Pathogen protection and virulence: Dark side of fungal membrane protein revealed

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Researchers at the Virginia Bioinformatics Institute (VBI) at Virginia Tech and Montana State University have discovered a fungal protein that plays a key role in causing disease in plants and animals and which also shields the pathogen from oxidative stress. The researchers have found that the fungal protein TmpL is critical for the infection of host tissue and helps these pathogens regulate oxidative stress responses that are caused by the presence of destructive reactive oxygen species, a natural feature of the adaptive response to infection.

Dr. Chris Lawrence, Associate Professor at VBI and the Department of Biological Sciences at Virginia Tech and leader of the project, remarked: "The critical roles of reactive oxygen species in fungal development and virulence have been well established over the past half century. However little is known about how these molecules are produced or how the balance is achieved between their cell signaling roles on the one hand and their potentially destructive properties on the other. I believe we now have a unique opportunity to study a common fungal disease-associated mechanism in plants and animals that appears to be inextricably linked to the oxidative stress of the host-pathogen environment."

The scientists looked at two different fungal pathogens: *Alternaria brassicicola*, which causes widespread damage in crops like canola, cabbage and broccoli, and *Aspergillus fumigatus*, a human pathogen that often leads to fatal disease in immunocompromised patients. Infection with *A. fumigatus* can lead to invasive pulmonary aspergillosis, the

leading cause of death due to invasive fungal infections in humans.

Said Lawrence: "This transdisciplinary project has involved bioinformatics, functional genomics, molecular biology, biochemistry, plant pathology, immunology, and medical mycology. We first discovered the gene encoding TmpL by computational screening and analysis of the A. brassicicola genome at VBI. Further investigation of the tmpL sequence and functional genomics analysis has allowed us to build up a picture of what the protein may look like in many filamentous fungi."

Dr. Biswarup Mukhopadhyay, an expert in microbial enzyme biochemistry at VBI, remarked: "The scientific evidence suggests that TmpL is a flavin-containing enzyme that possesses six membrane-spanning domains. Flavins are molecules that are involved in biological oxidation and reduction reactions. It seems likely that TmpL deploys electrons from NADPH, a key energy currency in the cell, via a flavin molecule to somehow modify subsequent signaling events that are linked to the overall oxidative state of the cell."

Dr. Robert Cramer, director of the Aspergillus-associated aspects of the research and Assistant Professor of Fungal Pathogenesis in the Department of Veterinary Molecular Biology at Montana State University, said: "We suspect that TmpL may act as a detoxifier of intracellular reactive oxygen species. These toxic molecules are highly elevated in the fungus at the initial stages of infection and during various developmental stages. While these molecules are toxic at inappropriate levels, they are also likely key signaling molecules involved in directing fungal development." He added: "TmpL-deficient mutants were also more sensitive to external oxidative stress which suggests that TmpL may aid the onset of disease by protecting the pathogen from the host's defense mechanism. Experiments with oxidative-burst-deficient mice suggest that the intracellular regulation of reactive oxygen species in the

fungus is most likely more important for pathogenesis than resistance to host-derived oxidative stress. However, more work is needed to confirm this hypothesis and to dissect the precise molecular mechanism that is involved."

The current study shows that TmpL is essential for both types of fungi to cause disease in their host cells. Dr. Kwang-Hyung Kim at VBI, the lead author on the paper and a scientist working on the project, remarked, "TmpL appears to be located in the membrane of the Woronin body, a specialized peroxisomal organelle found specifically in the cells of hyphae in filamentous fungi. When the function of TmpL is impaired or its gene inactivated, both A. brassicicola and A. fumigatus show drastic decreases in their ability to cause disease in our experimental host systems of cabbage, Arabidopsis, and mouse. Deletion of the tmpL gene also makes the fungi extremely sensitive to oxidative stress in the laboratory. Whether or not TmpL aids in protection against host-derived oxidative stress remains to be elucidated and will be one of our primary research areas in the future."

Lawrence said: "In this study, we provide the first evidence that a certain type of disease-related protein from both a plant and animal fungal pathogen is critical for protecting the pathogen and for causing infection of healthy host tissue. As such, this protein represents a good starting point as a potential target for the development of efficient and novel therapeutics for both plant and animal fungal diseases."

More information: The results will be published in *PLoS Pathogens:* Kim K-H, Willger SD, Park S-W, Puttikamonkul S, Grahl N, Cho Y, Mukhopadhyay B, Cramer RA, Lawrence CB (2009) TmpL, a transmembrane protein required for intracellular redox homeostasis and virulence in a plant and an animal fungal pathogen. *PLoS Pathogens*, doi: 10.1371/journal.ppat.1000653

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