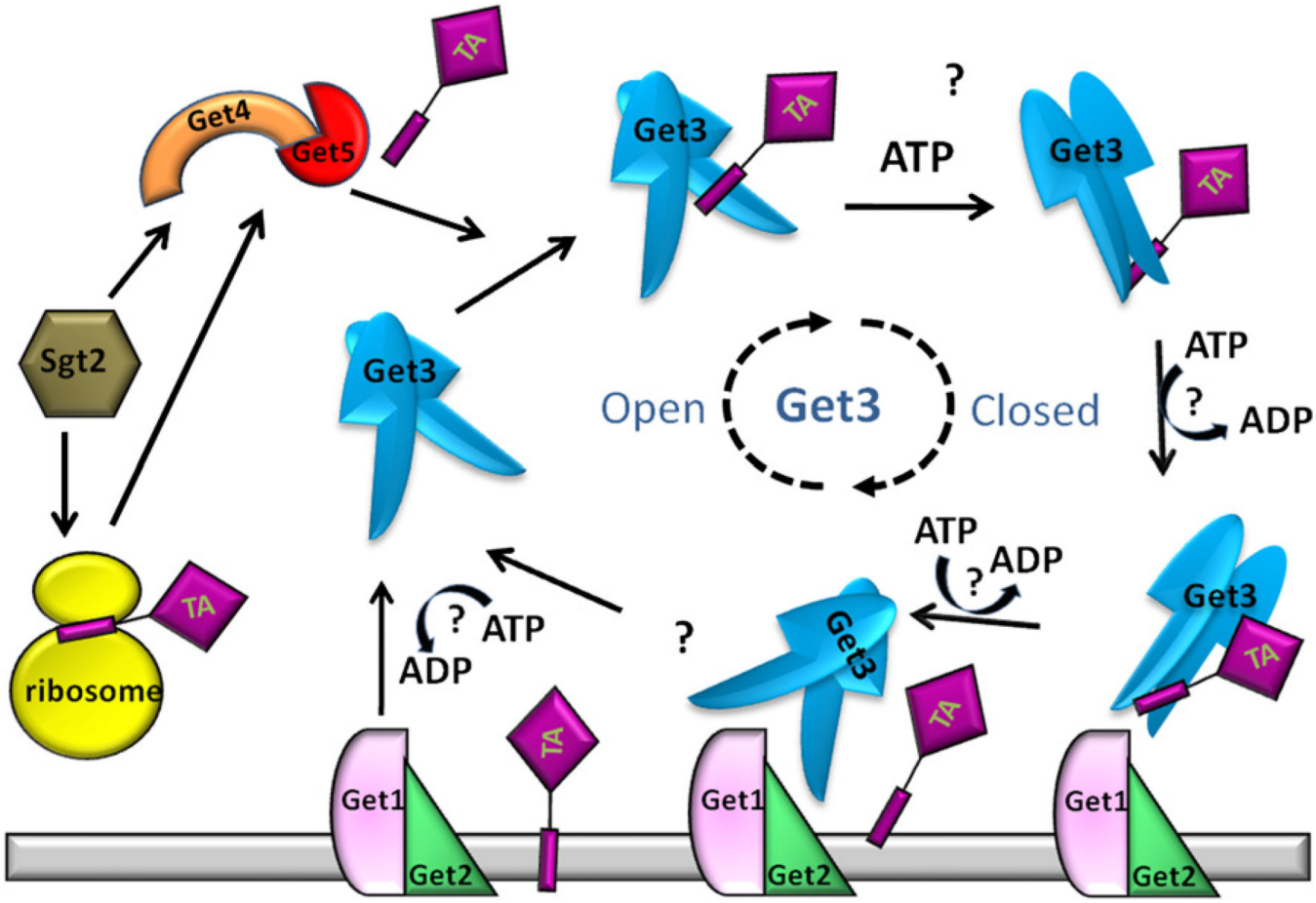

Figure 3. Schematic Representation of Our Current Understanding of the GET Pathway

Aside from the interaction of TMDs with Get3, we have no molecular information on modes of binding between proteins in this pathway. Schematic representations should therefore be interpreted as binding events between the pictured proteins rather than specific regions thereof. Potential nucleotide binding/hydrolysis steps are indicated; a detailed description of the possible ways in which the nucleotide cycle couples to TA-protein insertion into membranes can be found in the body of the text.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Table 1

GET Pathway Components

Protein (*S. cerevisiae*)	Size/aa	Mammalian Homolog (*H. sapiens*)	% Identity (aa overlap)	Properties
Get1	285	Unknown Suggested: WRB protein	N/A 25 (60)	Membrane CHD5 (coiled-coil)
Get2	235	Unknown	N/A	Membrane
Get3	354	TRC40/Asna-1	46 (342)	SIMIBI ATPase
Get4	312	Conserved edge-expressed protein (CEE)	33 (135)	TPR-like
Get5	212	Ubl4a/GDX	33 (77)	Ubiquitin-like domain
Sgt2	346	SGTA	36 (202)	TPR
Unknown	1126	Bat3	N/A	Ubiquitin-like domain BAG domain Nuclear localization signal

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Table 2

A Summary of Get3 Structures Solved by X-ray Crystallography

Species	Nucleotide	Res/Å	R, R_{free}	Form	Authors	PDB
S. cerevisiae	ADP.AlF_4^-	2.0	0.178,0.213	closed	Mateja et al. (2009)	2WOJ
S. pombe	none	3.0	0.240, 0.288	open	Mateja et al. (2009)	2WOO
A. fumigatus	ADP	3.2	0.214,0.251	open	Suloway et al. (2009)	3IBG
C. thermophilum	AMPPNP-Mg^{2+}	3.0	0.230, 0.271	closed	Bozkurt et al. (2009)	3IQW
C. thermophilum	ADP-Mg^{2+}	3.5	0.232, 0.295	closed	Bozkurt et al. (2009)	3IQX
S. cerevisiae	none	2.3	0.223, 0.247	open	Hu et al. (2006)	3H84
S. cerevisiae	none	2.8	0.237,0.281	open	Yamagata et al. (2010)	3A36
S. cerevisiae	ADP	3.0	0.253, 0.282	open	Yamagata et al. (2010)	3A37