CHAPTER 6

SIGNALLING IN BOTRYTIS CINEREA

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Abstract. The cellular environment plays an important role in growth and differentiation of fungi. Signal transduction cascades mediate communication between environmental signals and the cellular machinery regulating developmental programmes. Fungal pathogens of plants have to ‘recognize’ their susceptible hosts, penetrate any physical barriers, overcome host defences and proliferate in the invaded tissues. Recent work has established that cyclic AMP (cAMP) and conserved MAP kinase signalling pathways play crucial roles during pathogenesis in several plant-infecting fungi, including Botrytis cinerea. In all fungal pathogens analyzed so far, it has been demonstrated that the knock-out of genes whose products encode components of signaling cascades interferes with pathogen development. This chapter summarizes the recent progress in studying the function of genes that code for signalling components in B. cinerea.

1. Introduction

Botrytis cinerea affects nearly all species of dicotyledons including most vegetable and fruit crops, flowers, woody ornamentals and greenhouse-grown crops (Chapters 14-19). Thus, the fungus must have evolved strategies to ‘recognize’ suitable hosts, penetrate and invade plant tissues and overcome host defences. To perform these tasks, the fungus is capable of perceiving chemical and physical signals from different host plants and responding with the appropriate metabolic activities required for pathogenic development. In general, such metabolic adaptations include adhesion of conidia to the plant surface, directed germ-tube growth, differentiation of infection structures and secretion of lytic enzymes and phytotoxins (Knogge, 1996). All of these responses require a network of signal transduction pathways, such as the activation of G proteins (Bölker, 1998), cyclic adenosine monophosphate (cAMP) signalling (Mitchell and Dean, 1995) and mitogen-activated protein kinase (MAPK) cascades (Xu, 2000) to communicate the perceived external signal to the fungal genome so that the appropriate gene, or sets of genes, can be activated to build the developmental response required by the pathogen.

Enormous progress was made in recent years in the study of single components of signalling pathways, their functional analysis and interaction with other
components of the same or different signalling cascades. Some model filamentous fungi, *Aspergillus nidulans* and *Neurospora crassa*, and plant pathogens, such as *Magnaporthe grisea*, *Ustilago maydis* and *Cryphonectria parasitica*, have been most extensively studied. These studies reveal a high degree of conservation between different fungi even between divergent organisms, and illustrate conserved basic principles in the molecular determination of life (see Lengeler et al., 2000). However, despite the high degree of sequence conservation, signalling components can have different functions. Thus, the replacement of the highly homologous (98% amino acid identity) Gβ subunits CPG1 of *C. parasitica* and GNA1 of *N. crassa* resulted in the loss of conidiation in *C. parasitica* (Gao and Nuss, 1996), but had no effect on conidiation in *N. crassa* (Ivey et al., 1996). Furthermore, CPG1 inhibits and GNA1 activates the activity of the adenylate cyclase. These examples clearly demonstrate that the complicated networks of signalling cascades, and their functions and cross-talks, must be studied in each fungus to gain a clear insight to their structures and performance. In *B. cinerea* some progress has been made recently in studying signalling components and their role in plant-fungus interaction; the identified components are summarized in Table 1.

2. Gα subunits of heterotrimeric G proteins

Heterotrimeric guanine nucleotide-binding proteins (G proteins) are involved in regulating a variety of cellular functions in eukaryotic cells. They act as transducers between activated cell-surface receptors and intracellular effectors. In *B. cinerea*, two Gα subunit genes, *bcg1* and *bcg2*, have been identified (Schulze Gronover et al., 2001). The deduced amino acid sequence of BCG1 has the highest level of identity with the Gα subunits from other phytopathogenic fungi, such as CPC1 from *C. parasitica* (Gao and Nuss, 1996), CTG1 from *Colletotrichum trifolii* (Truesdell et al., 2000), CGA1 from *Cochliobolus heterostrophus* (Horwitz et al., 1999) and MAGB from *M. grisea* (Liu and Dean, 1997). All these Gα subunits are homologous to the mammalian Gia family. On the other hand, BCG2 is quite similar to GNA2 from *N. crassa* (Turner and Borkovich, 1993) and MAGC from *M. grisea* (Liu and Dean, 1997). RT-PCR experiments showed clearly that both genes are expressed *in planta* at very early stages of infection. Characterization of *bcg1* and *bcg2* deletion mutants revealed that both Gα subunits affect growth and fungal pathogenicity in different ways. BCG1 controls multiple functions, including vegetative growth, pigmentation, proteolytic activity and pathogenicity. On media with up to 1% sucrose Δbcg1 mutants grow slowly and form small, more compact colonies similar to cga1 mutants of *C. heterostrophus* and cpg1 mutants of *C. parasitica*. However, on media with higher sucrose concentrations, the phenotype is comparable to the wild-type (WT) (Schulze Gronover et al., 2001). Interestingly, 2 mM cAMP fully restored the WT colony morphology suggesting a stimulatory affect of BCG1 on adenylate cyclase, similar to GNA1 in *N. crassa* and MAGB in *M. grisea*. Apart from effects on vegetative growth and morphogenesis, BCG1 was shown to have a major role in the process of colonization of host tissue. Germination of conidia and penetration ceases after formation of primary lesions. After 48 h, on leaves infected