Proc. KMB Annual Meeting
June 24-26, 2003, Muju, Korea

Viral Regulation of Fungal Protein Kinases in *Cryphonectria parasitica*

Dae-Hyuk Kim

Institute for Molecular Biology and Genetics, Basic Science Research Institute, Chonbuk National University, Dukjindong 664-14, Chonju, Chonbuk 561-756, Korea

The chestnut blight fungus, Cryphonectria parasitica (Murr.) Barr, has been responsible for the virtual disappearance of the chestnut orchards in North America since the early 20th century. However its hypovirus infection is known to cause the hypovirulence, which makes a good example of biological control. Strains containing the double-stranded (ds) RNA Cryphonectria hypovirus (CHV) show the characteristic symptoms of hypovirulence, and display hypovirulence-associated changes, such as reduced pigmentation, sporulation, laccase production, and oxalate accumulation. Interestingly, the symptoms caused by hypoviral infection appear to be the result of the aberrant expression of specific sets of fungal genes in the hypovirulent strain, which may include the genes for cutinase, laccase, cryparin, and mating pheromones. Thus, the chestnut blight fungus C. parasitica and its hypovirus represent a useful model system to study the mechanisms of fungal gene regulation by mycoviruses. Since the phenotypic changes in the fungal host are pleiotropic, albeit coordinated and specific, it has been suggested that the hypovirus disturbs one or several regulatory pathways. Accordingly, several studies have shown the implementation of a signal transduction pathway during viral symptom development.

Several genes that encode components of the various signal transduction pathways in *C. parasitica* have been cloned and characterized. Included in this group are genes for the heterotrimeric G-proteins and their putative regulator, and a novel kinase. In addtion, many other genes are under investigations. However, the components of the mitogen-activated protein kinase (MAPK) signal transduction pathway have not yet been cloned and characterized.

The mitogen-activated protein kinase (MAPK) signal transduction pathway is utilized by eukaryotic cells to transduce a wide variety of cellular signals through a step-wise phosphorylation relay. This cascade appears to be well-preserved in a variety organisms, ranging from yeast to human, and consists of three functionally interlinked protein kinases: MEEK (MAP kinase kinase kinase), MEK (MAP kinase kinase), and MAPK. Several MAPKs have been cloned from various phytopathogenic fungi, including the cereal leaf pathogens *Magnaporthe grisea* and *Cochliobolus heterostrophus*, the cucumber leaf pathogen *Colleto-trichum lagenarium*, the maize pathogen *Ustilago maydis*, the broad-host-range necrotroph *Botrytis cinerea* and the soil-borne pathogens *Fusarium oxysporum* and *Nectria haematococca*. Studies on the biological functions of the fungal MAPK reveal that it is involved in many different pathways of growth, differentiation and pathogenicity in plant pathogenic fungi.

Here, we examined the biological function of *cpmk*1, which encodes a MAPK of *Cryphonectria parasitica* and its regulation by mycovirus. Sequence comparisons revealed that *cpmk*1 had highest homology with *osm*1, a *hog*1-homologue from *Magnaporthe grisea*. Growth defect was observed in the *cpmk*1-null mutant under hyperosmotic conditions, indicating that *cpmk*1 functionally belongs to a *hog*1 subfamily. Kinase assays and Northern blot analyses indicated that the CpMK1 pathway was affected specifically in hyperosmotic conditions by the hypovirus CHV1-EP713. Moreover, the virus-infected hypovirulent UEP1 strain also exhibited severe osmosensitivity compared with the virus-free isogenic strain EP155/2, thus

providing additional evidence for viral regulation of *cpmk*1. Besides osmosensitivity, disruption of *cpmk*1 resulted in several, but not all, hypovirulence-associated changes, such as reduced pigmentation, conidiation, laccase production, and cryparin expression. However, the *cpmk*1-null mutant exhibited an increased accumulation of pheromone gene transcripts. Virulence assays of the *cpmk*1-null mutant revealed reduced canker area, but not as severe as that of UEP1. These results suggest that mycoviruses modulate the MAPK and thereby provoke the aberrant expression of target genes for viral symptom development.