British Mycological Society promoting fungal science

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


Review

# Orchestration of morphogenesis in filamentous fungi: conserved roles for Ras signaling networks

*Jarrod R. FORTWENDEL**

*Department of Microbiology and Immunology, College of Medicine, University of South Alabama, 5851 USA Drive North, MSB 2102, Mobile, AL 36688, AL, USA*

ARTICLE INFO

*Article history:*
Received 14 October 2014
Received in revised form
27 April 2015
Accepted 28 April 2015

*Keywords:*
Fungal development
Fungal pathogenesis
Fungal virulence
Hyphal morphogenesis
Ras
Signal transduction

ABSTRACT

Filamentous fungi undergo complex developmental programs including conidial germination, polarized morphogenesis, and differentiation of sexual and asexual structures. For many fungi, the coordinated completion of development is required for pathogenicity, as specialized morphological structures must be produced by the invading fungus. Ras proteins are highly conserved GTPase signal transducers and function as major regulators of growth and development in eukaryotes. Filamentous fungi typically express two Ras homologs, comprising distinct groups of Ras1-like and Ras2-like proteins based on sequence homology. Recent evidence suggests shared roles for both Ras1 and Ras2 homologs, but also supports the existence of unique functions in the areas of stress response and virulence. This review focuses on the roles played by both Ras protein groups during growth, development, and pathogenicity of a diverse array of filamentous fungi.



## 1. Introduction

The Ras super-family is composed of membrane-associated GTPase proteins serving as major signal transduction elements in eukaryotic cells. Ras proteins act as molecular switches, toggling between active and inactive states, bound to either guanosine triphosphate (GTP) or guanosine diphosphate (GDP), respectively. Ras signaling is activated by interaction with guanosine nucleotide exchange factors (GEFs), a protein–protein interaction that aids Ras in the release of GDP allowing subsequent binding of the more cytoplasmically available GTP. Inactivation is accomplished by interaction of GTP-bound Ras with GTPase activator proteins (GAPs) that accelerate the slow intrinsic GTPase activity characteristic of Ras proteins. Within the Ras super-family is a sub-family of proteins characterized as "prototypical" Ras proteins, displaying high structural homology with mammalian H-ras (Wennerberg *et al.*, 2005). Members of the Ras sub-family have conserved domains required for protein activation, effector interactions and membrane association. The latter is required for a series of post-translational modifications that can promote specific signaling events through compartmentalization of Ras proteins to either the endomembranes of the endoplasmic reticulum (ER) and Golgi or to the plasma membrane (Fig. 1). The localization domains of Ras sub-family proteins include a classic CAAX motif necessary for prenylation, found in all Ras homologs, and typically one of two additional membrane association motifs found in the hypervariable region (HVR): 1) a conserved cysteine

* Tel.: +1 251 460 6681.
E-mail address: jfortwendel@southalabama.edu
http://dx.doi.org/10.1016/j.fbr.2015.04.003
1749-4613/

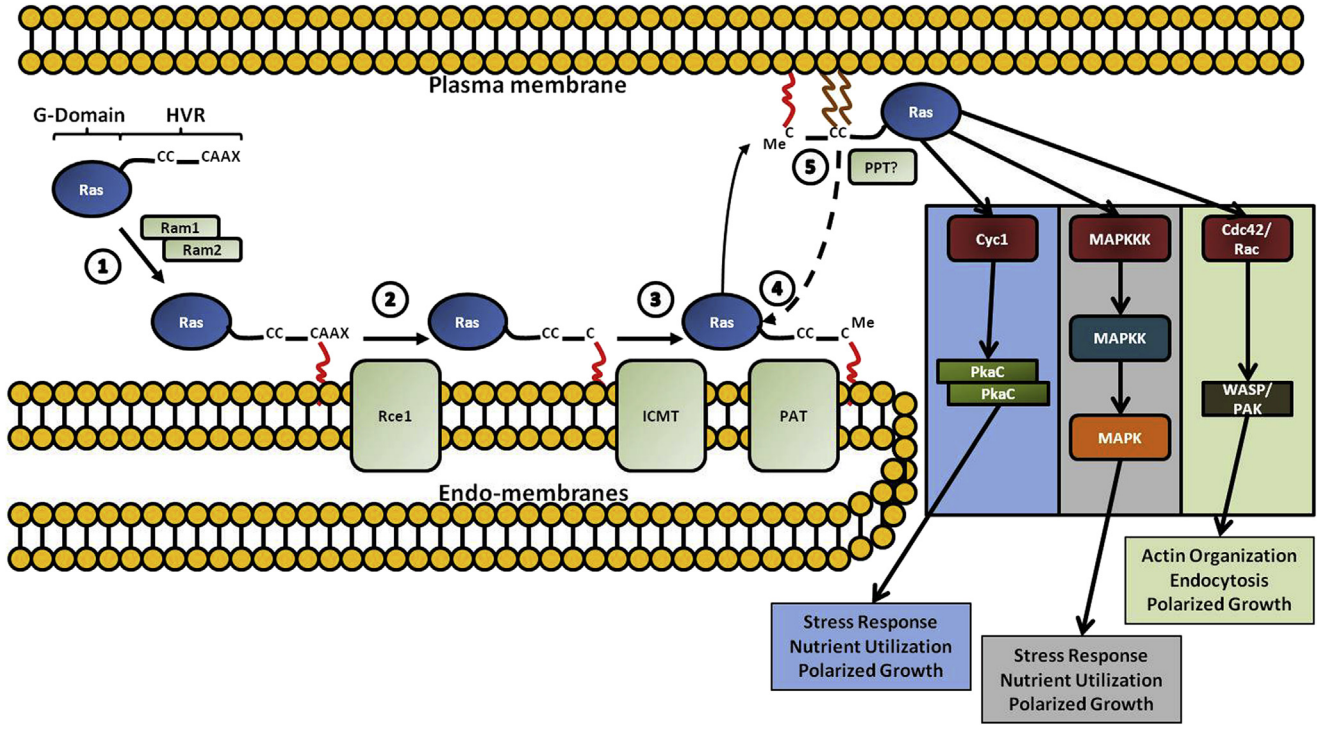


**Fig. 1 – Putative pathways for Ras localization and signaling in fungal organisms. Nascent Ras proteins contain C-terminal "CAAX" motif (C = cysteine, A = aliphatic amino acid, X = any amino acid) that serves as a recognition sequence for a cytoplasmic farnesyltransferase (Ram1/Ram2 in *S. cerevisiae*). Step 1: The farnesyltransferase enzyme modifies the CAAX motif by addition of a farnesyl lipid moiety to the cysteine residue. Step 2: Ras is then further processed by the activity of Ras converting enzyme (Rce1 in *S. cerevisiae*), which catalyzes the proteolytic cleavage of the "–AAX" residues from the farnesylated CAAX motif. Step 3: The farnesylated cysteine is then carboxymethylated by action of an Isoprenylcysteine carboxymethyltransferase (ICMT, Ste14 in *S. cerevisiae*). For Ras1-like proteins to complete transit via the exocytosis machinery of the ER/Golgi complexes and become associated with the plasma membrane (PM), another ER resident protein, a palmitoyltransferase (PAT), must modify the Ras protein through addition of a palmitoyl lipid moiety. Step 4: The palmitoyl modification occurs at a conserved cysteine residue located just upstream of the CAAX motif, within the hypervariable domain. Step 5: In higher eukaryotes, Ras palmitoylation is reversible and PM localized Ras has been shown to cycle back to the ER/golgi membranes through the activity of a palmitoyl-protein thioesterase (PPT) at the PM. Reversible palmitoylation of Ras has not yet been shown in fungal organisms. Once properly localized, Ras proteins transmit signals via PKA, MAPK, and Rho-GTPase pathways to control growth, development and stress response. Pathways shown are those known for the model yeast organisms, *Sacharomyces cerevisiae* and *Schizosaccharomyces pombe*, as well as the human pathogen, *Cryptococcus neoformans*, and are discussed in the Introduction.**

residue(s) utilized for palmitoylation or; 2) a stretch of polybasic residues generating a positively charged C-terminus (Konstantinopoulos *et al.*, 2007). Prenylation of the CAAX motif can be accomplished via either farnesylation or geranylgeranylation and the composition of the C-terminus reliably predicts the specific prenylation event (Konstantinopoulos *et al.*, 2007).

In filamentous fungi, prototypical Ras homologs can usually be divided into two sub-groups: 1) a "Ras1-like" subgroup that has a CAAX motif predicted to be farnesylated coupled with a "dual cysteine" motif for palmitoylation; or 2) a "Ras2-like" subgroup that is predicted to be geranylgeranylated and encodes an HVR with multiple basic residues (Fig. 2). It is important to note that the Ras1-like and Ras2-like homologs of filamentous fungi typically encode these differing localization motifs (Fig. 2), as this dichotomy suggests the potential for differential sub-cellular localizations and, therefore, potentially unique signaling partners under physiological conditions. In support of this possibility, Ras2-like homologs have been deleted in many filamentous fungi whereas deletion of Ras1-like homologs has only been achieved for *A. fumigatus*, *T. reesei* and *B. cinerea* (Fortwendel *et al.*, 2008; Minz Dub *et al.*, 2013; Zhang *et al.*, 2012a). The Ras1-and Ras2-like homolog paradigm is in contrast to the *S. cerevisiae* model, where Ras1p and Ras2p are highly homologous and have identical prenylation and palmitoylation domains, or *S. pombe* where only one Ras homolog exists (Weeks and Spiegelman, 2003).

Fungal Ras protein signaling has been studied most extensively in the model yeast organisms, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, and in the human pathogens, *Candida albicans* and *Cryptococcus neoformans*. Ras-mediated morphogenetic signaling has been reviewed extensively in