The HUS-box is required for allosteric regulation of the Sec7 Arf-GEF

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ABSTRACT

The Golgi complex is the central membrane and protein sorting station in eukaryotic cells. Activation of ADP ribosylation factor (Arf) GTPases is essential for vesicle formation via recruitment of cargo adaptors and coat proteins necessary for Golgi trafficking. Arf activation is spatially and temporally regulated by distinct guanine nucleotide exchange factors (GEFs) at different Golgi compartments. The yeast Arf-GEF Sec7 is a conserved and essential activator of Arf1 at the trans-Golgi network. Sec7 contains a highly conserved regulatory region, the HUS-box, with an unknown mechanistic role. In this study we explore how the HUS-box, which is N-terminal to the catalytic domain, acts together with C-terminal regulatory domains in the allosteric activation of Sec7. We report that mutation of the HUS-box disrupts positive feedback and allosteric activation of Sec7 by the GTPase Ypt31, a yeast Rab11 homolog. Taken together, our results support a model in which the inter- and intramolecular interactions of the HUS-box and C-terminus are necessary for the allosteric activation of Sec7.

INTRODUCTION

Transport of proteins and membranes within eukaryotic cells requires the biogenesis of membrane transport carriers such as vesicles and tubules. The formation of many of these transport carriers requires and is regulated by a GTPase. The GTPases responsible for vesicle formation at the Golgi complex are primarily Arf1-5 in mammals and Arf1/2 in yeast (1-4). Arf activation via GTP-binding triggers the recruitment of effectors such as cargo adapters and coat proteins to the Golgi membrane, resulting in cargo sorting into nascent vesicles. Arf activation is performed by Arf-GEFs. The Arf-GEF responsible for Arf activation at the cis- and medial-Golgi is GBF1 in mammals and Gea1/2 in yeast, while BIG1/2 and Sec7 fulfill this role at the trans-Golgi, trans-Golgi network (TGN), and recycling endosomes in mammals and yeast, respectively (5-10). Arf-GEFs are master regulators of trafficking at the Golgi; therefore, understanding their regulatory mechanisms will illuminate the molecular logic underpinning the Golgi complex, the central sorting station of the secretory pathway.
The Sec7/BIG family Arf-GEFs are composed of seven conserved domains, while the Gea/GBF family Arf-GEFs contain six (Fig. 1A)(11). Several studies have shed light on the roles of the Golgi Arf-GEF regulatory domains upstream (DCB/HUS) and downstream (HDS1-HDS4) of the central catalytic GEF domain (12-23), and there are structures available of the GEF domain (24-26) and the N-terminal domains (15,27,28). The N-terminal regulatory domains of Sec7 fold into a single structural unit that is recruited to membranes via an interaction with the activated form of the related Arl1 GTPase, and also appears to interact with the activated form of Arf1(15,17,28). The C-terminal domains have many roles, including membrane-binding, homo-dimerization, positive-feedback, autoinhibition, and allosteric regulation by interaction with the Ypt1(Rab1) and Ypt31/32(Rab11) GTPases (14-16,23).

Outside of the GEF domain, the most highly conserved motif in the Golgi Arf-GEFs is a region in the HUS domain referred to as the HUS-box (Fig. 1B). This motif is found in both the Sec7/BIG and Gea/GBF families but its physiological role is poorly understood. In an initial study, it was speculated to be necessary for folding and/or thermal stability in Sec7 (29). A subsequent study determined that a single point mutation in the HUS-box of Gea2 was sufficient to disturb anterograde traffic (30). In GNOM, the plant homolog of Gea2, the same HUS-box mutation disrupted a heterotypic interaction with the DCB domain (31).

Here we present evidence in support of a model in which the HUS-box, which is N-terminal to the GEF domain, is important for the allosteric activation of Sec7 that results from a protein-protein interaction between the C-terminus of Sec7 and its regulatory Rab GTPase Ypt31. We find that the deleterious effects of a HUS-box mutation cannot be compensated for by increasing Sec7 expression, indicating the importance of proper regulation. We show that a HUS-box mutation perturbs a Sec7 positive-feedback interaction. Finally, we demonstrate that although the HUS-box is not required for interaction between Sec7 and Ypt31, it is required for the ability of Ypt31 to stimulate Sec7 GEF activity.

RESULTS

The HUS-box is important for Sec7 function in vivo

We previously reported that the HUS-box is important for Sec7 function, as the N653A mutation was lethal in a sensitized arf1Δ strain in which the levels of Arf1/2 at the Golgi have been reduced to 10% of the wild-type levels (15). To perform a more detailed assessment of the role of the HUS-box in Sec7 function, we generated additional HUS-box mutant versions of Sec7 and introduced these into yeast cells as the sole copy of the SEC7 gene, using strains with either normal (ARF1 ARF2) or reduced (arf1Δ ARF2) levels of Arf1/2 (Fig. 2). Both the N653A and D655A mutations resulted in normal growth in otherwise wild-type cells but resulted in lethality in arf1Δ cells. The D655A/C656A double mutation was lethal in both wild-type ARF1 and arf1Δ backgrounds. These results indicate that the HUS-box is important for Sec7 function and that the N653A and D655A single mutations represent alleles that are likely partially compromised for HUS-box function.

To address the possibility that mutation of the HUS-box simply reduced overall Sec7 activity, we tested whether overexpression of a HUS-box mutant could rescue the growth phenotype. We found that overexpression of the N653A Sec7 mutant using a multi-copy plasmid did not rescue the growth phenotype observed in arf1Δ cells (Fig. 2A,B). This result suggests that the HUS-box serves an important regulatory role. We therefore focused our subsequent efforts on the N653A mutation in
order to gain mechanistic insights into the function of the HUS-box in regulating Sec7 activity.

The HUS-box is not required for dimerization of the Sec7 N-terminus

Homo-dimerization of the Golgi Arf-GEFs is regulated by the N-terminal DCB/HUS domain and the C-terminal HDS4 domain (18,31-33). In GBF1, N-terminal dimerization was shown to be necessary for protein stability as two point mutations that disrupted dimerization also resulted in faster degradation, yet did not affect activity or localization (34). We therefore considered the possibility that the HUS-box could be contributing to dimerization of Sec7 via the DCB/HUS domains. To test this possibility, we purified wild-type and HUS-box mutant (N653A) versions of the Sec7ΔC (residues 203-1017, lacking the C-terminal HDS1-4 regulatory domains) construct for in vitro studies (Fig. 3A,B). The N653A mutant construct appeared to be stably folded, as evidenced by the similar purification yields and behavior compared to its wild-type counterpart. We observed that introduction of the N653A mutation did not disrupt dimerization of the Sec7ΔC construct, as measured by multi-angle light scattering (MALS) (Fig. 3C). Therefore, the HUS-box does not appear to be required for dimerization of the Sec7 N-terminal domains.

The HUS-box is required for HDS1-mediated membrane association

To test whether the HUS-box plays any role in regulation of Sec7 Arf-GEF activity, we utilized an established assay (35-37) which takes advantage of the increase in innate tryptophan fluorescence of Arf1 that accompanies its activation upon GTP-binding. This GEF assay was performed using the physiological myristoylated Arf1 substrate in the presence of synthetic liposome membranes that mimic the composition of the yeast TGN (38). We introduced the N653A mutation into different Sec7 truncation constructs (Fig. 3A,B): Sec7Δ1 (residues 203-2009) provides the full essential function of Sec7 in vivo; Sec7ΔC+HDS1 (residues 203-1220) is relieved of most autoinhibition and is subject to positive feedback, but cannot be allosterically activated by Rab binding; and Sec7ΔC (described above) is the most active in solution but has low activity on membranes and is completely cytoplasmic in vivo (14-16).

We previously reported that the N653A mutation did not diminish the in vitro GEF activity of the Sec7ΔC construct on membranes at room temperature, but there appeared to be a slight, though statistically insignificant, increase in the activity of the mutant compared to the wild-type construct (15). We repeated these experiments at 30°C and observed that the N653A mutation resulted in approximately 2-fold higher activity of the Sec7ΔC construct (Fig. 4A,B). Consistent with our previously published results (14), we observed the Sec7ΔC+HDS1 construct to be significantly more active than the Sec7ΔC construct (Fig. 4A,B), owing to the role of the HDS1 domain in recruitment of this construct to the membrane surface. Interestingly, the N653A mutation reduced the activity of the Sec7ΔC+HDS1 construct to a level similar to that of the Sec7ΔC construct (Fig. 4A,B). This suggested to us that the HUS-box may be required for the HDS1 domain to exert its positive-feedback regulatory role in membrane association of Sec7.

To test this hypothesis, we performed a membrane binding assay to determine if the HUS-box plays a role in recruitment of Sec7 to membranes by Arf1-GTP. As seen previously (14), Sec7ΔC+HDS1 was recruited to membranes by activated Arf1-GTP (Fig. 4C). Importantly, we observed that this recruitment was completely abolished when the same assay was performed with the N653A mutant (Fig.
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4C). This indicates that the HUS-box is required for the HDS1-domain dependent recruitment of Sec7 to membranes by Arf1.

In light of this result, we were surprised to find that the N653A mutation did not prevent membrane recruitment of the longer Sec7Δ construct by Arf1-GTP (Fig. 4D), indicating that the HDS2-4 domains enable recruitment of Sec7 to the membrane surface even when the HUS-box is compromised by mutation. Consistent with this finding, we previously found that the Arf1-GTP recruitment of the Sec7Δ construct to membranes was more robust than that of the Sec7ΔC+HDS1 construct (14).

To determine the role of the HUS-box in Sec7 recruitment to the Golgi membrane surface in vivo, we imaged GFP-tagged versions of several wild-type and mutant Sec7 constructs. As reported previously, the GFP-Sec7ΔC construct was completely cytoplasmic while GFP-Sec7ΔC+HDS1 was localized to the TGN (punctate structures) and the cytoplasm (Fig. 5A). Strikingly, the N653A mutation rendered the GFP-Sec7ΔC+HDS1 construct completely cytoplasmic (Figs. 5A, S1). This result is consistent with the in vitro membrane binding assay results and indicates that mutation of the HUS-box phenocopies loss of the HDS1 domain.

When we imaged full-length GFP-Sec7 constructs present as the sole copy of Sec7 in cells, we found that mutation of the HUS-box had no effect on localization to punctate structures (Fig. 5B). This result is also consistent with the in vitro membrane binding assay results and indicates that the HDS2-4 domains enable recruitment of Sec7 to the Golgi even when the HUS-box is compromised by mutation. The proper localization of the N653A and D655A GFP-Sec7 constructs likely explains why these mutants are viable in wild-type ARF1 ARF2 cells, yet suggests these mutants are mis-regulated in a manner that becomes lethal in arf1A ARF2 cells.

The HUS-box is required for allosteric activation of Sec7 by Ypt31

The consequences of HUS-box mutations described above were revealed when the levels of Arf1/2 were reduced or when the C-terminal HDS2-4 domains were removed. Sec7 activity is stimulated by binding to the active form of the Rab11 paralogs Ypt31/32 (16). This interaction requires the HDS2-3 domains and likely enables Sec7 to adopt a fully active conformation on the membrane surface (Fig. 6A). We therefore considered the possibility that the HUS-box is important for the allosteric regulation of Sec7.

To test whether the HUS-box was important for the allosteric activation of Sec7 by Ypt31/32, we performed GEF assays in the presence of the membrane-anchored Rab protein Ypt31 activated by binding to GTP. Whereas our previous versions of this assay utilized a His-tag membrane anchor for Ypt31 (16), here we utilized a more physiological prenyl group anchor. This method involves the enzymatic synthesis of a prenyl-Ypt31/GDI (guanine dissociation inhibitor) complex (39). The prenyl-Ypt31 was simultaneously GTP-loaded and membrane-anchored by first using EDTA to chelate magnesium in the presence of GTP and then adding additional magnesium to stabilize the GTP-bound form. This form of activated Ypt31, though not as potent as His-tagged Ypt31 (perhaps due to a lower concentration of activated Rab on the membrane), stimulated the activity of Sec7Δ by approximately threefold (Fig. 6B,C). In contrast, the activity of the N653A-Sec7Δ construct was not stimulated by prenyl-Ypt31-GTP (Figs. 6B,C). The N653A mutation had only modest effects on the basal activity of Sec7Δ both on membranes (Fig. 6B) and in solution (Fig. 6D). Furthermore, the N653A mutation did not interfere with the physical interaction between Ypt31 and Sec7Δ, as determined by a membrane-recruitment assay (Fig. 6E). Therefore, mutation of the HUS-box
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prevents the stimulation of Sec7 GEF activity by Ypt31 without preventing the stable interaction of Sec7 with Ypt31. Taken together, these results indicate that the HUS-box is required for the allosteric stimulation of Sec7 GEF activity by Ypt31.

DISCUSSION

The Sec7/BIG and Gea/GBF families of large ARF-GEFs are essential master regulators of Golgi trafficking. These proteins are found in all eukaryotes and possess conserved regulatory domains that are not found outside of this family. Regulatory domains surrounding the catalytic GEF domain are involved in recruitment of Sec7 to the membrane, and the C-terminus is required for autoinhibition and allosteric activation (12,14,16). The role of the highly conserved HUS-box has been undefined. A previous study reported that mutating the first aspartate in the HUS-box produced a temperature sensitive mutant of GEA2 (D485G) that impaired anterograde traffic (30). Another study reported that mutating the equivalent residue in GNOM (D468G), the Gea2 paralog in Arabidopsis thaliana, disrupted a heterotypic interaction between the HUS and DCB domains (31). Given the evidence that the DCB and HUS domains fold into a single structural unit (15), we reasoned that these mutants may perturb protein folding and therefore we sought additional HUS-box mutants in order to dissect the mechanistic role of the HUS-box.

We identified the N653A HUS-box mutation as a good tool for mechanistic studies. The N653A mutant was viable in otherwise wild-type cells, but became inviable when the levels of Arf were reduced at the Golgi. The N653A mutation did not appear to affect protein folding, as all constructs containing this mutation expressed well and did not aggregate. In addition, our MALS results indicated that the N653A mutation did not disrupt dimerization of the N-terminal domains.

Using a combination of in vitro assays and in vivo imaging, we found that the N653A mutant interfered with functions of the C-terminal HDS domains. The N653A Sec7ΔC+HDS1 construct was unable to be recruited to membranes by Arf1 in vitro or in vivo, indicating a loss of positive-feedback regulation. The GEF activity of the N653A Sec7t construct was not stimulated by Ypt31 binding. Taken together, we interpret these findings to mean that the N653A mutation appears to have decoupled membrane recruitment from allosteric activation by and Ypt31. These results indicate that the HUS-box, which lies N-terminal to the GEF domain, is important for the allosteric regulation of Sec7 by the C-terminal HDS domains.

Sec7 interacts with and is regulated by the activated forms of multiple GTPases at the Golgi, and appears to adopt different conformations with distinct levels of activity (14-16). Sec7 may therefore integrate these different GTPase signals, and likely relies upon coincidence detection in order to function at the right place and right time. The transition of Sec7 from a less active to more active state relies upon its interaction with its GTPase regulators, with Ypt31/32 (Rab11) playing a particularly important role. How do the regulatory domains of Sec7 control such a conformational change? An implication of the results presented in this study is that Sec7 may fold into a structure in which the N- and C-terminal regulatory domains interact, potentially influencing the conformation of the catalytic GEF domain or its positioning relative to the membrane surface. The homodimerization of Sec7 may enable these interactions to occur between domains on separate polypeptides. Alternatively, the HUS-box may interact directly with the GEF domain or with the substrate Arf1 to modulate the kinetics of the GEF reaction. In either case, our
findings indicate that the HUS box is required for Sec7 to adopt its most active state induced by binding of the HDS2/3 domains to Ypt31/32.

In this study have established an important role of the HUS-box in the regulation of Sec7 by inter- and intramolecular interactions. Ultimately, structural studies will be needed to reveal the active and inactive conformations of Sec7 in order to fully understand its regulatory mechanisms.

EXPERIMENTAL PROCEDURES

Strains and plasmids

All new plasmids used in this study were verified by sequencing. Plasmids and yeast strains used in this study are reported in Tables 1 and 2.

Purification of constructs

Sec7ΔC+HDS1 and Sec7ΔC constructs were purified as previously described (14,15,40). These constructs were expressed with an N-terminal His6 tag in 8L cultures of E. coli in Terrific Broth (TB), and expression of proteins was induced by the addition of 250µM IPTG at 18°C. After overnight induction, cells were harvested and lysed by sonication. The soluble fraction was collected after centrifugation and proteins were purified via Ni.NTA resin (Qiagen) in batch, followed by MonoQ ion exchange and Superdex 200 gel filtration (GE Healthcare), with a final buffer composition of 20 mM HEPES pH 7.5, 500 mM NaCl, and 2 mM DTT. ΔN17Arf1 was purified similarly to Sec7 constructs in 2 L TB, protein expression was induced by the addition of 100µM IPTG, and with additional 2 mM MgCl2 added to all buffers.

Sec7i constructs were expressed and purified as described previously (40). Full-length Arf1 was co-expressed with S. cerevisiae NMT1 to produce myristoylated Arf1, which following lysis was purified via batch incubation with DEAE-sephacel, batch incubation with ToyoPearl phenyl resin, and a final clean up step by gel filtration using a Superdex 200 (41). Purification of the prenylated-Ypt31/GDI complex was performed as described previously (39).

Gel filtration coupled to Multi-angle light scattering (MALS)

Gel filtration-MALS was performed as described previously (15). Purified proteins were exchanged into 20 mM HEPES pH 7.5, 500 mM NaCl, and 2 mM DTT buffer to a final concentration of 5 mg/ml, and run through a Wyatt WTC-050S5 or WTC-050N5 gel filtration column coupled to DAWN HELEOS-II light scattering and Optilab T-rEX refractive index detectors (Wyatt Technology) at room temperature. Data were analyzed via ASTRA 6 software to obtain the molecular weight of the sample, and compared to that predicted from the sequence to determine oligomeric state.

Arf1 nucleotide exchange kinetics

All GEF activity assays were performed at 30°C in HKM buffer (20 mM HEPES pH 7.5, 150 mM KOAc, 2 mM MgCl2), 200 µM liposomes, 100 nM Sec7 construct, 1 µM Arf1 construct, and 200 µM GTP were added in sequence to a final volume of 150 µl with 1-5 minutes in between components. Data collection and normalization was performed as described earlier (14,16).

GEF assays measuring the allosteric activation of Sec7 by Ypt31 were performed as described in (13) with the exception that
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The Ypt31 was prenylated instead of His-tagged.

Triplicates of each condition were collected for statistics; error bars represent 95% confidence as determined by ANOVA with Tukey’s test in postprocessing, as appropriate.

Yeast plasmid shuffle

The mutant sec7 alleles were cloned with an N-terminal GFP tag into a centromeric LEU2 vector (pRS415). These plasmids were then transformed into sec7Δ yeast strains (CFY409 and CFY863) maintained viable by a URA3 plasmid harboring wild-type SEC7. Cells were grown overnight in –Leu synthetic media, spotted on growth plates, and grown for three days at 30°C or 37°C as indicated.

Microscopy

Cells were grown in –Leu synthetic media and imaged in log phase (OD_{600} ~ 0.5). For Fig. 5A, GFP-constructs were transformed into SEY6210.1 cells (42) and live cells were imaged on a DeltaVision RT wide-field deconvolution microscope (Applied Precision). Images were deconvolved using SoftWoRx 3.5.0 software (Applied Precision).

All images were further processed in ImageJ, adjusting only min/max light levels for clarity, and using equivalent processing for all images within an experiment.

Liposome preparation and flotation experiments

Liposomes mimicking TGN composition were prepared as described previously (14,38,40). Membrane binding was assayed by liposome flotation experiments as described (14,40,43).
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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest with the contents of this article.

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FIGURE LEGENDS

Figure 1. The HUS-box is a highly conserved region of the Golgi Arf-GEFs

(A) Relative domain organization of the Golgi Arf-GEFs with HUS-box location indicated by red line. DCB: dimerization and cyclophilin binding. HUS: homology upstream of Sec7. HDS: homology downstream of Sec7. GEF, guanine exchange factor (catalytic domain). HUS, homology downstream of Sec7. The DCB/HUS domain is a single structural unit (15). Sec7 is dimeric but shown in this and subsequent figures as a monomer for simplicity.

(B) Sequence alignment of the region of polypeptides containing the HUS-box motif (red box). Φ indicates a bulky hydrophobic residue.

Figure 2. The HUS-box is important for Sec7 function

(A) GFP-Sec7 constructs driven by the endogenous promoter on a centromeric plasmid: WT, D655A, DC655AA(D655A/C656A), and N653A; or overexpressed on a high-copy 2-micron plasmid: 2µ WT or 2µ N653A, were introduced into a sec7Δ strain (CFY409) or a sec7Δ/arf1Δ strain (CFY863) and tested for their ability to complement the sec7 deletion at 30°C. Growth on 5-FOA plates counter-selects against a URA3 SEC7 plasmid covering the sec7 deletion. Experiments were performed at least three times for WT, vector and N653A mutation. All others have been done in duplicate.

(B) Same as A, but grown at 37°C.

Figure 3. The HUS-box mutation does not prevent N-terminal dimerization

(A) Schematic of Sec7 constructs used in this study. The red star indicates the location of the N653A HUS-box mutation. Sec7f: residues 203-2009, Sec7ΔC+HDS1: residues 203-1220, Sec7ΔC: residues 203-1017.

(B) Left: Recombinant Sec7f WT and Sec7f N653A were purified from insect cells, resolved on an 8% SDS-PAGE gel, then coomassie-stained. Right: Recombinant Sec7ΔC+HDS1 (WT and N653A), Sec7ΔC (WT and N653A), myristoylated Arf1 and ΔN17-Arf1 were purified from E. coli and resolved on a 15% SDS-PAGE gel. The asterisk indicates a contaminant.

(C) Multi-Angle Light Scattering (MALS) coupled to gel filtration results for Sec7ΔC WT (left) and N653A mutant (right). Both constructs are dimeric.

Figure 4. N653A Mutation hinders GEF activity on membranes

(A) Representative nucleotide exchange (GEF activity) data for the activation of myr-Arf1 by different Sec7 constructs in the presence of liposome membranes. The intrinsic tryptophan fluorescence of Arf1 increases upon GTP-binding.

(B) Quantified rates of nucleotide exchange of myr-Arf1 by WT and N653A Sec7ΔC+HDS1 and Sec7ΔC constructs in the presence of membranes. Quantification was performed
The HUS-box mediates Sec7 allostery from reaction rates (n=3) from curves that were fit to a single exponential and normalized for measured GEF concentration to obtain the overall reaction rate. Points indicate actual data values. Error bars represent 95% confidence intervals; significance was measured by one-way ANOVA with Tukey’s test for multiple comparisons. ***, p < 0.001

(C) Liposome flotation assays showing membrane recruitment of WT Sec7ΔC+HDS1, but not N653A Sec7ΔC+HDS1, by activated myr-Arf1-GTP.

(D) Liposome flotation assays showing recruitment of both WT and N653A Sec7ΔC+HDS1 to membranes by activated myr-Arf1-GTP.

Figure 5. The N653A mutation disrupts localization of Sec7ΔC+HDS1

(A) Localization of an extra copy of GFP-tagged Sec7 constructs in live yeast cells. Constructs were expressed under the endogenous SEC7 promoter on centromeric plasmids in wild type cells (CFY188) and imaged in log phase. Dashed circles indicate cell boundaries. Scale bar, 2 microns. See also Figure S1.

(B) Localization of a single copy of GFP-tagged wild-type and mutant full-length Sec7 constructs expressed under the endogenous promoter on centromeric plasmids after shuffling. Plasmids were introduced into the sec7Δ shuffling strain (CFY409) and then plated on 5-FOA. Live cells were then imaged in log phase. Dashed circles indicate cells. Scale bar, 2 microns.

Figure 6. The HUS-box is required for allosteric activation of Sec7 by Ypt31

(A) Model of Sec7 recruitment and potentiation by GTP-Ypt31. (1) Ypt31-GTP physically interacts with Sec7 and this interaction requires the HDS2/3 domains. (2) This interaction is sufficient to recruit Sec7 to membranes in vitro and results in (3) enhancement of Sec7 activity via allosteric conformational change.

(B) Quantification of WT and N653A Sec7ΔC+HDS1 GEF activity on myr-Arf1 in the presence of membranes.

(C) Quantification of WT and N653A Sec7ΔC+HDS1 GEF activity on myr-Arf1 in the presence of activated membrane-bound prenyl-Ypt31-GTP.

(D) Quantification of WT and N653A Sec7ΔC+HDS1 GEF activity on soluble ΔN17-Arf1 in the absence of membranes.

(E) Liposome flotation assays showing membrane recruitment of both WT and N653A Sec7ΔC+HDS1 by prenyl-Ypt31-GTP.

Quantification was performed from reaction rates (n=3, except (B), for which n=4) from curves that were fit to a single exponential and normalized for measured GEF concentration to obtain the overall reaction rate. Points indicate actual data values. Error bars represent 95% confidence intervals; significance was measured by one-way ANOVA with Tukey’s test for multiple comparisons. ***, p < 0.001; *, p < 0.05; n.s., not significant.
The HUS-box mediates Sec7 allostery

Figure 7. Model for the role of the HUS-box in allosteric activation of Sec7

Sec7 is dimeric but shown as a monomer here for simplicity. The HUS-box is depicted here by a red line.

(A) (1) WT Sec7 is recruited to and stabilized on the membrane surface by interactions with multiple GTPases, including Arf1-GTP and Ypt31-GTP. (2) Sec7 adopts its most active conformation (represented as a fully open conformation) due to allosteric activation by Ypt31.

(B) (1) The N653A HUS-box mutation (indicated by a red star) impairs allosteric activation by both Arf1-GTP and Ypt31-GTP. (2) The mutant Sec7 can still interact with Arf1-GTP and Ypt31-GTP, but full allosteric activation is impaired, as represented by the partially closed conformation.
Figure 1

A

BIG/Sec7
- Sec7 (S. cerevisiae)
- BIG2 (H. sapiens)

GBF/Gea
- Gea2 (S. cerevisiae)
- GBF1 (H. sapiens)
- GNOM (A. thaliana)

B

N(Φ)DC(D/N)
- Sec7 (S. cerevisiae) ...
- BIG2 (H. sapiens) ...
- Gea2 (S. cerevisiae) ...
- GBF1 (H. sapiens) ...
- GNOM (A. thaliana) ...

... : * **: :: :: :: * :: :
Figure 3

A

Sec7

DCB/HUS

GEF

HDS1

HDS2

HDS3

HDS4

Sec7f

Sec7ΔC+HDS1

Sec7ΔC

B

C

UV signal (normalized)

Molecular weight (Da)

Retention Volume

UV signal (normalized)

Molecular weight (Da)

Retention Volume
Figure 7

(A) WT

1. ARF1 GTP
2. Ypt31 GTP

HUS-box

DCB/HUS GEF HDS1 HDS2 HDS3 HDS4

TGN MEMBRANE

(B) HUS-box Mutant

1. ARF1 GTP
2. Ypt31 GTP

HUS-box

DCB/HUS GEF HDS1 HDS2 HDS3 HDS4

TGN MEMBRANE
The HUS-box is required for allosteric regulation of the Sec7 Arf-GEF
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