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REVIEW

Golgi GRASPs: moonlighting membrane tethers

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Abstract: The identification of mammalian Golgi reassembly stacking proteins (GRASPs) 15 years ago was followed by experiments implicating them in diverse functions, including two differing structural roles in Golgi biogenesis and at least two distinct roles in the secretion of proteins. GRASP55 and GRASP65 are localized to cis and medial/trans Golgi cisternae, respectively. They are both required for stacking of Golgi membranes in a Golgi reassembly assay. Depletion of either GRASP from cultured cells prevents the linking of Golgi membranes into their normal ribbon-like network. While GRASPs are not required for transport of secretory cargo per se, they are required for ER-to-Golgi transport of certain specific cargo, such as those containing a C-terminal valine motif. Surprisingly, GRASPs also promote secretion of cargo by the so-called unconventional secretory pathway, which bypasses the Golgi apparatus where the GRASPs reside. Furthermore, regulation of GRASP activity is now recognized for its connections to cell cycle control, development, and disease. Underlying these diverse activities is the structurally conserved N-terminal GRASP domain whose crystal structure was recently determined. It consists of a tandem array of atypical PSD95–DlgA–Zo–1 (PDZ) domains, which are well-known protein–protein interaction motifs. The GRASP PDZ domains are used to localize the proteins to the Golgi as well as GRASP-mediated membrane tethering and cargo interactions. These activities are regulated, in part, by phosphorylation of the large unstructured C-terminal domain.

Keywords: GRASP, review, membrane, tether, PDZ domain, secretory chaperone, unconventional secretion

Introduction

Proteins translated in association with the endoplasmic reticulum (ER) are packaged into vesicles for export to the Golgi apparatus. This protein cargo may bear exit signals promoting selective concentration during exit.[1]

In mammalian cells, ER exit sites are both centrally located and distributed throughout the cell periphery. Vesicles leaving the ER coalesce into the ER-Golgi intermediate compartment (ERGIC).[2] From the ERGIC, there is both recycling to the ER and transfer of cargo-bearing membrane to the centrally located Golgi apparatus.[3]

The Golgi apparatus is structured to promote sequential processing of the arriving cargo.[4] Flattened membranes called cisternae are in close apposition to one another, forming a stack. The first Golgi enzymes to act on the cargo are enriched in the entry, or cis-cisternae. Enzymes enriched in the medial cisternae of the stack carry out the next processing steps. The last events occur in the trans-cisternae and trans-Golgi network (TGN). In the TGN, the processed cargo is packaged for exit from the Golgi apparatus.

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The stacked structure of the Golgi apparatus is evident in most eukaryotes.

Mammalian Golgi stacks are positioned near the centrosome-based microtubule-organizing center and they are laterally linked to form a ribbon-like membrane network.[5] Dynamic tubular membranes connect adjacent analogous cisternae within this network promoting uniform, yet cisternae-specific distribution of Golgi enzymes across the entire Golgi ribbon. Discontinuities, if prolonged, lead to nonuniform enzyme distribution and under-processing of secretory cargo.[6] Positioning of Golgi membranes near the centrosome allows the secretion to be efficiently directed to the most proximal plasma membrane domain or cell leading edge.[7] This directed secretion is needed for cell polarity and cell migration for wound repair. It is also involved in muscle differentiation, immunological synapse formation, and neuritic process extension.[8–10]

In preparation for mitosis, the mammalian Golgi ribbon is unlinked, and the resulting stacks then vesiculate, leaving dispersed vesicles and clusters of vesicles.[11–13] These vesicles and vesicle clusters are partitioned equally into daughter cells, whereupon they reassemble into the stacked and laterally linked interphase membrane network. The Golgi ribbon is also extensively fragmented during apoptosis, and unlinking of the ribbon is thought to occur transiently during reorientation of the organelle as cells define a new leading edge.[14,15]

Interestingly, certain secretory cargo molecules entirely bypass the Golgi apparatus en route to secretion.[16,17] Examples include fibroblast growth factors 1 and 2,[18,19] interleukins 1α and β,[20,21] integrin αPS1,[22] acyl-CoA binding protein,[23–26] and galectins (1 and 3).[27–30] Unconventional secretion occurs by multiple routes. For some unconventional cargo, secretion begins with transmembrane proteins translated in association with the ER or cytosolic proteins internalized into autophagic membranes.[31] Membranes derived from the ER or the autophagic membranes then dock and fuse directly with the plasma membrane to transfer their content.

Since their discovery 15 years ago, experimental evidence indicates that mammalian Golgi reassembly stacking proteins (GRASPs) may participate in a surprising number of the processes just described (Figure 1). Mammalian cells express two GRASPs and both are encoded by distinct genes. GRASP65 is localized to cis-Golgi cisternae and GRASP55 is localized to medial and trans-Golgi cisternae.[32] The GRASPs contain a conserved N-terminal GRASP domain. Simpler eukaryotes express a single GRASP, which is also defined by the presence of this domain. The key feature of the GRASP domain is its two PSD95–DlgA–Zo–1 (PDZ) domains, which interact with PDZ ligands in partner proteins (Figure 2A and B). Following the GRASP domain, GRASPs have a nonconserved, serine and proline rich (SPR) domain comprising about half of the protein (Figure 2A). A simplifying assumption is

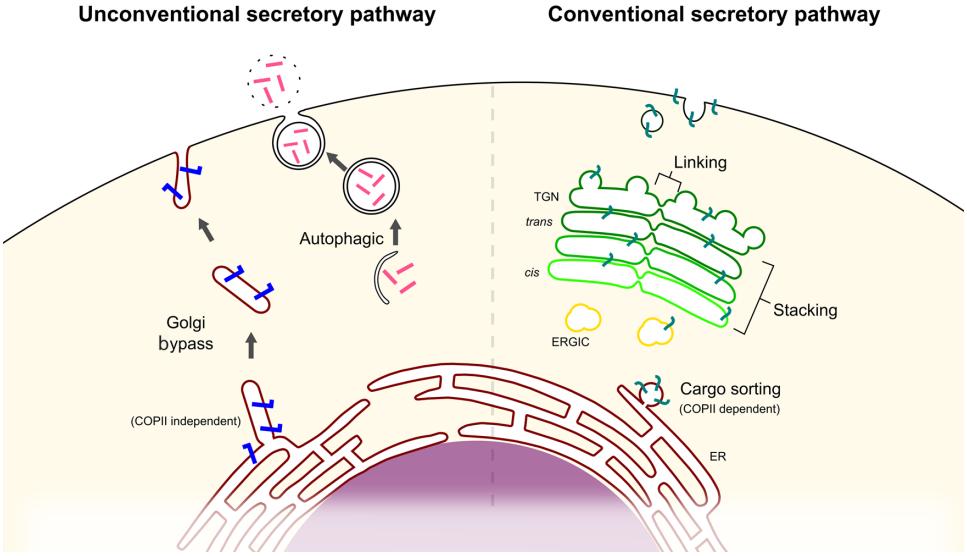

Figure 1 Overview of unconventional and conventional secretory pathways.
Notes: Three types of cargo are shown. Unconventional transmembrane cargo (blue) is shown exported from the ER in a COPII independent fashion followed by fusion of its carrier with the plasma membrane. Cytosolic cargo (pink) is first internalized in autophagic membranes. Traditional cargo (teal) undergoes COPII-mediated budding, transfer from the ER Golgi intermediate compartment to the Golgi, and export from the Golgi to the plasma membrane. Linking and stacking are, respectively, homotypic and heterotypic membrane interactions that contribute to Golgi ribbon architecture.
Abbreviations: ER, endoplasmic reticulum; ERGIC, ER-Golgi intermediate compartment; TGN, trans-Golgi network.

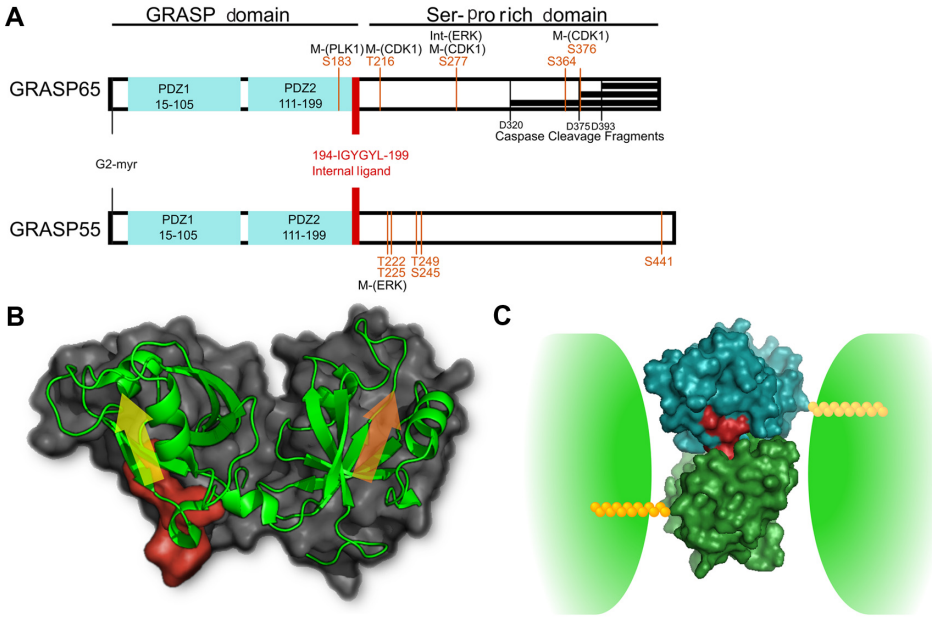

Figure 2 Mammalian GRASP65 and GRASP55. (**A**) Schematic of mammalian GRASP functional domains and features. N-terminal myristoylation (on glycine at position 2), PDZ1 and PDZ2 (blue), phosphorylation sites (orange), internal ligand (red), and caspase cleavage sites (black) are shown with indicated residue numbers. The implicated kinases active at interphase or mitosis are also indicated. (**B**) A cartoon representation of the GRASP domain of GRASP55 1–208 overlaid on space fill model. PDZ1 (orange arrow) and PDZ2 (yellow arrow) binding grooves are highlighted. The internal ligand is highlighted in red. (**C**) Schematic of GRASP domain tethering mechanism. Two apposed Golgi cisternal rims are shown (green) each bearing a GRASP protein. The internal ligand (red) of one GRASP domain is docked in the PDZ1 binding pocket of the second GRASP domain. Myristoylation (yellow) anchors the domains and is hypothetically placed.
Abbreviation: GRASP, Golgi reassembly stacking protein.

that the GRASP domain mediates functional interactions regulated by the SPR domain.

GRASP structural features

The GRASP domain

The GRASP domain is a striking feature conserved across GRASP homologues. It is comprised mostly of two PDZ domains in tandem (Figure 2B). PDZ domains share a conserved globular structure in which an α-helix and β-strand form a hydrophobic binding groove. Many proteins contain PDZ domains, and most PDZ-containing proteins contain multiple such domains that are present in arrays. The domain mediates protein–protein interactions and, as such, its presence in multiple copies confers the ability to scaffold the assembly of large complexes.[33] Typically, the ligands that bind the groove fall into one of three categories. Canonical ligands are present at a protein's C-terminus. The four C-terminal residues insert into the PDZ groove, forming a β-strand that completes a β-sheet. The carboxyl group at the C-terminus is coordinated within the PDZ binding pocket by a Gly–Leu–Gly–Phe sequence and water molecule.[34] The second and third types are internal sequences. They may form a β-finger with a tight hairpin and usually an acidic amino acid to mimic the free C-terminus[35] or they might simply be flexible sequence stretches that contain glycines, for example, that decrease steric constraints and project side chains into the pocket.[36,37]

The structure of the GRASP55 GRASP domain was recently solved[38] and unexpectedly revealed that the GRASP PDZ domains lack the typical βββαββαβ secondary structure organization of eukaryotic PDZ domains. Instead, the two PDZ domains are circularly permuted, which has two important consequences. First, although not evident among metazoan PDZ domains, circular permutation is present in prokaryotic PDZs. In fact, the overall structures of the GRASP PDZ domains align remarkably well with prokaryotic PDZ domains as well as with one another.[18] Second, this unusual arrangement of a metazoan PDZ revealed that the key β2 strands of the binding grooves lay outside the previously predicted PDZ-like regions. This calls into question the interpretation of experiments based on incorrect assignment of the domains. In fact, a recent study failed to include the key β2 strands in its GRASP PDZ domain constructs even though this work was published after the structure was known.[39] Inexplicably, the constructs were binding competent, but this binding could not have been due to a PDZ interaction.

Although the two PDZ domains in the GRASP domain are similar in overall structure, there are significant differences in the binding grooves, which reflect the specificities of their interactions. The interface between the second α-helix

and second β-strand of the PDZ1 binding domain contains a deep depression resembling a pocket.[38] In contrast, the PDZ2 groove contains a phenylalanine occluding the pocket. Evidence suggests that while PDZ2 of GRASP65 binds a C-terminal ligand present in the coiled-coil Golgi protein GM130, PDZ1 interacts with a novel type of internal PDZ ligand. Remarkably, this ligand is present on the surface of the PDZ2 domain and forms a conspicuous surface protrusion that appears to fit into the pocket of PDZ1 (Figure 2C). As described in more detail below, the separation of function of the two PDZ domains in GRASP65 allows targeting of the protein to the Golgi via PDZ2 and homodimer formation via PDZ1. Together these activities allow the protein to mediate homotypic membrane tethering. Residues flanking PDZ2 may also stabilize the interaction of PDZ2 with GM130. Mutation of these residues blocks binding to GM130,[40] and flanking regions are known to stabilize the ligand-pocket interaction of other PDZ domains by making the PDZ domain less dynamic.[33] Paralleling the activities of GRASP65, GRASP55 self-interacts and binds a coiled-coil Golgi protein, golgin45. Nevertheless, it remains to be determined whether these activities map to PDZ1 and PDZ2, respectively, and whether the golgin45 interaction mediates Golgi localization of GRASP55.[41]

N-terminal membrane attachment

The mammalian GRASPs are myristoylated at their N-termini. This modification is required along with golgin binding for their localization to the Golgi.[42] Thus, dual contact with the membrane stabilizes membrane binding. The N-terminal myristic acid is immediately adjacent to the PDZ1 module. Mutation of the glycine residue that becomes myristoylated blocks membrane tethering by PDZ1, even if the protein is stably anchored to the membrane by other means. Substituting a transmembrane domain for the myristoylation site restores activity, which indicates that the N-terminus of PDZ1 must be membrane anchored for PDZ1-mediated membrane tethering.[43] Interestingly, membrane binding of the N-terminus is conserved, even though certain species express GRASPs without the myristoylation sequence.[44,45] The GRASP homologue in *Plasmodium falciparum* expresses a splice variant with an N-terminal signal anchor, and the *Saccharomyces cerevisiae* version has an amphipathic helix that is acetylated to mediate membrane association.

There appears to be additional significance to the dual-anchoring of GRASP65 by the N-terminal myristic acid and GM130 binding.[6] The two contact points of membrane binding might orient the GRASP65 homotypic binding interface to promote trans interactions across two membranes.[46] Subsequent membrane fusion then imparts a torque on the complex because of the dual anchors. In other words, membrane rearrangement forces an unfavorable cis configuration leading to partner dissociation. Because they are relatively weak, PDZ interactions[47] might lend themselves to regulation by membrane dynamics. Interestingly, by their shared requirement for dual anchoring, localization of the tether and tether activity are linked. Thus, only dually anchored GRASP65 molecules are on the Golgi, thereby ensuring that cis interactions do not interfere with trans pairing.

GRASP55 has at least one additional fatty acid modification. It is palmitoylated[48] although the functional relevance of this modification is unclear. The longer acyl chain could affect the distribution and preference of GRASP55 in the membrane, making it prefer the thicker, late Golgi membranes.[49]

Internal ligand

GRASP55 and GRASP65 share a conserved sequence stretch IGYGYL at the end of PDZ2 that binds PDZ1[38,50] (Figure 2A–C). The glycines likely contribute to rotational flexibility, allowing the surface projecting tyrosine and leucine side chains to fit precisely into the deep pocket present in the PDZ1-binding groove.[38] Extensive van der Waals contacts may provide the stability to the interaction, nullifying the need for coordination of a carboxyl group seen in other PDZ interactions. Because the proteins do not bind one another, it is puzzling that the surface projecting ligand is identical in GRASP65 and GRASP55. There may be residues flanking the ligand sequence that play an important role in ligand specificity. Importantly, the GRASP ligand is a target of mitotic regulation. In GRASP65 a nearby serine is phosphorylated during mitosis likely inducing a conformational change that blocks the ligand from binding PDZ1.[50]

Serine proline-rich (SPR) domain

The C-terminal half of the GRASP proteins is enriched in serine and proline residues. This region appears to be unstructured. In striking contrast to the GRASP domain, the SPR domain is not conserved at the sequence level. Noteworthy in this region are multiple phosphorylation and caspase cleavage sites.[11,14,51–56] Cyclin-dependent kinase 1/cyclin B (CDK1), a MEK/ERK cascade, and Polo-like kinase 1 (PLK1) each phosphorylates one or both GRASP proteins.[51–53] ERK directly phosphorylates GRASP55, and inhibition of its upstream activator MEK1 blocks both GRASP55 phosphorylation and G2-phase Golgi unlinking.[11,52,53] Furthermore, mutation of ERK phosphorylation sites in GRASP55 to mimic the phosphorylated

state blocks GRASP55 activity in both Golgi ribbon formation and self-association.[52] GRASP65 is directly phosphorylated at multiple sites by CDK1, ERK, and PLK1, and phosphorylation blocks its homo-oligomerization in vitro.[53,57–59] Interestingly, the PLK family of kinases initially binds substrates and becomes activated through their Polo box domains. Once activated, the kinases can phosphorylate distant sites.[60–62] In the case of GRASP65, mitotic phosphorylation of the SPR domain creates a PLK1 binding site.[63] The activated PLK1 then phosphorylates a site adjacent to the internal GRASP65 PDZ ligand and blocks its ability to mediate GRASP65 self-interaction.[50]

Structuring the Golgi

The role of GRASPs in Golgi structure

Stacking of Golgi cisternae is heterotypic in that Golgi stacks consist of cisternae of differing membrane compositions. Stacking connections might be initiated as new cis-cisternae form and might be broken as the membranes of trans-cisternae remodel, giving rise to budding membranes. During mammalian cell mitosis, as the Golgi vesiculates, the stacked architecture of the Golgi is lost, which suggests inhibition of the stacking mechanism. In contrast to stacking, Golgi ribbon formation is a homotypic reaction. Analogous cisternae among Golgi stacks are dynamically linked to one another by tubular membrane projections that undergo fusion and fission. Specificity of fusion in ribbon formation is needed to preserve compositional differences between cis-, medial-, and trans-cisternae. The lateral ribbon-forming contacts between analogous cisternae are disrupted as cells prepare for cell division and rebuilt after cell division.[64]

Originally, GRASP65 and then GRASP55 were shown to be required individually for the in vitro assembly of stacked Golgi membranes.[42] Surprisingly, however, knockdown of either GRASP65 or GRASP55 in cultured cells leaves the Golgi with normal stacks.[6,11] Only depletion of both proteins perturbs the stacked architecture.[65] GRASP proteins have the ability to link membranes through their homotypic interactions, but the membrane junctions evident when GRASP proteins are used to tether mitochondrial membranes to one another are morphologically distinct from those of Golgi cisternae.[43] Even more perplexing, Saccharomyces cerevisiae does not have a stacked Golgi and yet expresses a GRASP homologue, and *Pichia pastoris* has a stacked Golgi, yet its stacked architecture does not depend on GRASPs.[66–68] In *Drosophila*, depletion of the lone GRASP homologue dGRASP decreases but does not abolish cisternal stacking.[69] Overall, it seems that another mechanism is primarily responsible for Golgi stacking, the GRASP proteins providing an additional contribution.

Mammals and other vertebrates that form Golgi ribbon networks express two GRASP proteins. Depletion of either breaks the ribbon into individual Golgi stacks. Because each protein interacts only with itself, the proteins could act in parallel reactions: GRASP65 supports membrane fusion to link laterally and elongate cis-cisternae, and GRASP55 maintains these contacts in medial cisternae. From an evolutionary perspective, functional divergence that gives rise to compartment specific tethering by the GRASP proteins might be for the maintenance of Golgi subcompartments in the face of microtubule-based motility, thereby bringing Golgi ministacks into close proximity in the region of the microtubule organizing center. In lower eukaryotes, a single GRASP gene is present and Golgi membranes, even when present as stacked cisternae, are neither confined to a central position nor fused laterally to form a ribbon-like membrane network. Possibly, ribbon formation in vertebrates is a more extreme form of cisternal elongation carried out by simpler eukaryotes.[70] If so, homotypic membrane-tethering mediated by membrane-anchored PDZ1 could represent the fundamental mechanism of GRASP65 action. The physical distance separating Golgi elements in simpler eukaryotes may prevent lateral fusion which, given the presence of only a single GRASP, might otherwise impair maintenance of subcompartment identity.

GRASP-tethering mechanism

GRASPs contribute to Golgi structure by homotypic oligomerization, an ability that was first observed for purified GRASP65 either in isolation or on the surface of beads.[57,58] The GRASP domain mediates oligomerization. In order to understand the mechanism of GRASP-mediated tethering on the surface of membranes and in physiological conditions, GRASP65 has been targeted to the outer membrane of mitochondria.[43] Paralleling its activity at the Golgi, GM130 recruits GRASP65, and GRASP65 is necessary and sufficient for mitochondrial tethering, which depends on the PDZ1-binding groove and the PDZ2 surface-projecting ligand. The same mutations of these elements that block mitochondrial clustering also block GRASP-mediated Golgi ribbon formation. Thus, the mechanism involves an internal PDZ ligand within a GRASP65 partner on one membrane binding to the first of two PDZ binding pockets in a GRASP65 partner on the opposing membrane (Figure 3A). The binding groove of PDZ2 is expendable for tethering membranes, but it is needed to localize GRASP65 to cis-cisternae by binding GM130. Both the N- and C-termini of the GRASP domain require anchoring to the membrane for efficient tethering, the former by myristoylation and the latter by a golgin.

This orients the internal ligand and PDZ1 pocket into a conformation that promotes a trans-interaction between GRASPs on separate membranes.[46] Loss of either anchor point allows free rotation of the GRASP molecule in the plane of the membrane, which allows interactions in cis and decreases the propensity for functional trans-pairing.

As mentioned above, the crystal structure of the GRASP domain yields a model for the tethering interface. The internal ligand present on the surface of PDZ2 projects its side chains into the deep binding pocket of PDZ1.[38] A significant feature of the GRASP domain structure is that the internal ligand for self-interaction is on the surface opposite that of the PDZ1 groove to which it binds. This provides a structural explanation for the observed tendency of GRASP65 to form multimers[58] and suggests that interdigitation of the molecules may occur during tethering.

Regulation of GRASP activity

Mitotic regulation of GRASP tethering activity

The homotypic tethering activity of both GRASP65 and GRASP55 is regulated during the cell cycle. As mentioned, both GRASP65 and GRASP55 are phosphorylated during mitosis. GRASP65 is a major target of Plk-1.[42] GRASP55 is phosphorylated by MEK/ERK.[11,52,53] In the case of GRASP65, a two-step phosphorylation process inhibits its homotypic tethering activity (Figure 3B). First, the SPR region is phosphorylated by ERK (in late G2) or CDK1 (in M phase), resulting in a docking site for Plk-1.[15,63] Plk-1 then phosphorylates Ser-189, which has been shown to block homotypic tethering and cause unlinking of the Golgi ribbon.[50] The proximity of Ser-189 to the internal ligand suggests that phosphorylation of Ser-189 causes a conformational change in the ligand that prevents its binding to the PDZ1 pocket. GRASP55 also has a serine at 189 but whether it is mitotically phosphorylated at this position is not known. GRASP55 does not appear to interact with Plk-1 during mitosis.[63]

GRASP regulation during cell motility

Certain cell types respond to an external motility cue by repositioning their microtubule-nucleating centrosome. Golgi membranes also move, presumably because cytoplasmic dynein keeps them near the minus ends of microtubules. Intriguingly, expression of a version of GRASP65 that cannot be phosphorylated by ERK blocks cellular reorientation to a scratch wound.[15] Because GRASP65 maintains

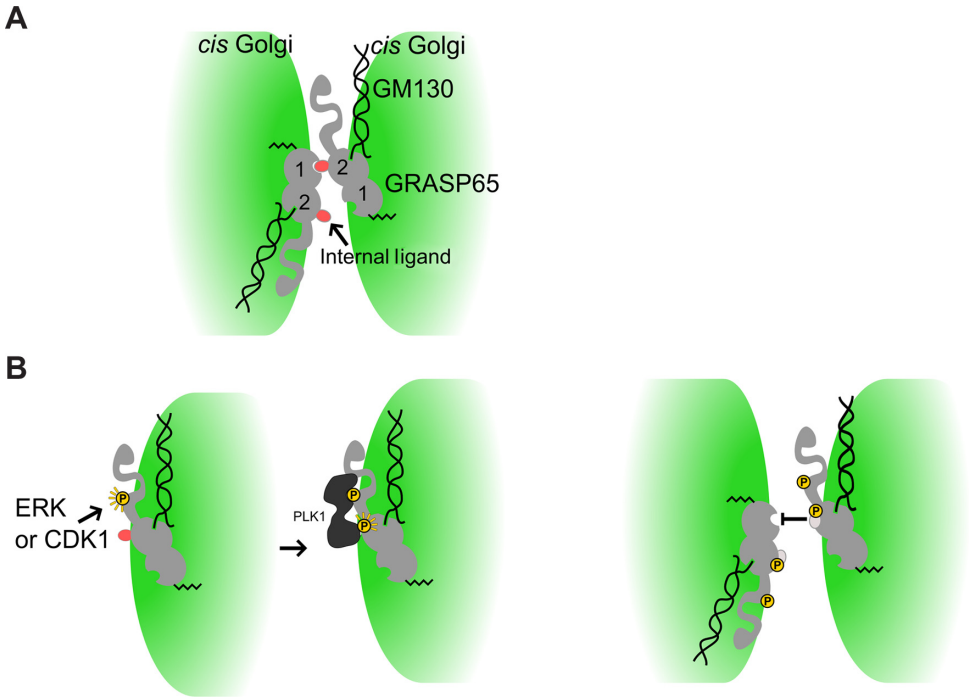

Figure 3 GRASP65 tethering and 2 step phosphoregulation. (**A**) Schematic diagram of GRASP65 tethering at the rims of two cis-Golgi cisternae (green). The internal ligand (shown as a red protrusion) of one GRASP65 binds to the PDZ1 pocket of the other. Each is anchored to the membrane through N-terminal myristoylation and a PDZ2-GM130 interaction. (**B**) Two step model for GRASP65 inactivation. ERK1/2 or CDK1 phosphorylates a site in the SPR domain creating a binding site for PLK1. PLK1 (shown in dark grey) docks and phosphorylates Ser-189 near the internal ligand. This phosphorylation causes a conformational change in the ligand, which prevents its binding to the PDZ1 pocket.

Abbreviation: GRASP, Golgi reassembly stacking protein.

Golgi structure and is phospho-inhibited, it is thought that GRASP65 is phosphorylated under these conditions to break transiently the Golgi membrane network, thereby removing a physical barrier that Golgi membranes might exert over centrosomal movement. This view is supported by the finding that Golgi disassembly by brefeldin A treatment bypasses the requirement for GRASP65 phosphorylation.[15]

GRASP regulation during development

In migrating granule neurons of the developing nervous system, polyubiquitination of GRASP65 PDZ1 by the Golgi-localized Cul7-Fbxw8 E3-ubiquitin ligase decreases GRASP65 protein levels.[71] Interestingly, reducing GRASP65 levels causes an increase in dendritic size and branching. Because GRASP65 knockdown increases Golgi fragmentation, possibly increasing its secretory ability,[72] it may be that ubiquitination of GRASP65 is a regulated step that governs morphogenesis of the Golgi apparatus and development of dendrites in the brain.

GRASPs and AIDS progression

Hiyoshi and colleagues have shown that GRASP65 is a critical downstream target in human immunodeficiency virus (HIV) to acquired immunodeficiency syndrome (AIDS) progression in macrophages. HIV-1 Nef is a steric activator of Hck, a Src kinase in macrophages. By reducing the surface level of the receptor Fms, Hck inhibits macrophage colony stimulating factor signaling.[73] The reduced signaling disables the anti-inflammatory state,[74] thereby allowing more viral replication.[75] The reduction in Fms surface levels is attributed to Golgi accumulation of a hypo-N-glycosylated form of the protein.[48] The cause of this accumulation appears to be disruption of the Golgi ribbon through phosphorylation of GRASP65 by ERK,[76] which is consistent with other experiments showing reduced glycosylation upon perturbation of the Golgi ribbon.[6] In sum, inhibition of GRASP65-mediated cisternal linking appears to play a role in the virulence of AIDS. These experiments also show that glycosylation defects arising from perturbed Golgi ribbon maintenance can have physiologically significant effects.

Chaperoning secretion and processing

C-terminal valine motif cargoes

Although GRASPs are not required for traffic of model cargo, such as the viral protein VSVG, the situation is different for processing and transport of specific proteins. One type of protein that is dependent on GRASPs contains a C-terminal valine motif. These include CD8-α, Frizzled4,[77] and TGF-α.[348] Their efficient transport is thought to involve GRASP65 chaperoning ER-to-Golgi transit and GRASP55 chaperoning intra-Golgi trafficking (Figure 4).

The C-terminal valine motif matches the minimal consensus for a C-terminal PDZ-ligand, and these cargos bind PDZ1 of both GRASP65 and GRASP55.[48,77] In the absence of GRASP65, the cargo molecules accumulate at ER exit sites, whereas GRASP55 depletion results in the retention of these cargoes at the Golgi.[77] The interaction with GRASP65 might serve as an exit signal, as the yeast GRASP has been shown to localize to exit sites and interact with the inner COPII coat complex sec23/24.[44,68] However, it is unknown how GRASP55 depletion decreases exit from the Golgi of C-terminal valine motif cargoes.[77] One hypothesis is that interaction with GRASP55 is needed to free these cargoes from their interaction with GRASP65, which holds them in the cis-Golgi.

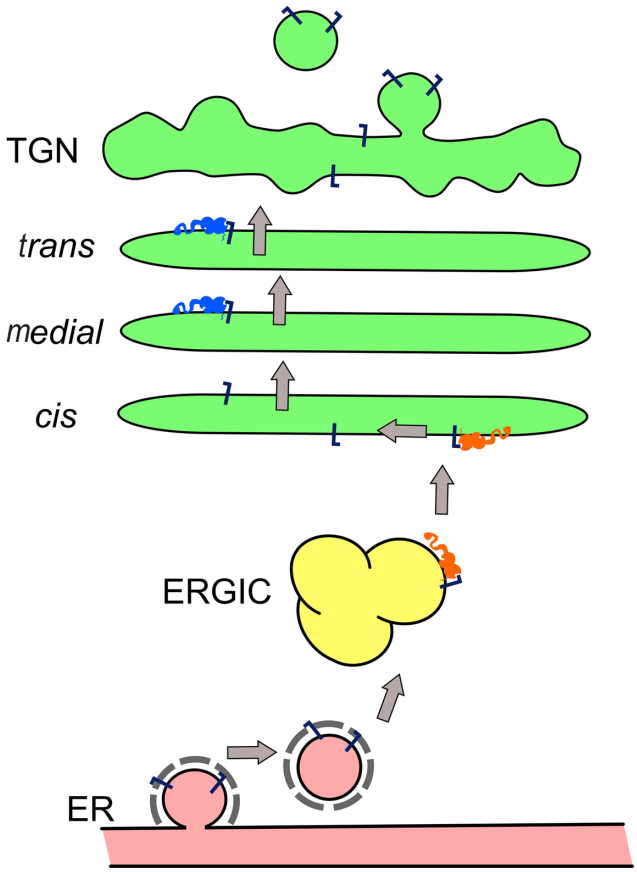

Figure 4 GRASP65 and GRASP55 work sequentially to promote C-terminal valine motif cargo through the secretory pathway.
Notes: Cargo with a C-terminal valine motif interacts with GRASP65 (orange) at the ERGIC to promote transport to the cis Golgi somehow. Once at the Golgi, GRASP55 (blue) binds the cargo and somehow promotes its transport through the stack.
Abbreviations: ERGIC, ER-Golgi intermediate compartment; GRASP, Golgi reassembly stacking protein; TGN, trans-Golgi network.

An intriguing aspect of these findings is that cargo levels could influence Golgi ribbon integrity.[78,79] Increasing occupancy of PDZ1 by C-terminal valine-bearing cargo would prevent homotypic GRASP interactions. Consequently, the ribbon would transiently fragment, thereby exposing greater surface areas for the purpose of cargo transit.

Cargo adaptors

GRASPs also interact with the p24 family of cargo receptors.[80] This family of cargo receptors is important for secretion of lumenal cargo, and they interact with both the COPI and COPII vesicle coats.[81] Interestingly, their cytoplasmic C-termini also match a minimal consensus for PDZ ligands, and they bind the GRASP proteins. Loss of GRASP binding by mutation of the C-terminal VV to AA results in an increased surface expression of these cargo receptors[80] and would presumably decrease the secretion efficiency of p24-specific cargoes. GRASP65 was found in complexes containing both GM130 and p24 family members. GRASP55 was found in complexes with either golgin-45 or p24 family members, but not both at the same time.

Processing efficiency

Interactions between GRASPs and cargo can also increase cargo-processing efficiency. A minor population of GRASP55 is found in cells interacting with the cytoplasmic domain of furin and the cytoplasmic domain of a matrix metalloproteinase (MT1-MMP).[82] The latter involves the sequence LLY near the C-terminus of MT1-MMP possibly binding PDZ2 of GRASP55. Furin also binds PDZ2, which suggests that dimerization of GRASP55 through PDZ1 generates a scaffold that brings furin and MT1-MMP together. This allows furin to cleave to the N-terminal inhibitory prodomain peptide of MT1-MMP. Because MMPs are often regulated during development,[83] binding of GRASP55 to MT1-MMP could be developmentally regulated.

Unconventional secretion

While the majority of secreted proteins follow the canonical secretory pathway, some proteins follow an unconventional pathway. This pathway includes both cytoplasmic and membrane spanning cargo. The former lack a signal sequence and do not incorporate into the ER during synthesis, whereas the latter are translated in association with the ER. Transport of both types for secretion excludes the Golgi apparatus, and despite being predominately Golgi localized, GRASP proteins are required in this process (Figure 5). Unconventional secretion is regulated by stress.[17]

Autophagic secretion

In *Dictyostelium discoideum*, knockout of its GRASP homologue GrpA blocks secretion of acyl coenzyme A binding protein (AcbA) causing decreased sporulation.[25] AcbA lacks a signal sequence, which indicates that it is secreted unconventionally. AcbA secretion is induced by the spore differentiation factor 2 (SDF-2), and there is cytoplasmic build-up of AcbA in the absence of GrpA. Further analysis shows that stimulation with SDF-2 results in AcbA incorporation into vesicles prior to secretion and that GrpA is required for the fusion of these vesicles, not their formation.[26] Significantly, the secretion of the AcbA homologue in yeast, Acb1, requires the yeast GRASP Grh1, as well as autophagosomal and early endosomal machinery.[23,24] It also requires Bug1, the yeast homologue to GM130, which suggests that, in this pathway too, the GRASP requires a golgin for its attachment to membranes.

During the inflammatory response in macrophages, caspase-1 is activated and cleaves the precursor to the interleukin cytokine IL-1β. The cytokine is processed in the cytosol and secreted, bypassing the normal secretory pathway. Induction of autophagy stimulates secretion causing a partial redistribution of GRASP55 from the Golgi to the autophagic membranes.[21] Knockdown of GRASP55 reduces the number of autophagic punctae and reduces secretion. The mechanism of GRASP55 in autophagy related unconventional secretion is currently unknown.

Golgi bypass

During *Drosophila* embryogenesis, dGRASP is required for unconventional secretion of αPS1 integrin to confer adhesion to a specific plasma membrane zone during epithelial remodeling.[22] The remodeling event causes a triangular void where the cells pull apart. At this stage, dGRASP and dGM130 change their localization from the Golgi to the zone of contact and nearby transitional ER, and the gap is repaired. Embryos homozygous at loss of dGRASP show a block in αPS1 secretion and a disorganized epithelium. Because dGRASP becomes localized to the plasma membrane, it may function there as a membrane tether that links membranes of unconventional secretory pathway to the plasma membrane.[17] Because dGM130 is also present, it may act, as it does at the Golgi, to localize dGRASP.[22] It has been speculated that unconventional secretion provides a means of modulating integrin adhesivity because glycosylation increases integrin adhesion to the extracellular matrix.

Additionally, GRASPs may play a role as chaperones of cargo, recruiting them to the unconventional secretory pathway.

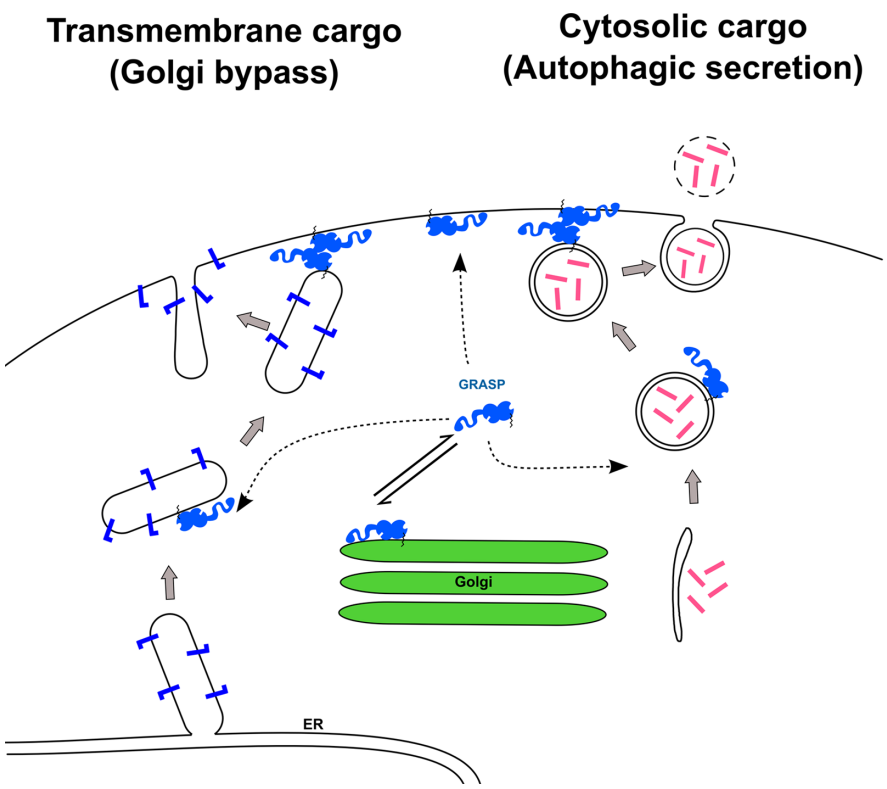

Figure 5 Models of GRASP function in unconventional secretion.
Notes: Unconventionally secreted transmembrane cargo exits the ER through a COPII independent process (left). GRASPs are recruited to the unconventional carrier as well as the plasma membrane. GRASPs then tether the membranes prior to fusion. Cytosolic cargo is first internalized into autophagic membranes (right). GRASPs are recruited to these membranes and the plasma membrane. GRASPs function as a tether prior to fusion.
Abbreviations: ER, endoplasmic reticulum; GRASP, Golgi reassembly stacking protein.

This could be similar to the binding of C-terminal valine motif cargoes in the conventional secretory pathway.[77] GRASP55 depletion during interleukin-1β secretion decreases autophagic punctae, but not the maturation of these punctae,[21] which suggests that GRASP55 plays a role early in the unconventional secretory pathway, presumably in the organization of membrane and capture of cargo.

Therapeutic target?

The tantalizing possibility of using GRASP55 to enhance unconventional secretion as a means of treating cystic fibrosis recently emerged.[39] It is well known that a common form of the disease is caused by a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR), which prevents its exit from the ER even though the protein retains activity. Interestingly, CFTR has a C-terminal PDZ ligand consensus sequence and undergoes unconventional secretion during the stress of an unfolded protein response. GRASP55 is required for this pathway of CFTR secretion. Furthermore, GRASP55 overexpression enhanced surface expression of the mutated CFTR, even rescuing growth defects in a mouse model.[39]

Conclusion

The GRASP proteins are important components in maintaining the integrity of the Golgi apparatus. In this capacity, they sustain the ribbon-like membrane network, which is important for Golgi processing and secretion kinetics, and they contribute to Golgi stacking. GRASPs are regulated during the cell cycle, in development, and in disease, which has a significant impact on the Golgi membrane network and the processes that depend on this network. Additionally, GRASPs play an important, if less understood, role in specialized secretion events. In several of these cases, there is a strong indication that PDZ interactions occur between the GRASP and the secretory cargo. However, the purpose of these interactions remains an exciting and potentially clinically significant area for future work.

Disclosure

The authors report no conflicts of interest in this work.

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