Fungal Genetics and Biology 73 (2014) 69–82

Contents lists available at ScienceDirect

Fungal Genetics and Biology

journal homepage: www.elsevier.com/locate/yfgbi

Mutations in proteins of the Conserved Oligomeric Golgi Complex affect polarity, cell wall structure, and glycosylation in the filamentous fungus *Aspergillus nidulans*

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ARTICLE INFO

Article history:
Received 25 June 2014
Accepted 5 October 2014
Available online 12 October 2014

Keywords:
Polarity maintenance
COG complex
Glycosylation
Cell wall
Aspergillus nidulans

ABSTRACT

We have described two *Aspergillus nidulans* gene mutations, designated *podB1* (polarity defective) and *swoP1* (swollen cell), which cause temperature-sensitive defects during polarization. Mutant strains also displayed unevenness and abnormal thickness of cell walls. Un-polarized or poorly-polarized mutant cells were capable of establishing normal polarity after a shift to a permissive temperature, and mutant hyphae shifted from permissive to restrictive temperature show wall and polarity abnormalities in subsequent growth. The mutated genes (*podB* = AN8226.3; *swoP* = AN7462.3) were identified as homologues of COG2 and COG4, respectively, each predicted to encode a subunit of the multi-protein COG (Conserved Oligomeric Golgi) Complex involved in retrograde vesicle trafficking in the Golgi apparatus. Down-regulation of COG2 or COG4 resulted in abnormal polarization and cell wall staining. The GFP-tagged COG2 and COG4 homologues displayed punctate, Golgi-like localization. Lectin-blotting indicated that protein glycosylation was altered in the mutant strains compared to the wild type. A multicopy expression experiment showed evidence for functional interactions between the homologues COG2 and COG4 as well as between COG2 and COG3. To date, this work is the first regarding a functional role of the COG proteins in the development of a filamentous fungus.

1. Introduction

In the filamentous fungi, growth begins when a spore leaves a state of dormancy and begins to swell isotropically. During swelling, specific sites inside the spore are selected for polarized growth, and information about those locations is communicated to the rest of the cell (Momany, 2002). Next, the production, modification, and transport of cellular materials for new growth at those sites commence. Each selected site of polarity gives rise to a filamentous germ tube that grows apically. Polarity is maintained as the colony forms hyphal branches and eventually a mycelial network. Development of hyphal growth in the filamentous fungus *Aspergillus nidulans*, specifically regarding polarity establishment and maintenance, has been addressed in reviews by Harris and Momany (2004) and Harris et al. (2009).

Numerous mutations in *A. nidulans* have been identified that affect the process of spore swelling, polarity establishment, and polarity maintenance (Harris et al., 1999; Momany et al., 1999; Osherov et al., 2000; Shaw and Upadhyay, 2005; Upadhyay and Shaw, 2006). In several cases, specific genes have been identified, and their products have been associated with specific functions (reviewed by Harris et al., 2009). Among these are proteins involved in cell wall metabolism including PkcA (Ronen et al., 2007), the Rho GTPase RhoA (Guest et al., 2004), and three *O*-mannosyltransferases (Kriangkripipat and Momany, 2009), as well as proteins involved in the vesicle trafficking in Golgi cisternae including the tethering complex TRAPPII (Shi et al., 2004) and the vesicle coat protein COPI (Yang et al., 2008). A recent paper by Pinar et al. (2013) highlighted the connection between polarity maintenance and the Golgi in *A. nidulans*; temperature-sensitive

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http://dx.doi.org/10.1016/j.fgb.2014.10.005
1087-1845/

mutations in the two Golgi proteins, the Rab GTPase RabO and the t-SNARE Syntaxin, involved in retrograde transport between the cis-Golgi and the endoplasmic reticulum, led to a loss of apical extension and disorganization of the Golgi. In a commentary on Pinar et al. (2013), Harris (2013) suggested that this disruption of Golgi cisternae in filamentous fungi may negatively affect the continuous supply of vesicles needed at the polar tip or that key proteins required for polarized growth may lack appropriate glycosylation for correct function.

In this work we describe an *A. nidulans* temperature-sensitive mutation designated *swoP1* (swollen cell), which displays extended isotropic swelling followed by the formation of one or more abnormal germ tubes, and we demonstrate that this mutation lies within the *A. nidulans* orthologue of the *Saccharomyces cerevisiae* gene encoding the COG4 subunit of the Conserved Oligomeric Golgi (COG) Complex. In addition, we present evidence that a separate previously reported polarity-defective mutation *podB1* (Harris et al., 1999) lies within a separate COG subunit orthologue, COG2. Numerous experiments in yeast and animal cells indicate that the COG complex plays an essential role in retrograde transport in the Golgi, and mutations or deletions of some subunits of the COG complex have led to a reduction or absence of cell growth as well as mis-glycosylation of cellular proteins (reviewed by Miller and Ungar, 2012). The current study will present the first data demonstrating a role of members of the COG complex in polarity maintenance, cell wall production and maintenance, and protein glycosylation in a filamentous fungus.

2. Materials and methods

2.1. Strains, media formulations, and basic culturing methods

Strains used in this study are listed in Table 1. Complete medium (CM) consisted of 1% glucose, 0.2% peptone, 0.1% yeast extract, 0.1% casamino acids, 5% nitrate salts, 1% trace elements, 0.1% vitamin mix, and 1.2 mM L-arginine. Minimal media (MM) consisted of 1% glucose, 5% nitrate salts, 1% trace elements, 0.001% thiamine hydrochloride, and 25 ppb biotin. Vitamin mix and nitrate salts were prepared according to the appendix of Kafer (1977), and trace element solution according to Hill and Kafer (2001). When necessary, media were supplemented with 0.1 mg/ml of riboflavin or 10 mM uracil and 5 mM uridine. Solid media contained 1.5% agar. All media were supplemented with 50 mg/ml ampicillin.

2.2. Mutagenesis, mutant screening, and Mendelian methods

The *swoP1* mutant strain was generated by mutagenizing conidia of the *A. nidulans* strain A28 to 50% mortality with 4-nitroquinoline-1-oxide (NQO) as described by Harris et al. (1994). Survivors were screened for aberrant growth morphology when grown at the restrictive temperature of 42 °C. One new mutant strain named RCH-67 showed a swollen cell phenotype at 42 °C; therefore, the mutation was named *swoP1* after a series of previously described swollen cell mutants (swo) (Momany et al., 1999). Strain RCH-67 was crossed with strain A773, which has wild-type growth at 42 °C, using standard genetic methods (Kafer, 1977; Kaminskyj, 2000). Strain R441 (*pyrG89*; *pyroA4 swoP1*) resulted from this cross. Analysis of the resulting R441 progeny showed that the polarity defect segregated in a 1:1 ratio, and the trait is recessive in a diploid created between strains RCH-67 and A773. Diploids constructed between RCH-67 and all *swo* mutant strains identified to date (Lee and Shaw, 2007; Momany et al., 1999; Shaw and Upadhyay, 2005; Upadhyay and Shaw, 2006) confirmed that the RCH-67 mutation is in an independent locus. For this reason we have designated the allele *swoP1*, following the nomenclature recommendations of Clutterbuck and Arst (1995).

The *podB1* mutant strain used in this study was generated previously by Harris et al. (1999) following identical procedures for mutagenesis and allele characterization. However, the gene locus of the mutation was not identified in that study, nor was any cellular level function reported.

Table 1
Aspergillus nidulans strains used in this study.

Strain	Genotype	Source
A28	*pabaA6 biA1*	FGSC[a]
A773	*pyrG89*; *wA3*; *pyroA4*	FGSC
A1145	*pyrG89*; *nkuA*::*argB*; *pyroA4*; *riboB2*	FGSC
A655	*pabaA1*; *acuK248*	FGSC
ASH83	*pabaA6*; *podB1 pyroA4*	Harris et al. (1999)
ASH84	*pyrG89*; *podB1*; *wA2*	Harris et al. (1999)
RCH-67	*pabaA6 biA1*; *swoP1*	This study
R441	*pyrG89*; *pyroA4 swoP1*	This study[b]
R550	*pyrG89*; *pyroA4 swoP1*; [AN7462::*AfpyrG*]	This study[c]
R763	*pyrG89*; *podB1*; *wA2*; [AN8226::*AfpyrG*]	This study[d]
R764	*pyrG89*; *podB1*; *wA2*; [AN4886::*AfpyrG*]	This study[e]
R766	*pyrG89*; *podB1*; *wA2*; [AN7462::*AfpyrG*]	This study[f]
R768	*pyrG89*; *pyroA4 swoP1*; [AN8226::*AfpyrG*]	This study[g]
R785	*pyrG89*; *pyroA4*; *nkuA*::*argB*; *riboB2*; *argB2* + *AfpyrG*::*alcA(p)*::*COG2*	This study[h]
R794	*pyrG89*; *pyroA4*; *nkuA*::*argB*; *riboB2*; *argB2* + *AfpyrG*::*alcA(p)*::*GFP*::*COG2*	This study[h]
R915	*pyrG89*; *pyroA4*; *nkuA*::*argB*; *riboB2*; *argB2*; *AfpyrG:alcA(p)*::*GFP*::*COG2*; *copA*::*mRFP*::*Afribo*	This study[h]
G61	*pyrG89*; *pyroA4*; *nkuA*::*argB*; *riboB2*; *argB2* + *AfpyrG*::*alcA(p)*::*GFP*::*COG4*	This study[h]
G66	*pyrG89*; *pyroA4*; *nkuA*::*argB*; *riboB2*; *argB2* + *AfpyrG*::*alcA(p)*::*COG4*	This study[h]

Genes enclosed by square brackets were introduced as components of non-integrating plasmids.
[a] FGSC = Fungal Genetics Stock Center, University of Missouri, Kansas City, MO, USA (McCluskey, 2003).
[b] Resulting from a cross between A773 and RCH-67.
[c] Resulting from a transformation of R441 with pDL160.
[d] Resulting from a transformation of ASH84 with pSG102.
[e] Resulting from a transformation of ASH84 with pSG103.
[f] Resulting from a transformation of ASH84 with pDL160.
[g] Resulting from a transformation of R441 with pSG102.
[h] A1145 was the transformed recipient strain.