GBF/Gea mutant with a single substitution sustains fungal growth in the absence of BIG/Sec7

Herbert N. Arst Jr. a,b,1, Miguel Hernandez-Gonzalez b,1, Miguel A. Peñalva b, Areti Pantazopoulou b,*

Section of Microbiology, Department of Medicine, Imperial College London, London SW7 2AZ, United Kingdom
Centro de Investigaciones Biologicas, CSIC, Madrid 28040, Spain

1 H.N. Arst and M. Hernandez-Gonzalez contributed equally to this work.

ABSTRACT
Golgi Arf1-guanine nucleotide exchange factors (GEFs) belong to two subfamilies: GBF/Gea and BIG/Sec7. Both are conserved across eukaryotes, but the physiological role of each is not well understood. Aspergillus nidulans has a single member of the early Golgi GBF/Gea-subfamily, geaA, and the late Golgi BIG/Sec7-subfamily, hypB. Both geaA and hypB are essential. hypB is conditionally blocks secretion. We sought extragenic hypB suppressors and obtained geaA1. geaA1 results in Tyr1022Cys within a conserved GBF/Gea-specific S(Y/W/F)(L/I) motif in GeaA. This mutation alters GeaA localization. Remarkably, geaA1 suppresses hypBΔ, indicating that a single mutant Golgi Arf1-GEF suffices for growth.

1. Introduction
ADP-ribosylation-factor (Arf) GTPases regulate membrane traffic by organizing vesicle budding. Their activation depends on the GEF (guanine nucleotide exchange factor) domain of their N-terminal myristoylated amphipathic helix and facilitating recruitment of specialized effectors [1,2].

Arf1, an essential Golgi regulator, is activated by the GBF/Gea and the BIG/Sec7 GEF subfamilies [1]. These two subfamilies comprise related proteins sharing the highly conserved catalytic Sec7 domain (Sec7d) and five conserved regions denoted DCB, HUS, HDS1, -2 and -3 domains [3–5]. GBF/Gea and BIG/Sec7 are both considered essential for Golgi function in Saccharomyces cerevisiae, Dro sophila melanogaster and mammalian cells [6–14]. Moreover, they are the only Arf-GEF subfamilies common to all eukaryotes [3]. Thus, although both GBF/Gea and BIG/Sec7 activate Arf1 at the Golgi, they have some non-overlapping essential functions. Apart from triggering Arf1-mediated effector recruitment, Arf1-GEFs each engage specific protein interactors, resulting in variable outcomes from the activation of a single Arf. This possibly underlies non-overlapping Arf1-GEF functions. For example, Gea1p/GBF1 interacts with Sec21p/γ-COP [15].

Arf GEFs are peripheral membrane proteins; their membrane recruitment is tightly regulated to ensure precise spatiotemporal responses [16–19]. However, the mechanistic bases of this regulation are incompletely understood [reviewed in [20]]. It is widely accepted that the BIG/Sec7 subfamily members act at the late/trans-Golgi, while the GBF/Gea subfamily members act at the early/cis–Golgi [1]. An HDS1-mediated interaction of S. cerevisiae Sec7p with membrane-bound Arf1-GTP contributes to Sec7p recruitment to, and activation at late Golgi compartments via a positive feedback loop [21]. The Arf-like protein Arl1 is necessary for recruitment of the Sec7-orthologues BIG1/2 at the mammalian trans-Golgi [22]. Mammalian GBF1 is a Rab1 effector [23]. Its cis-Golgi recruitment occurs in response to an increase in membrane-associated Arf-GDP [24]. The HDS1 domain of its S. cerevisiae homologue Gea1p was implicated in lipid droplet binding and Golgi recruitment [25] and in interaction with the early Golgi resident Gm1p [26]. Coincidence detection might additionally link the above observations and determine the Arf1-GEF localizations at the Golgi.

* Corresponding author at: Centro de Investigaciones Biologicas, CSIC, Biologfa Celular y Molecular, Lab.247, Ramiro de Maeztu 9, 28040 Madrid, Spain. Fax: +34 91 536 04 32.
E-mail address: apantazopoulou@cib.csic.es (A. Pantazopoulou).
1 H.N. Arst and M. Hernandez-Gonzalez contributed equally to this work.

Article info
Received 24 July 2014
Revised 7 November 2014
Accepted 10 November 2014
Available online 20 November 2014
Edited by Felix Wieland

Keywords:
Golgi Arf1-GEFs
GBF/Gea-subfamily
BIG/Sec7-subfamily
Fungal secretion

Article history:
Available online 20 November 2014
Accepted 10 November 2014
Revised 7 November 2014
Received 24 July 2014

http://dx.doi.org/10.1016/j.febslet.2014.11.014
0014-5793/© 2014 The Authors. Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/3.0/).
2. Materials and methods

2.1. Deletion of hypB

hypB is AN6709 (http://www.aspgd.org/). We constructed by fusion PCR (primers 14–19, Supplementary Table II) [27,28], a cassette (Fig. 1) for substituting the hypB ORF with Aspergillus fumigatus pyrG in wt (MAD1739-List of strains in Supplementary Table I) and gea1 backgrounds (MAD5107). The cassette was cloned in pGEM (plasmid p2001) and checked by sequencing. A p2001 linearized (Ncol/NsiI) fragment was used in transformations for gene replacement. We assessed the lethality of hypB deletion using the heterokaryon rescue [29]. Diploids, heterokaryons and homokaryotic mini-colonies (hypBΔ) or non-sporulating colonies (hypBΔ gea1) of transformants were genotyped by PCR (primers 14 and 19) and/or by Southern blots.

2.2. Ultraviolet light (UV) induced mutagenesis and molecular characterization of suppressors

40 UV-induced hypB5 suppressors were selected in MAD3574 for growth at 42 °C. In all but one case, reversion occurred within hypB (Table I). Meiotic crosses verified that this suppressor mutation (suA1hypB5) is extragenic. Haploidization analysis [30] localized the suppressor mutation to chromosome VIII. We meiotically mapped suA1hypB5 to a position between nudA and nirA (in the process of mapping suA1hypB5, we identified the mutational lesion of se15 as a frameshift in trxA, see Supplementary Materials and Methods). AN0112, encoding GeaA, was identified as the most likely candidate in this interval and was sequenced (primers 20–27).

2.3. Reconstruction of the hypB5 gea1 strain by transformation

To confirm that the suppression phenotype in suA1hypB5 is indeed due to the mutation identified in gea1 (gea1-1), we PCR-amplified (from MAD4041 gDNA using primers 22 and 23) an ~1.5 kb region of gea1 carrying the A3065G substitution (approximately in the middle of the fragment) and used this molecule to transform hypB5 strain MAD3574. We directly selected transformants for growth at 42 °C, the restrictive temperature for hypB5, and verified by sequencing that all transformants contained gea1-1. This procedure yielded MAD4836.

2.4. In silico analyses

Sequences used for in silico analyses that detected the GBF/Gea-subfamily specific motif were identified by Blast using as queries the S. cerevisiae Gea1p (GenBank CAAB9558.1)/Sec7p (NCBI NP_010454.3), Aspergillus nidulans GeaA (AN0112)/HypB (AN6709) or Homo sapiens GBF1 (GenBank AA17683.1)/BIG1 (NCBI NP_006412.2). Alignments used T-Coffee (http://tcoffee.crg.cat/apps/tcoffee/do:regular), while their visualization and editing were performed with GeneDoc.

2.5. GFP-tagging and microscopy

Using PCR (primers 30 through 39), we fused part of the gea1 ORF (starting downstream the nucleotide that is mutated in gea1-1) to gfp in frame, the 3’UTR of gea1 and pyrG of A. fumigatus (Fig. 3).
This fragment was cloned in pGEM generating p2198 and was sequenced to verify the absence of mutations. p2198 was linearized (BglII/SpeI) to transform MAD1739 for obtaining geaA::gfp or MAD5107 for geaA::gfp. Transformants were analyzed by Southern blot to verify in locus integration.

In vivo microscopy used an inverted Leica DMi6000B microscope [optics/procedures as previously described in [31]]. Deconvolution was done with Huygens Professional (www.svi.nl). Further image processing was with MetaMorph (Molecular Devices). FM4-64 staining was as previously described [32].

Further image processing was with MetaMorph (Molecular Devices). FM4-64 staining was as previously described [32].

Co-localization was studied with Li’s Intensity Correlation Analysis [33] and the Pearson’s correlation coefficient using the JACoP plugin [34] of ImageJ (http://imagej.nih.gov/ij/). Maximal intensity projections of 3-plane deconvolved z-stacks (total width = 600 nm) were used. Regions for co-localization assays were selected as described in [31]. Due to the difference in intensities of the channels, image stacks in each channel were acquired consecutively using different exposure times. Co-localization was nevertheless possible because low motility of Golgi cisternae makes the time required for shifting between the two channels irrelevant. Statistical analyses of correlation coefficients was based on [35] and used the GraphPad Prism 6 (www.graphpad.com).

### Table 1

| Strain       | HypB amino acid sequencea |
|--------------|---------------------------|
| hypB         | A881P                     |
| Revertants   |                           |
| Revertant type | Times obtained |
| True revertant | 1                       |
| First site S | 19                       |
| First site T | 12                       |
| First site T and second site | 1                |
| Second site  | 2                        |
| Second site  | 1                        |
| Second site  | 1                        |
| Extragenic (hypB geaA) | 1             |
| Total = 40                                           |

a Changes in comparison with the wild type strain MAD2. Only the nucleotide sequence coding for F825 to D961 of HypB was determined (amplified with primers 1 and 2), except in the case of the “extragenic” suppressor, where the whole hypB was sequenced (primers 1 to 13 and 20) and found identical to hypB parent.

### 3. Results and discussion

The A. nidulans genome codes for a single member of each of the two Arf-GEF subfamilies. AN0112 encodes the GBF/Gea homologue, GeaA. HypB (encoded by AN6709, www.aspgd.org) is the Big/Sec7 homologue [36], co-localizing with the Arf1- and Ptn4p-binding, late-Golgi marker mRFP-PH (37,38). HypB/ mRFP-PH (38)-containing cisternae are resolvable from Sed5-containing early Golgi cisternae in the unstacked A. nidulans Golgi [39,40]. Late Golgi cisternae are transient and mature to RabERab11 post-Golgi carriers [41].

Fungal life is crucially dependent on exocytosis, required to maintain the cell wall. The hypBΔ null mutation severely impairs hyphal growth [36]. Using heterokaryon rescue [29] (Fig. 1A), we found that hypBΔ spores give rise to aconidial microcolonies on solid medium at 30 and 37 °C, but they do not grow at 42 °C (Fig. 1A and B). We conclude that hypBΔ is virtually lethal. Spores carrying the geaA null mutation are also inviable (Supplementary Fig. 1) and, thus, both HypB and GeaA are essential for fungal growth.

hypBΔ is a ts mutation [42,43], resulting in Ala881Pro in the catalytic Sec7d [36] (Fig. 2A). hypBΔ strains grow well at 30 °C but form aconidial microcolonies at 42 °C (Fig. 1B). Ala881 lying in a conserved region of the Sec7d α-helix F contributes to a hydrophobic network situated across from the catalytic hydrophobic groove that contacts Arf1 [44]. By interfering (putatively) with the folding of α-helix F, Ala881Pro might decrease HypB stability at elevated temperatures. Shifting hypBΔ cells from 28 °C to 37 °C results in cessation of apical extension and mislocalization of exocytic-carriers from an apical crescent to intracellular structures [31,41,45], showing that exocytosis is prevented.

To determine whether the essential late Golgi function of HypB for secretion can be bypassed, we sought suppressors of hypBΔ at 42 °C. Out of 40 strains able to grow at 42 °C, one extragenic mutation, suA1hypBΔ, was obtained (Table 1). Suppression of hypBΔ is partial (Fig. 1B) and suA1hypBΔ itself results in constricted growth (Fig. 1B) and is dominant in diploids. Genetic analyses localized it between AN0118 (nudA) and AN0098 (nirA). The nudA to nirA 75 kb-interval contains 24 autocalled genes including AN0112 encoding the early Golgi Arf1-GEF homologue, GeaA. Sequencing of AN0112 in suA1hypBΔ revealed the presence of a missense A3065G mutation (geaA1) resulting in Tyr1022Cys (GeaA1). Reconstruction by transformation of the geaA1 mutation in a hypBΔ strain showed that geaA1 suppresses hypBΔ5x (i.e., geaA1 is indeed suA1hypBΔ) (Fig. 1B).

To determine whether geaA1 also suppresses hypBΔ, we deleted hypB in a geaA1 strain and, in contrast to the heterokaryotransformants obtained when hypB was deleted in the wild type background (Fig. 1A), we recovered homokaryotic hypBΔ geaA1 double mutants, showing that HypB is dispensable in the geaA1 genetic background (Fig. 1B).

GeaA-Tyr1022 lies between the HDS1 and HDS2 domains (Fig. 2A and B) [3,4]. Using fungal GBF/Gea and Big/Sec7 homologues in multiple alignments, we found that Tyr1022 lies in a previously unidentified Gea-specific motif that makes Gea-specific functions dispensable. Members of the GBF/Gea subfamily, although predominating at the early Golgi [4], have been recently reported to localize also at the trans-Golgi and TGN [46,47]. An attractive hypothesis was that Tyr1022 shifts GeaA localization towards the late Golgi, perhaps by reducing its affinity for an early Golgi receptor, and that this suffices to bypass HypB partially.

We investigated GeaA localization, replacing the endogenous gea4 coding region by the gea4::gfp fusion allele (Fig. 3A). GeaA-GFP is functional, as shown by almost wild type
growth of a strain carrying solely the geaA::gfp allele (Fig. 3B). GeaA-GFP labels punctate cytosolic structures resembling in shape and polarization towards the tip the Golgi cisternae (Fig. 3C). Upon brefeldin A (BFA) treatment triggering the collapse of the A. nidulans Golgi network into large aggregates [38], GeaA-GFP structures collapse into large aggregates (Fig. 3E). We constructed strains simultaneously expressing GeaA-GFP and the early Golgi marker mCherry-Sed5 [38]. GeaA-GFP structures collapse into large aggregates (Fig. 3E). We constructed strains simultaneously expressing GeaA-GFP and the early Golgi marker mCherry-Sed5 [38]. GeaA-GFP structures collapse into large aggregates (Fig. 3E).

Fig. 2. GeaA1-Y1022C substitution lies in a region between HDS1 and HDS2 and alters a conserved GBF/Gea-specific motif. (A) Upper panel: scheme of HypB sequence homology domains [named DCB, HUS, Sec7, HDS1–HDS4 [3–4], identified by T-coffee alignment with yeast and mammalian homologues sequences]. HypB5 (A881P) affects a conserved amino acid residue within the catalytic Sec7d. Lower panel: scheme of GeaA sequence homology domains. GeaA-Y1022C lies between HDS1 and HDS2. (B) Summary of multiple alignments of GBF/Gea homologues from Opisthokonta and Arabidopsis thaliana in the region between HDS1 and HDS2, where GeaA1-Y1022C lies (sequences displayed are from: A. nidulans XP_657716, Saccharomyces cerevisiae CAA89558, Pichia pastoris XP_002495063, Ustilago maydis XP_757309, Rhizopus delemar EIE78027, Mus musculus BAD32197, Homo sapiens NP_004184, Danio rerio XP_694714, Strongylocentrotus purpuratus XP_003728128, Caenorhabditis elegans NP_001255140, Drosophila melanogaster NP_610761, A. thaliana NP_198766). We looked for robust alignment of HDS1 and 2. Then we inspected and manually adjusted the less conserved region between HDS1 and HDS2. 11 sequences of Aspergillus GBF/Gea invariably contain serine, tyrosine (Y1022 in GeaA), leucine (SYL) in the region between HDS1 and 2. In 27 further fungal sequences, including basidiomycetes, zygomycetes, ascomycetes, the motif is SY (L/I/V), with few exceptions (P. pastoris: SFM, Candida albicans: SFL). >30 sequences of vertebrates (including mammals, zebrafish, xenopus) contain a SFSVLW sequence, corresponding to a duplication of the motif. Echinodermata have SYF. We conclude that a serine-aromatic-hydrophobic aminoacid motif, SFLF (yellow box), is conserved in GBF/Gea proteins in Opisthokonta. The A. thaliana GBF-subfamily member GLN1, functioning at the Golgi [53], possesses SFI between HDS1 and HDS2. On the contrary, among 30 BIG/Sec7 subfamily fungal members tested, only S. cerevisiae Sec7p and the Ashbya gossypii and Candida albicans Sec7 homologues contain SFF, a GBF/Gea-motif related sequence, in the region between HDS1 and HDS2 (no SFF motif variant is found in any GBF/Gea member), while no motif was found in BIG members of vertebrates/eucaryotes.
To assess the localization of GeaA1-GFP in a hypB5 background, we co-cultivated a geaA1::gfp hypB5 double mutant and a hypB5 single mutant. Double mutants are easily identifiable because of their green fluorescence, but also because at 28°C geaA1::gfp hypB5 hyphae are thicker than hypB5 hyphae. After a temperature shift to 37°C, growth of hypB5 cells ceased as previously described [31], whereas geaA1::gfp hypB5 strains continued to grow, confirming that geaA1::gfp suppresses hypB5 in these conditions. Importantly, GeaA1::GFP apical localization is maintained in the double mutant background at 37°C, consistent with the possibility that this localization is involved in suppression.

It is largely unknown why two Arf1-GEF subfamilies, asymmetrically located in the Golgi, are conserved among eukaryotes. Our finding that an Arf1-GEF mutation bypasses this asymmetry, rendering a single Arf1-GEF capable of maintaining growth, is unprecedented. GeaA1 partially bypasses HypB. Moreover, geaA1 is hypomorphic (or GeaA1 is somewhat deleterious, as geaA1 single mutants grow less than wild type). This implies that acquisition of HypB-bypassing capability by GeaA1 occurs at the expense of its physiological role. We have demonstrated that Tyr1022Cys induces a shift in the localization of GeaA1 at the expense of its early Golgi localization. This change in localization appears to be a forward displacement within the secretory pathway, as GeaA1 is, in part, redistributed towards the apical plasma membrane, where exocytic carriers are preferentially delivered. This localization shift might reflect the mutationaly altered Golgi dynamics.

Fig. 3. Intracellular distribution of GeaA-GFP and GeaA1-GFP. (A) C-terminal tagging of geaA or geaA1 with gfp was achieved by in loco integration by transformation of a linear cassette containing part of the geaA open reading frame fused to gfp, the geaA 3'UTR and the A. fumigatus pyrG as a selection marker, resulting in strains carrying a single gfp-tagged copy of the geaA or geaA1 allele. (B) Growth test showing that GFP tagged GeaA and GeaA1 are functional. Note that GeaA1-GFP suppresses hypB5 thermosensitivity, like the untagged allele. (C) Maximal intensity projections of deconvolved z-stacks. GeaA-GFP localizes at Golgi cisternae. GeaA1-GFP, although still localizing at Golgi cisternae, also labels an apical crescent and an apex-associated accumulation. The apical localization is maintained in the hypB5 background after shift to 37°C for 40 min (n: nucleus, bar: 3 μm). (D) Fluorescence intensity profiles across the hyphae in (C) (n: position of the nucleus). Note the shift towards the apex displayed by GeaA1-GFP, compared to GeaA-GFP. (E) Hyphae treated with 200 μg/ml BFA for the indicated time, a treatment that provokes the collapse of the Golgi into large aggregates. Note that in GeaA1-GFP some labeling of the apical crescent is still visible in these conditions. (F) Staining of the plasma membrane with FM4-64 in a GeaA1-GFP-expressing strain shows that GeaA1 in the apical crescent is at or very closely associated with the plasma membrane.
and/or Golgi exit, resulting in re-routing a portion of GEA1 towards the plasma membrane. Alternatively, the GEA1 localization shift might reflect a direct role of the Ser-X-U motif on GEA localization, in which case the change in localization per se would effect the suppression, perhaps by GEA1 acquiring function at its new apical locale. In this case, one highly speculative possibility is that GEA1 reorganizes the exocytic pathway in such a way that the Golgi is partially bypassed. Reorganization of the secretory pathway by manipulation of the early Golgi Arf1-GEF has precedents in intracellular membrane remodeling via GFB1 function alteration by RNA viruses [51]. However, despite its minor localization at the late Golgi, we cannot discard the possibility that GEA1 would have gained function at this compartment. According to this hypothetical possibility, GEA1 would be capable of performing HypB essential functions at the late Golgi and Arf-GEFs would be, to some extent, interchangeable.

At present we can only speculate on the mechanism by which Tyr1022Cys shifts GEA localization. The substitution might impair a Ser-X-U motif-mediated interaction of GEA with a ‘recycling’ factor/adaptor hypothetically required to restore GEA localization to the early Golgi during cisternal maturation. This implies that if GEA1 is inefficiently recycled retrogradely, it must be rapidly

---

**Fig. 4.** Intra-Golgi distribution of GEA-GFP and GEA1-GFP. Maximal intensity projections of deconvolved z-stacks, using strains co-expressing GEA-GFP and the early Golgi marker mCherry-SedV (left panel) or the late Golgi marker mRFP-PHOSBP (right). Note the high degree of overlap (yellow color in the “merge” image) between GEA-GFP and mCherry-SedV, contrasting the low overlap of GEA-GFP and mRFP-PHOSBP. Coincidence in shape of fluorescent structures indicated true co-localization (examples included in the green and red boxes). Bar = 2 μm. (B) As in (A), but with strains expressing mutant GEA1-GFP. (C) Li’s green channel intensity correlation analysis of the indicated pair of markers. The analysis was done for a region of the cells displayed in (A and B). The y-value reflects the intensity distribution in the GFP channel. The x-value depends on co-variance of the two channels. The cloud of points towards the right of the y axis (positive values) reflects positive correlation of the two channels (co-localization). The horizontal dotted green line intersects the y axis at the mean intensity value. Note that, in the GEA/SedV graph, the majority of the “above the average intensity” pixels form a cloud on the right of the y axis, indicating localization of GEA to the early Golgi. The opposite is true for GEA/PHOSBP, where high intensity pixels have negative x-values, indicating inverted correlation (ICQ: the intensity correlation quotient, ICQ approaches 0.5 in complete co-localization or 0.0 in random staining; Ai: gfp pixel intensity, a: gfp average pixel intensity, Bi: rfp pixel intensity, b: rfp average pixel intensity). (D) Graph showing the dispersion and the mean value of the Pearson’s correlation coefficient (PCC) calculated from co-localization analysis in (n) examples for each pair of markers. Blue bars indicate the standard error of the mean. The difference between the mean PCC of the pair GEA/SedV versus the mean PCC of GEA/PHOSBP is large and very significant, showing that GEA-GFP mainly localizes at the early Golgi. The difference in PCC between GEA1/SedV versus GEA1/PHOSBP is also large, suggesting that the mutant GEA1-GFP also predominates at the early Golgi. However, a drop in the mean PCC of GEA1/SedV compared to GEA/SedV suggests that localization of mutant GEA1 at the early Golgi is reduced. This correlates with GEA1 localization shift to the apical crescent in (B). A small increase in PCC of GEA1/PHOSBP versus GEA/PHOSBP is also observed, although the correlation between the two markers is still weak in the mutant GEA1.
sorted into exocytic carriers, as we have only detected a small increase in Gea1 residence at the late Golgi. A slightly modified alternative is that the motif might bind one key component of a coincidence detection module restricting GeaA localization to the early Golgi. Loss of interaction with this component could make Gea1 localization largely dependent on a second component with broader distribution. It is notable that a GBF1 HD51 and HD52 module containing the Ser-Ω-Φ motif has been shown to redirect GBF1 to the leading cell edge through phosphoinositide binding \cite{52}. Similarly, the Gea1 mutation might modify a lipid-recognizing module, such that loss of affinity for a Golgi-specific lipid would be accompanied by gain of affinity for an apical crescent-specific lipid, resulting in the shift in Gea1 localization. In any case, future studies addressing how the Gea1 substitution has such an impact on its localization constitute a promising tool to understand the mechanisms by which Gea specifically localizes to the Golgi.

Acknowledgements

We thank Elena Reoyo for excellent technical assistance. This work was supported by MINECO-Spain (grant BIO2012-30965) and Comunidad de Madrid (grant S2010/BMD-2144) to MAP and by Wellcome Trust-United Kingdom Grant 084660/Z/08/Z to H.N.A. and Joan Tilburn. M.H.-G. is holder of an FPI fellowship (Spanish Government).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2014.11.014.

References

[1] Gillingham, A.K. and Munro, S. (2007) The small G proteins of the Arf family and their regulators. Annu. Rev. Cell Dev. Biol. 23, 579–611.
[2] Donaldson, J.G. and Jackson, C.L. (2011) ARF family G proteins and their regulators: roles in membrane transport, development and disease. Nat. Rev. Mol. Cell Biol. 12 (6), 362–375.
[3] Mouratov, B. et al. (2005) The domain architecture of large guanine nucleotide exchange factors for the small GTP-binding protein Arf. BMC Genomics 6, 20.
[4] Bui, QT. Golinielli-Cohen, M.P. and Jackson, C.L. (2009) Large Arf guanine nucleotide exchange factors: evolution, domain structure, and roles in membrane trafficking and human disease. Mol. Genet. Genomics 282 (4), 295–313.
[5] Cox, R. et al. (2004) Phylogenetic analysis of Sec7-domain-containing Arf nucleotide exchange factors. Mol. Biol. Cell 15 (4), 1487–1505.
[6] Spang, A. et al. (2001) The ADP-ribosylation factor-nucleotide exchange factors Gea1p and Gea2p have overlapping, but not redundant functions in retrograde transport from the Golgi to the endoplasmic reticulum. Mol. Biol. Cell 12 (4), 1035–1045.
[7] Citerio, C. et al. (2008) Unfolded protein response and cell death after depletion of brefeldin A-inhibited guanine nucleotide-exchange protein GBF1. Proc. Natl. Acad. Sci. U.S.A. 105 (8), 2877–2882.
[8] Ishizaki, R. et al. (2008) Redundant roles of BIG2 and BIG1, guanine-nucleotide exchange factors for ADP-ribosylation factors in membrane traffic between the trans-Golgi network and endosomes. Mol. Biol. Cell 19 (6), 2650–2660.
[9] Grizm, P. et al. (2010) Early embryonic lethality in gene trap mice with disruption of the Arfgef2 gene. Int. J. Dev. Biol. 54 (8–9), 1259–1266.
[10] Achstetter, T. et al. (1988) SEC7 encodes an unusual, high molecular weight protein required for membrane traffic from the yeast Golgi apparatus. J. Biol. Chem. 263 (24), 11711–11717.
[11] Saen, J.B. et al. (2009) Golgicide A reveals essential roles for GBF1 in Golgi assembly and function. Nat. Chem. Biol. 5 (3), 157–165.
[12] Armbruster, K. and Lusch, S. (2012) The drosophila Sec7 domain guanine nucleotide exchange factor protein Gartenzwerg localizes to the cis-Golgi and is essential for epithelial tube expansion. J. Cell Sci. 125 (Pt 5), 1318–1328.
[13] Mummery-Widmer, J.L. et al. (2009) Genome-wide analysis of notch signaling in drosophila by transgenic RNAi. Nature 458 (7241), 987–992.
[14] Peyroche, A. et al. (2001) The ARF exchange factors Gea1p and Gea2p regulate Golgi structure and function in yeast. J. Cell Sci. 114 (Pt 12), 2241–2253.
[15] Deng, Y. et al. (2009) A COPII coat subunit interacts directly with an early-Golgi localized ARF exchange factor. EMBO Rep. 10 (1), 58–64.
[16] Kahn, R.A. (2009) Toward a model for Arf GTPases as regulators of traffic at the Golgi. FEBS Lett. 583 (23), 3872–3879.
[17] Szul, T. et al. (2005) Dissection of membrane dynamics of the ARF-guanine nucleotide exchange factor Rab6 in living cells. Traffic 6 (5), 374–385.
[18] Niu, T.K. et al. (2005) Dynamics of GBF1, a brefeldin A-sensitive Arf1 exchange factor at the Golgi. Mol. Biol. Cell 16 (3), 1213–1222.
[19] Chantalat, S. et al. (2013) Targeting of the Arf-GEF GBF1 to lipid droplets and Golgi membranes. J. Cell Sci. 126 (Pt 20), 4794–4805.
[20] Chantalat, S. et al. (2003) A novel Golgi membrane protein is a partner of the ARF exchange factors Gea1p and Gea2p. Mol. Biol. Cell 14 (6), 2357–2371.
[21] Abenza, J.F. et al. (2004) Dose-dependent PCR: A PCR-based molecular tool for gene manipulations in filamentous fungi. Fungal Genet. Biol. 41 (11), 973–981.
[22] Szewczyk, E. et al. (2006) Fusion PCR and gene targeting in Aspergillus nidulans. Nat. Protoc. 1 (6), 3111–3120.
[23] Guzman, A.H., Oakley, B.R. and Osmani, S.A. (2006) Identification and analysis of essential Aspergillus nidulans genes using the heterokaryon rescue technique. Nat. Protoc. 1 (5), 2517–2526.
[24] Boit, S. and Cordelieres, P.F. (2006) A guided tour into subcellular colocalization analysis in light microscopy. J. Microsc. 224 (Pt 3), 4070–4081.
[25] Pantazopoulou, A. and Penalva, M.A. (2014) Characterization of Aspergillus nidulans Rab1/Rab8/Rab11 exocytic post-Golgi carriers visualized in vivo. Mol. Cell. Biol. 25 (16), 2428–2433.
[26] Harris, S.D., Morrell, J.L. and Hamer, J.E. (1994) Identification and analysis of the Sec7 Arf-GEF to direct traffic at the trans-Golgi network. Dev. Cell 30 (6), 4335–4347.
[27] Pantazopoulou, A. and Penalva, M.A. (2013) Acute inactivation of the Aspergillus nidulans Golgi membrane fusion machinery: correlation of apical extension arrest and tip swelling with cisternal disorganization. Cell Mol Life Sci 71 (18), 3419–3438.
[28] Abenza, J.F. et al. (2009) Long-distance movement of Aspergillus nidulans early endosomes on microtubule tracks. Traffic 10 (1), 57–75.
[29] Pinar, M. et al. (2013) Statistical tests for measures of colocalization in biological microscopy. J. Microsc. 252 (3), 295–302.
[30] Yang, Y. et al. (2008) Aspergillus nidulans hyp5 encodes a Sec7-domain protein important for hypoxia tolerance and rapamycin sensitivity. Fungal Genet. Biol. 45 (5), 749–759.
[31] Penalva, M.A. (2009) Colocalization and dynamics of the Aspergillus nidulans Golgi during apical extension and mitosis. Mol. Biol. Cell 20 (20), 4315–4327.
[32] Pantazopoulou, A. and Penalva, M.A. (2014) Maturation of late Golgi cisternae into Rab11-positive compartments: evidence for distinct functions in protein traffic. J. Cell Sci. 127 (Pt 2), 354–364.
[33] Bouvet, S. et al. (2013) Targeting of the Arf-GEF GBF1 to lipid droplets and Golgi membranes. J. Cell Sci. 126 (Pt 20), 4794–4805.
[34] Christis, C. and Munro, S. (2012) The small G protein Arl1 directs the trans-Golgi-specific targeting of the Arf exchange factors BIG1 and BIG2. J. Cell Biol. 196 (3), 327–335.
[35] Monetta, P. et al. (2007) Rab11b interacts with GBF1 and modulates both ARF1 dynamics and COPII association. Mol. Biol. Cell 18 (7), 2400–2410.
[36] Quilty, D. et al. (2014) Arf activation at the Golgi is modulated by feed-forward stimulation of the exchange factor GBF1. J. Cell Sci. 127 (Pt 2), 354–364.
[37] Citerio, C. et al. (2008) Unfolded protein response and cell death after depletion of brefeldin A-inhibited guanine nucleotide-exchange protein GBF1. Proc. Natl. Acad. Sci. U.S.A. 105 (8), 2877–2882.
[49] Taheri-Talesh, N. et al. (2008) The tip growth apparatus of *Aspergillus nidulans*. Mol. Biol. Cell 19 (4), 1439–1449.

[50] Penalva, M.A. (2005) Tracing the endocytic pathway of *Aspergillus nidulans* with FM4-64. Fungal Genet. Biol. 42 (12), 963–975.

[51] Hsu, N.Y. et al. (2010) Viral reorganization of the secretory pathway generates distinct organelles for RNA replication. Cell 141 (5), 799–811.

[52] Mazaki, Y., Nishimura, Y. and Sabe, H. (2012) GBF1 bears a novel phosphatidylinositol-phosphate binding module, BP3K, to link PI3Kgamma activity with Arf1 activation involved in GPCR-mediated neutrophil chemotaxis and superoxide production. Mol. Biol. Cell 23 (13), 2457–2467.

[53] Teh, O.K. and Moore, I. (2007) An ARF-GEF acting at the Golgi and in selective endocytosis in polarized plant cells. Nature 448 (7152), 493–496.