

Autoregulation of Sec7 Arf-GEF activity and localization by positive feedback

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Members of the highly conserved Arf family of small GTPases serve as master regulators of vesicular transport. In yeast, Arf1 acts at the Golgi and trans-Golgi network (TGN) to recruit vesicular coat proteins and other effectors for both anterograde and retrograde transport. Arf1 is activated at the TGN by Sec7, the founding member of the Sec7 family of guanine nucleotide exchange factors (GEFs) and a close homolog of the human ARFGEF2 implicated in congenital defects in cerebral cortex development. Through the use of purified Sec7 in biochemical assays, we recently discovered that autoinhibition of Sec7 is relieved by stable recruitment to lipid membranes by activated Arf1. This interaction is mediated by a conserved domain proximal to, but not including, the GEF domain, creating a positive feedback loop in the activation of Arf1 at the TGN. We further demonstrated that this stable interaction with Arf1 plays a role in localizing Sec7 to the TGN. We elaborate here on the implications of these results to small GTPase-mediated cellular processes and coincidence detection models of GEF localization.

A primary challenge faced by eukaryotic cells is the separate regulation of events at distinct intracellular membranes. Fusion of a vesicle with only its target membrane is essential for proper targeting and distribution of cellular proteins as well as for orderly posttranslational modifications of secreted proteins, and such critical functions as regulated neurotransmitter release cannot be affected by crosstalk from constitutively active vesicular fusion

elsewhere in the cell. Similarly, vesicles budding from their membranes of origin require both unique identifiers to “mark” their destination and unique cargo selection functions. As such, different membranes in the cell must possess different regulatory proteins; conversely, regulatory proteins must possess a way to uniquely be recruited to their particular target membrane(s). Small GTPases, which are easily converted between GDP-bound, often soluble, “off” states and GTP- and membrane-bound “on” states, serve as the core of this process. In the case of the secretory pathway, sequential small GTPase conversion is thought to play a key role in establishing a gradient of differently marked membranes, enabling the construction of an orderly vesicular flow.¹

At the penultimate stage of secretion, occurring at the trans-Golgi network, the key players in the initiation of yeast vesicular transport are the Arf1 small GTPase and its activating guanine exchange factor (GEF) Sec7.^{2,3} These correspond respectively to their mammalian Golgi-localized homologs Arf1–5 and BIG1/2. Fluorescently tagged Sec7 is commonly used as a TGN marker; however, its means of specifically restricting its localization to the TGN, as well as details of its regulation of Arf1, were until recently unknown. Taking a primarily biochemical approach, we recently presented evidence⁴ for several autoregulatory activities of Sec7, most notably a conversion from a GEF-inhibited form in solution to a GEF-enhanced form when bound to membranes. This regulatory switch is mediated by the GEF-proximal HDS1 domain,⁵ and depends upon a direct interaction with activated

Keywords: Arf1, Sec7, positive feedback, Golgi, TGN, Arf-GEF, autoregulation, GTPase

Abbreviations: GTP, guanosine triphosphate; TGN, trans-Golgi network; GEF, guanine nucleotide exchange factor; PH, pleckstrin homology

Submitted: 06/29/12

Accepted: 08/14/12

<http://dx.doi.org/10.4161/sntp.21828>

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Commentary to: Richardson BC, McDonold CM, Fromme JC. The Sec7 Arf-GEF is recruited to the trans-Golgi network by positive feedback. *Dev Cell* 2012; 22:799-810; PMID:22516198; <http://dx.doi.org/10.1016/j.devcel.2012.02.006>.

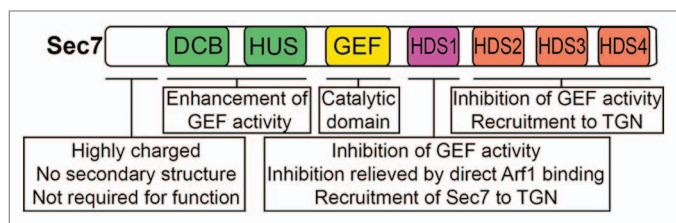


Figure 1. Summary of domain functions of Sec7 from Richardson et al.⁴ Domain identifications are as per Mouratou et al.⁵

and membrane-recruited Arf1 itself, forming the basis for a positive feedback loop of Arf1 activation. Under our in vitro experimental conditions, the N-terminal DCB and HUS domains exhibit a constitutive enhancing activity, whereas the GEF-distal C-terminal HDS2-4 domains exhibit a constitutive inhibiting activity (Fig. 1).

This work extends several observations of related peripheral GEFs to the essential large GEFs involved in constitutive secretion. Most prominent among the former is the plasma-membrane-localized Arno, consisting of a short N-terminal coiled-coil motif, a Sec7/GEF domain and a C-terminal PH domain and polybasic sequence.⁶ The PH domain of Arno/Grp1/cytohesin, in addition to its canonical lipid-binding function, inhibits the protein's GEF activity in a manner relieved by direct binding of activated Arf6 to the PH domain.⁷ Similarly, autoinhibition of the unrelated Ras GEF SOS is relieved allosterically by binding of Ras-GTP to a secondary site of the Cdc25/GEF domain.^{8,9} Common to each is an inhibition/activation cycle linked to but separate from simple membrane recruitment. Notably, each example is structurally unrelated: the primarily β -sheet PH domain of Arno is distinct from the α -helical HDS1 of Sec7, and SOS contains a different GEF domain entirely. The cellular network "logic" of this mechanism for Arno and SOS is clear, as both participate in functions requiring strict and large-scale binary control: SOS regulates Ras, a cell cycle regulator and proto-oncogene,¹⁰ and Arno participates primarily in the regulation of large-scale plasma membrane rearrangements and must be prevented from simultaneously activating Arf1 at the Golgi.⁷ As Arf1-mediated Golgi export does not appear to be subject to especially tight cell-cycle

control, the necessity of its binary regulation by positive feedback is somewhat less clear. This regulation of Sec7 is perhaps most relevant in the context of a cisternal maturation/small GTPase conversion model in which speed is a particularly critical component of the system. A recent study of the temporal sequence of clathrin cargo adaptor recruitment to the TGN demonstrates that the GGA adaptor recruits via Arf1 nearly coincident with Sec7 itself, signaling for the recruitment mere seconds later of the separate AP-1 adaptor.¹¹ Under such time constraints, the Sec7/Arf1 feedback loop may serve to build up Arf1 levels at the TGN as quickly as possible upon TGN development to facilitate the rapid recruitment of numerous vesicle coats. Another attractive (and at present, highly speculative) possibility is that it may serve as a means to localize and concentrate Arf1 activation to specific sites on the trans-Golgi as a precursor to concentration-dependent Arf1-mediated membrane curvature for vesiculation.¹²

Our recent data⁴ indicate that the direct interaction between HDS1 of Sec7 and membrane-bound Arf1 not only regulates the "switch" behavior of HDS1, but also assists in recruitment of Sec7 to membranes in vitro and the TGN in vivo. Indeed, while HDS1 clearly exhibits regulated autoinhibition of the GEF domain, it is difficult to determine whether the additional activation seen over constructs lacking the domain represents direct allosteric activation or simply reflects Sec7's recruitment to its site of action, the membrane surface. Arf1-mediated localization taken by itself, while required, does not suffice to generate Sec7's well-established TGN localization, as Arf1 is present throughout the Golgi. In principle, the role of Arf1 in early Golgi traffic provides a means by which Sec7 may be excluded from the early

Golgi – namely, through competition with the COPI coat for binding Arf1. Binding of COPI to Arf1, stabilized by interaction with retrograde-bound cargo,^{13,14} could thereby prevent Sec7 from stably binding Arf1 if and only if early Golgi resident proteins are available for retrograde transport. Under this model, the presence of free Arf1-GTP at the late Golgi could serve as a signal that the early Golgi has fully matured, triggering recruitment of Sec7 and amplification of Arf1 required for export by vesiculation. However, the increasingly common themes of coincidence detection and GTPase conversion in the identification of membranes suggest that full TGN localization may involve multiple interactions acting in concert, and several lines of evidence suggest that this is in fact the case. From our own study,⁴ the partial mislocalization seen when domains C-terminal to HDS1 are removed (Fig. 2) indicates these domains play a critical role in addition to the direct Arf1-HDS1 interaction. While difficulty in purifying these domains in the absence of HDS1 precluded detailed analysis in our work, an interaction between the Sec7 C-terminus and the Rsp5 ubiquitin ligase has been proposed to be involved in Sec7 localization.¹⁵

Perhaps the most convincing specific candidate to date for an Arf1 coincidence partner is Arl1, recently determined by the Munro lab to bind the N-terminus of the human and fly Sec7 homologs and to cause their delocalization when knocked down.¹⁶ A particularly attractive feature of Arl1 as a recruiting partner is the continued proper localization of GBF1 earlier in the Golgi upon Arl1 knockdown, suggesting an additional means of distinguishing between early and late Golgi pools of Arf1 on the basis of coincidence detection with Arl1. While Arl1 deletion in yeast fails to cause significant Sec7 mislocalization,¹⁷ it is synthetically lethal with *arf1* Δ (albeit in a strain-dependent manner), consistent with a parallel activity to that of Arf1.^{18,19} The discrepancy in the localization phenotype between yeast and higher eukaryotes may reflect differential regulation of Sec7 between systems, or may simply reflect differences in the relative levels of Arf and Arl proteins. In mammalian cells in particular, the expanded number of Arf

proteins with overlapping roles²⁰ may lead to a greater dependence on Arl1 as a unique TGN identifier and makes a full combinatorial analysis of Arf depletion on Sec7 localization problematic. As Sec7 consists of a very long α -helical armadillo repeat, giving it an expected highly elongated structure, the identification of N-terminal, central and potential C-terminal binding proteins cooperating for full localization of Sec7 builds a picture of Sec7 as a trans-Golgi scaffold; while its activation of Arf1 is its only presently identified effector role in yeast, Sec7-dependent recruitment of additional TGN-localized proteins would not be surprising.

The lipid composition and biophysical properties of the membrane itself frequently play a role in membrane identification and protein recruitment. Indeed, relief of Sec7 autoinhibition is dependent on the presence of membranes.⁴ While it has been proposed that the trans-Golgi phospholipid PI4P or its kinase Pik1 may also be a relevant partner in coincidence detection by Sec7,²¹ several studies, including ours, have failed to identify a significant role of membrane composition in Sec7 recruitment or activity, and Daboussi et al. convincingly place the role of PI4P in vesiculation downstream of Sec7 activity.¹¹ As such, it seems more likely that the membrane dependence of Sec7 GEF activity in this particular case is more relevant to the prevention of unproductive GEF activity in free cytosol than it is to the specific localization of activity to the TGN. With respect to membrane behavior, we note that the membrane-associating helix of Arf1, which is frequently removed for biochemical convenience, is known to affect membrane curvature,²² underscoring the importance of using complete constructs whenever possible in the study of small GTPases.

Many questions remain in characterizing the activity, localization and regulation of the large Golgi-localized GEFs. There is no conservation of Sec7 and the related BIG/GBF families with the Arno/Grp1/cytohesin family outside the GEF domain or with SOS, and Sec7 currently lacks high-resolution structural data analogous to those facilitating analysis of SOS and Arno.^{8,23,24} How does Sec7 differ from these in the relationship between

inhibition, activation and recruitment? How do Arf1, Arl1 and potentially other proteins interrelate for recruitment by coincidence detection? What are the roles of the remaining conserved domains of Sec7, and do they play a role in recruiting proteins to the TGN independent of Arf1? How do the related Gea1/2 proteins instead localize to the early Golgi, and how is their intermediate position in the secretory pathway reflected in their activity? This initial characterization of the regulation of Sec7 will hopefully provide a foothold for addressing these questions and more.

Acknowledgments

We thank J. Lees and C. McDonold for helpful comments on the manuscript. The authors are supported by NIH/NIGMS grant R01GM098621.

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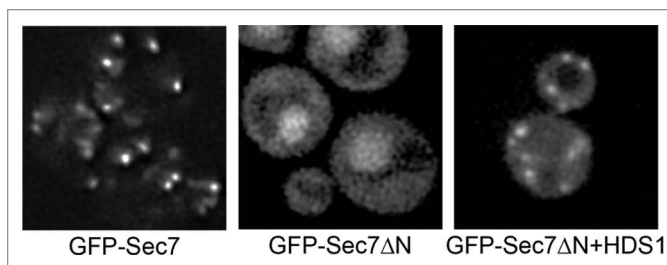


Figure 2. Localization of truncated Sec7 constructs. The indicated GFP-tagged Sec7 constructs were expressed in wild-type yeast on low-copy vectors under the control of their endogenous promoter as per Richardson et al.;⁴ alternative yeast pictures to those previously used are shown here. “Sec7” represents full-length Sec7. “Sec7 Δ N” represents Sec7(1–1017) containing the DCB, HUS and GEF domains. “Sec7 Δ N+HDS1” represents Sec7(1–1215) containing the DCB, HUS, GEF and HDS1 domains.

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