Modulation of a Fungal Signaling by Hypovirus

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The chestnut blight fungus, Cryphonectria parasitica, and its hypovirus are a useful model system in the study of the mechanisms of hypoviral infection and its consequences, such as a biological control of fungal pathogens. Strains containing the double-stranded (ds) RNA virus Cryphonectria hypovirus 1 show characteristic symptoms of hypovirulence and display hypovirulence-associated changes, such as reduced pigmentation, sporulation, lactase production, and oxalate accumulation. Interestingly, symptoms caused by hypoviral infection appear to be the result of aberrant expression of a number of specific genes in the hypovirulent strain. Several viral-regulated fungal genes are identified as cutinase gene, Lac1, which encodes an extracellular lactase, Crp, which encodes an abundant tissue-specific cell-surface hydrophobin that mediates physical strength, and Mf2/1 and Mf2/2, which encode pheromone genes involved in poor sporulation in the presence of hypovirus. Since the phenotypic changes in the fungal host are pleiotropic, although coordinated and specific, it has been suggested that the hypovirus disturbs one or several regulatory pathways (Nuss, 1996). Accordingly, several studies have shown the implementation of a signal transduction pathway during viral symptom development. Although further studies are required, hypovirulence and its associated symptom development due to the hypoviral regulation of a fungal heterotrimeric G-protein have been suggested. In addition, recent studies have shown the presence of a novel protein kinase gene cppk1 and its transcriptional upregulation by hypovirus. In this review, the presence of important components in signal transduction pathway, their putative biological function, and viral-specific regulation will be addressed.

Keywords: Cryphonectria parasitica, hypovirulence, signal transduction pathway.

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1998). Theses studies all suggest that hypovirus modulates fungal gene expression in a specific manner. Since the hypovirus causes diverse phenotypic changes, but in a coordinated and specific way, that slow the development of the fungus without killing it, it has been suggested that the hypovirus disturbs one or several regulatory pathways. Therefore, it can be said that the signal transduction pathway in C. parasitica appears to be the main target of hypoviruses.

Eukaryotic organisms recognize and respond to various external environments through a variety of signal transduction pathways, which involve protein phosphorylation at some critical step. This is a highly evolved and sophisticated process that is critical for nearly all aspects of physiological regulation in eukaryotes, including filamentous fungi. In filamentous fungi, signaling pathways that act via phosphorylation may be involved in the following: 1) response to extracellular signals; 2) regulation of cell cycle-related processes; and 3) response to nutritional and environmental stresses (Dickman and Yarden, 1999). Filamentous fungi have signaling systems that are similar to those of the higher eukaryotes. Theses systems include G-proteins, mitogen activated protein kinases (MAPK), protein kinase C (PKC), phospholipase C (PLC), and protein kinase. Several genes involved in C. parasitica signal transduction have been identified: cpg-1, cpg-2, cpgb-1, bdm-1, and cppk1. These genes encode two Gα sub-units, a Gβ sub-unit, a beta disruption mimic factor, and a novel Ser/Thr protein kinase, respectively (Choi et al., 1995; Kasahara and Nuss, 1997; Kasahara et al., 2000; Kim et al., 2002). However, among many regulatory genes of C. parasitica, not many of them were both viral-regulated and specific symptom-related i.e., they were either viral-regulated but reproduce all symptoms (Choi et al., 1995; Gao and Nuss, 1996), or specific symptom-related but viral independent (Wang et al., 1998). Accordingly, it is not easy to depict the details of molecular hierarchy explaining how mycovirus regulates the fungal gene expression to result in specific viral symptoms.

This review explored whether the hypoviruses affect a fungal signal transduction pathway and what the biological function of a specific pathway might be in terms of symptom development.

**Does the hypovirus affect the G protein-mediated signal transduction in C. parasitica?**

Several studies have shown the existence of genes encoding major components of signal transduction pathway. These include two Gα sub-unit genes (cpg-1 and cpg-2) and a Gβ sub-unit gene (cpgb-1), which seem to be among the very first G protein genes cloned from filamentous fungi. The deletion of cpg-1 showed a set of phenotypic changes similar to, but more severe than, those associated with hypovirus infection (Gao and Nuss, 1996). Changes included a marked reduction in fungal growth rate and loss of virulence, asexual sporulation, female sterility, and transcriptional induction of laccase gene (lac-1). However, cpg-2 disruption resulted in slight reductions in growth rate and sporulation. No other phenotypes were distinctive. These results provide definitive confirmation of a requirement of CPG-1-linked signaling for a number of fungal processes, including virulence and reproduction, while demonstrating that a second Gα, CPG-2, is dispensable for these processes (Fig. 1). However, the viral regulation of this gene is still cumbersome even if there was a subsequent report that the CPG-1 protein was reduced in virus-containing strain. Moreover, the various levels of CPG-1 expression by transgenic co-suppression correlated with phenotypic changes similar to the viral infection (Fig. 2). However, no changes among virus-containing and their virus-free isogenic strains were detected in the level of Gα protein using Gα-specific toxin (Fig. 3). In conclusion, Gα- and its related proteins are important for fungal processes, such as growth and development, but further studies are required to confirm whether the hypoviruses are directly involved in the regulation of these genes or gene products.
Fig. 2. Colony morphology and Western analysis of selected *C. parasitica* strains transformed with sense copies of *cpg-1*. (A) Colony morphology of selected *cpg-2* sense transformants. Eleven of 18 randomly selected *cpg-1* transformants from a single representative transformation exhibited altered phenotypic traits compared with the nontransformed virulent strain EP155. When cultured on PDA, many of the sense transformants grew slowly and had uneven colony margins, sparse aerial hyphae, an intense brown-orange pigmentation rather than the normal orange pigmentation, and absence of concentric orange growth rings, suggesting disruption of response to light/dark cycles. These alterations were stable and qualitatively similar among the transformants but varied in severity between transformants as shown by representative sense transformants 1310, 1316, 1317, and 1318. (B) Western analysis of selected sense transformants. Lysates prepared from nontransformed *C. parasitica* virulent (EP155) and hypovirulent (EP713) strains and *cpg-1* sense transformants 1310, 1316, 1317, and 1318 were probed with CPG-1 specific antiserum. Migration position of a M, 47,000 size marker is indicated on the left. Severity of phenotypic alterations exhibited by the four transformants when cultured on PDA correlated with the level of CPG-1 suppression (comparison A and B) (Choi et al., 1995).

Does the hypovirus affect the signal transduction in *C. parasitica*?

Kim et al. (2002) reported the presence of a novel Ser/Thr kinase, *cppkl*, the transcription of which is specifically regulated by the presence of hypovirus. The protein product of *cppkl* had a catalytic activity of protein phosphorylation and its transcriptional accumulation was specifically up-regulated by the presence of hypovirus, which appears to be the first example of the transcriptional up-regulation by hypovirus with a known function. Kinase assay using E. coli-expressed CPPK1 exhibited the presence of cellular target protein with a size of 50 kDa and 44 kDa, which strongly indicates that the *cppkl* is one of the components in fungal signaling cascade with many downstream target proteins. The transformation of virus-free strain with a vector containing *cppkl* derived by a constitutive promoter can reproduce some, but not all, viral symptoms, such as reduced pigmentation, asexual sporulation, and female sterility, in virus-free transformants with a concomitant increased accumulation of transcript and its protein product.

Fig. 3. Detection of the Ga sub-unit of *C. parasitica* by pertussis toxin ADP-ribosylation. Crude plasma membranes were extracted from virulent strains EP155 and EP67 and their respective isogenic CHV1-infected strains UEP1 and EP802, and labeled with pertussis toxin by the method of Turner and Borkovich (1993). (A) Protein labeled with [32P]NAD in the presence (+) or absence (−) of pertussis toxin. (B) Polyacrylamide gel of the same membrane preparation as in panel B stained with Coomassie blue. A total of threefold more protein was loaded in each lane of panel B than in each lane of panel A (Zhang et al., 1998).

Fig. 4. Phenotypic and molecular characteristics of selected *cppkl* overexpression transformants. (A) Colony morphology, when cultured on PDAmib, (B) Northern blot analysis of *cppkl* transcript, (C) Western blot analysis of CppK1, (D) Northern blot analysis of *Mf21* transcript, (E) Northern blot analysis of *Mf22* transcript. Panels B, C, D, and E contain samples prepared from 5-day old culture. Equal loading amount of protein preparations is normalized by Bradford assay, and Northern blot analyses are normalized with *Gpd* probe (F). Numbers 1, 2, 3, and 4 indicate strains of EP155/2, TPK13-1, TPK15-1, and UEP1, respectively (Kim et al., 2002).
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The cypk1 study clearly demonstrates that the hypovirus disturbs fungal signaling at least one part by transcriptional up-regulation of cypk1, which results in reduced pigmentation and conidiation, and female sterility. Disruption mutant of cypk1 gene has recently been constructed and it will be of interest to find the relationship between the biological function of gene in signaling and its involvement in symptom development.

As an alternative approach to prove the viral regulation of fungal signal transduction pathway, several genes belonging to representative components of the signal transduction pathway were cloned from C. parasitica based on the sequence homology, and the viral regulation of their expression were examined. One of the early examples can be a mitogen-activated protein kinase (MAPK). In this study, high-osmotic glycerol (HOG) homologue from C. parasitica was cloned, and it was proven that the induction of this gene product is also affected by the hypovirus infection, which is another example of hypoviral regulation of fungal gene expression (in review).

There are many examples that provide confirmative evidences for the viral regulation of fungal signaling. To understand the mechanism by which mycoviruses perturb fungal gene expression, knowledge of the host genes that are affected, as well as their roles in fungal development and virulence, are important. Assessing the roles of signal transduction pathways in symptom development, it is possible to infer the correlation between a specific signal pathway and its corresponding symptom, which will provide researchers with better strategies for the biological control of fungal pathogen without disturbing other desirable characteristics of a hypovirulence-inducing strain. In addition, it can provide valuable information on how mycovirus coordinates and regulates fungal gene expression, which suggests the general mechanism of viral regulation of host gene expression.

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References


