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

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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*5 conditionally blocks secretion. We sought extragenic *hypB*5 suppressors and obtained *geaA*1. *geaA*1 results in Tyr1022Cys within a conserved GBF/Gea-specific S(Y/W/F)(L/I) motif in GeaA. This mutation alters GeaA localization. Remarkably, *geaA*1 suppresses *hypB*Δ, 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)-catalyzed exchange of GTP for GDP, that leads to a conformational switch stabilizing membrane insertion 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*, *Drosophila 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 Gmh1p [26]. Coincidence detection might additionally link the above observations and determine the Arf1-GEFs localizations at the Golgi.

Abbreviations: Arf GTPases, ADP-ribosylation-factor GTPases; BFA, brefeldin A; BIG, brefeldin A-inhibited guanine nucleotide-exchange; GBF, Golgi-specific brefeldin A resistance factor; GEF, Guanine nucleotide exchange factor; PH^{OSBP}, pleckstrin homology domain of the oxysterol binding protein; Sec7d, Sec7 domain

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How different sets of interactors and/or localization determinants result in essentially distinct functions of the two Golgi Arf1-GEF subfamilies and how these functions serve cargo passage through the Golgi are unanswered questions.

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 *geaA1* backgrounds (MAD5107). The cassette was cloned in pGEM (plasmid p2001) and checked by sequencing. A p2001 linearized (NcoI/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 (*hypB*Δ) or non-sporulating colonies (*hypB*Δ *geaA1*) 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 *hypB*5 suppressors were selected in MAD3574 for growth at 42 °C. In all but one case, reversion occurred within *hypB* (Table 1). Meiotic crosses verified that this suppressor mutation (*suA*1*hypB*5) is extragenic. Haploidization analysis [30] localized the suppressor mutation to chromosome VIII. We meiotically mapped *suA*1*hypB*5 to a position between *nudA* and *nirA* (In the process of mapping *suA*1*hypB*5, we identified the mutational lesion of *sE*15 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 geaA1 strain by transformation

To confirm that the suppression phenotype in *suA*1*hypB*5 is indeed due to the mutation identified in *geaA* (*geaA*1), we PCR-amplified (from MAD4041 gDNA using primers 22 and 23) an ~1.5 kb region of *geaA*1 carrying the A3065G substitution (approximately in the middle of the fragment) and used this molecule to transform *hypB*5 strain MAD3574. We directly selected transformants for growth at 42 °C, the restrictive temperature for *hypB*5, and verified by sequencing that all transformants contained *geaA*1. This procedure yielded MAD4836.

2.4. In silico analyses

Sequences used for in silico analyses that detected the GBF/Gea-specific motif were identified by Blast using as queries the *S. cerevisiae* Gea1p (GenBank CAA89558.1)/Sec7p (NCBI NP_010454.3), *Aspergillus nidulans* GeaA (AN0112)/HypB (AN6709) or *Homo sapiens* GBF1 (GenBank AAI17683.1)/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 *geaA* ORF (starting downstream the nucleotide that is mutated in *geaA*1) to *gfp* in frame, the 3′UTR of *geaA* and *pyrG* of *A. fumigatus* (Fig. 3).

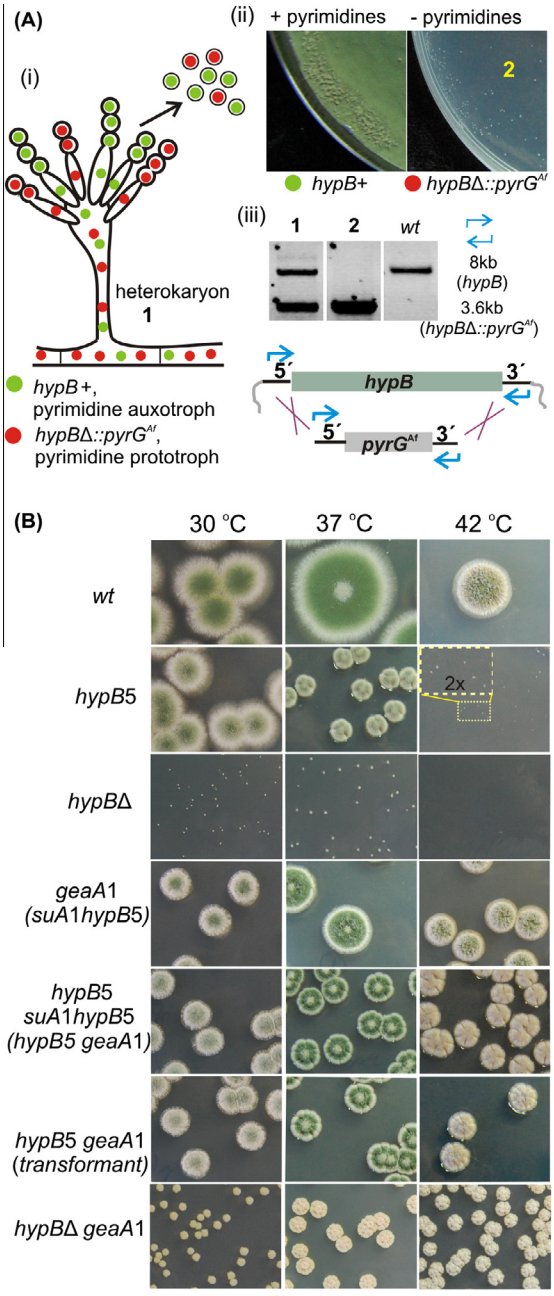

Fig. 1. (A) Deletion of the *hypB* gene encoding the only *A. nidulans* late-Golgi Arf1-GEF is virtually lethal. *hypB*Δ transformants, selected as pyrimidine prototrophs, were heterokaryotic (i), i.e., they carried both transformed (*hypB*Δ:: *pyrG*^{Af}) and wild type (*hypB* + *pyrG89*) nuclei. Spores produced by these heterokaryons are uninucleate. On medium without pyrimidines, untransformed nuclei-containing spores cannot grow due to pyrimidine auxotrophy, which permits testing for growth of the prototroph spores that contain the deletion mutation. *hypB*Δ spores form aconidial microcolonies (ii). PCR genotyping with DNA from the multinuclear primary transformant mycelium (1) yielded amplification bands corresponding to both the wild type *hypB* and the *hypB*Δ::*pyrG*^{Af} deletion cassette (iii), confirming that this was heterokaryotic. In contrast, only the band corresponding to the *hypB*Δ::*pyrG*^{Af} construct was amplified from DNA from the micro-colonies on the selective medium (2), indicating that these are homokaryotic *hypB*Δ::*pyrG*^{Af} colonies, which showed that HypB is virtually essential for *A. nidulans*. (B) GBF/Gea-subfamily mutant GeaA1 bypasses the requirement for the BIG/Sec7-subfamily HypB. Growth at the specified temperatures from spore dilutions of strains: *wt* (wild type, MAD2), *hypB*5 (MAD3574), *hypB*Δ (*hypB*Δ::*pyrG*^{Af} *pyrG*89; *nkuA*Δ::*bar pyroA*4), *geaA*1 (MAD4062), *hypB*5 *suA*1*hypB*5 (MAD4041-obtained by UV mutagenesis), *hypB*5 *geaA*1 transformant (MAD4836-obtained by transformation of a *hypB*5 strain with a DNA fragment carrying the *geaA*1 mutation) and *hypB*Δ *geaA*1 (MAD5130). All photos are at the same magnification (except the one indicated as 2×, this is a 2 times magnification of the yellow rectangular region).