Plant Pathology in the 21st Century

Dov Prusky
Maria Lodovica Gullino
*Editors*

# Post-harvest Pathology

## Plant Pathology in the 21st Century, Contributions to the 10th International Congress, ICPP 2013

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# Preface

## Recent Development in Postharvest Pathology

This collection of paper includes some of the presentation given at the International congress of Plant Pathology held in Beijing in 2013 in the session of Recent Development in Postharvest Pathology. Fruit production for human consumption is an important part of the market economy. Any waste during spoilage and pest infestation, in the field and the postharvest phase, results in significant economic losses which are more pronounced as the losses occur closer to the time of produce sale. Careful handling of perishable produce is needed for the prevention of postharvest diseases at different stages during harvesting. Improved handling, transport and storage are needed in order to preserve the high quality produce. The extent of postharvest losses varies markedly depending on the commodities and country estimated to range between 4 and 8 % in countries where postharvest refrigeration facilities are well developed to 30 % where facilities are minimal. Microbial decay is one of the main factors that determine losses compromising the quality of the fresh produce. For the development of an integrated approach for decay management, cultural, pre-harvest, harvest and postharvest practices should be regarded as essential components that influence the complex interactions between host, pathogen, and environmental conditions. Orchards practices including pre-harvest fungicide applications can also directly reduce the development of postharvest fruit decay. Among postharvest practices, postharvest fruit treatments with fungicide are the most effective means to reduce decay. Ideally, these fungicides protect the fruit from infections that occur before treatment, including pathogen causing quiescent infections, as well from infection that are initiated after treatment during postharvest handling, shipment and marketing. The implementation of these alternatives techniques often requires modifying currently used postharvest practices and development of new formulation for their applications.

The present chapters deal with the newest report related to postharvest pathology in the world.

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# Part I
# Fungal Pathogenicity

# Chapter 1
# Function of Rab GTPases in Regulating the Development, Protein Secretion and Virulence of Fungi

**Shiping Tian, Zhanquan Zhang, and Guozheng Qin**

**Abstract** Rab GTPases are small guanosine triphosphatases with organelle-specific localization, and very stable in size in fungi, ranging from 8 to 12 in most of the sequenced fungi. Extensive studies about Rab GTPases in model eukaryotic cells indicated that they are master regulators of membrane trafficking, responsible for many essential processes including exocytosis, endocytosis and cellular differentiation. However, the function of Rab GTPases in fungi, especially in the plant pathogenic fungi, needs to be explored in recent. Here, we mainly summarize the research advances on the function of Rab GTPases in the life progress of fungi.

**Keywords** Rab GTPase • Fungi • Development • Protein secretion • Virulence

## Introduction

Small GTPase family includes five subfamilies: Ras, Rho, Rab, Arf and Ran (Novick and Zerial 1997). As the largest branch of the small GTPases family, Rab GTPases have been considered to play a pivotal role in the secretory pathway (Punt et al. 2001). Small GTPases usually function by cycling between active and inactive GTP-bound states. Some protein effectors are involved in the regulation of Rab GTPase activity during this cycling, such as a GDP/GTP exchange factor (GEF) which catalyzes the GDP/GTP conversion, and GTPase-activating proteins which accelerate GTP hydrolysis (Stenmark and Olkkonen 2001). Each Rab GTPase has a specific subcellular localization and takes part in a specific step of the secretory pathway (Novick and Zerial 1997). Rab GTPases have some conservative domains: four GTP-interaction domains (G1–G4), five Rab-specific functional domains (F1–F5) and four subfamily-specific domains (SF1–SF4) (Pereira-Leal and Seabra 2000).

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Pathogenic fungi or industrially exploited fungi can secrete many kinds of enzymes and metabolites into the extracellular environment (Kim et al. 2008). Some of extracellular enzymes and metabolites from phytopathogens have the ability to induce hypersensitive response of hosts (Schouten et al. 2008; Frías et al. 2011; Noda et al. 2010). The cell wall degrading enzymes secreted by phytopathogen during early stages of infection are necessary for successful establishment and proliferation in plant tissues (ten Have et al. 1998; Li et al. 2003; Oeser et al. 2002). Extracellular proteins also play a role in the molecular dialogue associated with host-pathogen interactions (Esquerré-Tugayé et al. 2000), suggesting that a precise regulation of these proteins should exist during pathogenesis. Current evidence suggests that both exocytosis and cell growth are occurred at the hyphal tips of filamentous fungi, although not exclusively (Read 2011). Extracellular secretion is dependent on vesicle transport and Rab GTPases are well established regulators of this process (Novick and Zerial 1997). Rab GTPases have been shown to influence major steps in vesicle transport, such as vesicle budding, delivery, tethering and fusion of the vesicle membrane with the target compartment (Grosshans et al. 2006). Many studies on the functions of Rab GTPase during vesicle transport in mammalian cells and yeast have been reported (Walworth et al. 1989; Van den Hazel et al. 1996; Chen et al. 2001; Chanda et al. 2009), but a few of results related to the function of Rab GTPases in filamentous fungi, especially in plant pathogenic fungi.

## Results

### *The Rab GTPases in Fungi*

Some research results have indicated that *Arabidopsis* and mammals each have roughly 60 Rab GTPases (Pereira-Leal and Seabra 2001; Rutherford and Moore 2002). The model, unicellular organism, *Saccharomyces cerevisiae*, has 11 Rab family GTPases named as YPT or SEC4 (Pereira-Leal and Seabra 2001). The number of Rab GTPases usually ranges from 8 to 12 in most of the sequenced fungi (Pereira-Leal 2008), including some important plant pathogens. For example, *Botrytis cinerea* has ten Rab GTPases, *Magnaporthe grisea* has 11 Rab GTPases and *Ustilago maydis* has 12 Rab GTPases. Here, we summarize the numbers of Rab GTPases in different fungi (Table 1.1). Although few genes involved in vesicle secretion in filamentous fungi have been cloned, sequence information from these clones can be used to search for homologues that contribute to vesicle secretion in other organisms.

**Table 1.1** The numbers of Rab GTPases in some pathogenic fungi

| Name of fungi | Number of Rab protein |
|---|---|
| *Botrytis cinerea* | 10 |
| *Gibberella zeae* | 11 |
| *Magnaporthe grisea* | 11 |
| *Aspergillus fumigatus* | 10 |
| *Phaeosphaeria nodorum* | 10 |
| *Filobasidiella neoformans* | 11 |
| *Ustilago maydis* | 12 |
| *Candida glabrata* | 9 |
| *Candida albicans* | 9 |

## *Rab GTPases Affecting the Development of Fungi*

Rab GTPases have very important function in the life processes of model organisms. Mao et al. (1999) reported that the invalidation of Rab GTPase (SEC4) was lethal in *S. cerevisiae*. Similarly, Siriputthaiwan et al. (2005) indicated Rab GTPase CLPT1 to be essential for the differentiation of infectious structures in the bean pathogen *Colletotrichum lindemuthianum*. They found that the expression of the dominant-negative mutant mutation could impair appressorial differentiation, suggesting Rab GTPase acts an important function in the development of phytopathogens. Punt et al. (2001) also observed in *Aspergillus niger* to prove that the disruption of Rab family gene *srgA* led to a slower growth rate of the fungus. Based on an experimental model system of *Dictyostelium discoideum*, Powell and Temesvari (2004) found that the Rab8-like protein, Sas1, could participate in the formation of membrane extensions, and cell-cell adhesion during development. Carvalho et al. (2011) studied the function of another Rab GTPase srgC of *A. niger*, and found that deletion of the *srgC* gene resulted in strongly reduced growth and the inability to form conidiospores at 37 °C and higher, which suggested that srgC has an important role in maintaining the integrity of Golgi-like structures. In addition, Pantazopoulou and Peñalva (2011) proved that the rabC protein of *Aspergillus nidulans* was involved in the apical extension and Golgi network organization, like the function of srgC in *A. niger*. Powers-Fletcher et al. (2013) pointed out that the Rab GTPase srgA in *Aspergillus fumigates* contributed to the conidiation and hyphal growth in the opportunistic human mold pathogen. They found that the conidia released from the mutant *ΔsrgA* colonies were heterogeneous in size and shape compared to wild type. The growth of *ΔsrgA* were impaired at temperatures ranging from 30 to 40 °C, but the extent of growth inhibition was variable between strains (Powers-Fletcher et al. 2013). Our recent results demonstrate that disruption of a *Rab8*/*SEC4* like gene *Bcsas1* in *B. cinerea*, an aggressive fungal pathogen that

infects more than 200 plant species, has a striking effect on hyphal growth and morphology on solid medium. The mutants are characterized by smaller, compact colonies, as well as reduce sporulation on PDA plates (Zhang et al. 2014). All above results provide the evidences to confirm the role of Rab GTPases in the development of fungi.

## *Rab GTPases Regulating Protein Secretion in Fungi*

Pathogenic fungi always secrete a variety of extracellular proteins which were shown in a number of cases to contribute to pathogenicity (Novick and Zerial 1997). The secretion of these extracellular proteins should be under the control of some precise regulation. Punt et al. (2001) considered that Rab GTPases could regulate the secretory pathway in the model organisms. In *S. cerevisiae*, the secretion pathway has been extensively studied and proved many Rab family genes to be the key regulator of vesicular transport (Novick and Zerial 1997). Until now, a few studies focused on the secretion regulation of extracellular proteins in plant pathogenic fungi. Siriputthaiwan et al. (2005) indicated that the depression of the expression of *CLPT1*, a Rab family GTPase in *C. lindemuthianum*, resulted in the inhibition of the secretion of extracellular polygalacturonase and block the transport of vesicles. We find that the Rab8-like gene *Bcsas1* in *B. cinerea* also has striking effect on secretion of extracellular protein via regulating the vesicular transport, and prove that the deletion of *Bcsas1* results in significantly reduction of polysaccharide hydrolases and proteases (Zhang et al. 2014), some of these enzymes have close relationship with the development and virulence of phytopathogen. Other results previously reported by Punt et al. (2001) have shown that the Rab family gene *srgA* of *A. niger* has carbon source dependent effect on protein secretion. They thought that secretion of protein was strongly reduced in the *△srgA* strains during growth on glucose and it was similar during growth on maltodextrin, and gave a hypothesise, that there would exist two different secretory pathways: one depending strongly on *srgA* function and one less dependent on *srgA* function (Punt et al. 2001). Moreover, Pantazopoulou and Peñalva (2011) also found that *rabC△* mutants of *A. nidulans* showed the decrease in extracellular levels of the major secretable protease, suggesting that it impairs secretion. The previous reports indicated YPT1 and SEC4, two Rab GTPases from human pathogen *Candida albicans,* to be essential for the protease secretion (Lee et al. 2001; Mao et al. 1999).