[Plant Signaling & Behavior 3:7, 480-481; July 2008]; ©2008 Landes Bioscience

Article Addendum

Connections between polar growth and cell cycle arrest during the induction of the virulence program in the phytopathogenic fungus *Ustilago maydis*

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Key words: polar growth, phytopathogenic fungus, cell cycle, corn smut, *Ustilago maydis*, virulence

Elegant work by others has highlighted the importance of connections between polar growth and cell cycle regulation in budding and fission yeast. However, it is striking that little attention has been paid to the study of these connections in phytopathogenic fungi. In these crop pests, germination of spores, the main infective agent, requires a strict control of cell cycle regulation as well as polarity growth. Our finding that a cyclin-cdk pair controls both processes in the corn smut fungus *Ustilago maydis* supports the importance of such a regulation during the pathogenic development of fungi.

Plant diseases caused by foliar fungal pathogens are initiated when fungal spores attach to host surface and germinate. The process of germination implies the activation of a polarity axis and the emergence of a germ tube. As this process is crucial for the initiation of the infection, it is clear that the ability to form polarized infective hyphae may represent an "Achiles Heel" that can be exploited to limit fungal invasion of the plant tissue.[1] Corn smut fungus, *Ustilago maydis*, seems to be a paradigmatic model to understand these processes. In this fungus, the infective hypha is produced after the mating of two sporidial cells on the plant surface.[2] Strikingly, this infective hypha is composed of a single cell that is cell cycle arrested at G_2 phase while undergoes a strong polar growth.[3] Once the infective hypha enters the plant, this cell cycle arrest is abolished and the fungus proliferates. The reasons for this cell cycle arrest are not understood though seems to be a wide phenomenon, as many fungal pests do not undergo any cell division on the plant surface during the infective process.[4] It has been proposed that this cell cycle arrest may have a mechanistic reason.[3,5] In *U. maydis*, during the G_2 phase, the cytoskeletal growth machinery is set up to support polar growth, and then a prolonged G_2 phase is best suited to support tip growth during infective hyphae formation. Both processes, cell cycle arrest and strong polar growth, are directly related to the pathogenic development as they are triggered by the expression of the bW/bE heterodimer, the master transcriptional regulator of the pathogenic program in *U. maydis*.[6] In a recent report,[7] Flor-Parra et al identified the kinase Cdk5 and its regulatory subunit Pcl12 as a crucial element that could be mediating the connections between cell cycle regulation and polarity in this fungus.

The cyclin-dependent kinase Cdk5 is required for sustained polar growth in *U. maydis*.[8] Cdk5 belongs to a family of cyclin-dependent kinases (CDK) implicated in the regulation of morphogenesis in organisms ranging from yeast to human.[9] In *U. maydis* Cdk5 seems to have no role in cell cycle regulation and it has been involved in the correct localization of polarity determinants at the tip growth. As it happens with other CDKs, Cdk5 activity requires the interaction with proteins known as cyclins, which target the catalytic subunit to correct substrates. In *U. maydis*, one of the genes encoding these cyclins, *pcl12*, was recently reported to be dependent on bW/bE protein for its expression.[7] Furthermore, the ectopic expression in axenic conditions of *pcl12* mimics the *b*-dependent filament production[7]. Interestingly, this mimicry not only applies at the level of polar growth but also induces a G_2 cell cycle arrest. However, while the absence of Pcl12 strongly affects the ability to polarize growth during the induction of the infective filament, cell cycle is still arrested in these mutants. Though these results indicated that Pcl12 is not required for cell cycle arrest during the induction of the infective filament in *U. maydis*, one possibility is that redundant additional control systems ensuring a cell cycle arrest must exist. One of these plausible complementary mechanisms has been recently described by us, consisting in the downregulation of cyclin *clb1* expression by the transcriptional factor Biz1, which as *pcl7,* is activated by the presence of an active b heterodimer.[10] As it happens in the case of cells defective in *pcl7*, deletion of *biz1* did not abolish the *b*-induced cell cycle arrest, although its ectopic expression does induce a G_2 cell cycle arrest.[10] However, double *biz1 pcl7* mutant are severely impaired in filament formation, but they still arrest cell cycle upon bW/bE heterodimer expression (unpublished observations). These

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Submitted: 01/24/08; Accepted: 01/31/08

Previously published online as a *Plant Signaling & Behavior* E-publication: http://www.landesbioscience.com/journals/psb/article/5680

Addendum to: Parra IF, Castillo-Lluva S, Pérez-Martín J. Polar growth in the infectious hyphae of the phytopathogen Ustilago maydis depends on a virulence-specific cyclin. Plant Cell 2007; 19:3280–96; PMID: 17921314; DOI: 10.1105/tpc.107.052738.

results therefore open the possibility that either redundant additional mechanism exist to arrest cell cycle in response to *b* expression, or cell cycle arrest is a epiphenomenon associated to the induction of the virulence program.

Alternatively, it could be possible both polar growth and G_2 arrest are interdependent, in such a way that they are two sides of the same coin: G_2 cell cycle arrest has a consequence the activation of polar growth, but induction of polar growth generates a cell cycle delay/arrest in G_2 phase. This explanation is supported by different results that indicated that arresting the cell cycle in G_2, for instance by downregulation of crucial elements involved in G_2/M transition such as cyclin b or the phosphatase Cdc25, resulted in a sustained polar growth.[11,12] In the same way, induction of a strong polar growth forces the cell cycle to remain in G_2. For instance, we found that overexpression of *rac1*, a Rho-like GTPase that induces a strong polar growth in *U. maydis*[13] generates a G_2 delay. This is also the case when the *cln1* cyclin is overexpressed: the induction of a strong polar growth curses with a G_2 delay.[14]

Further research efforts will be needed to define the nature of these putative connections as well as their roles during the induction of the virulence program in phytopathogenic fungi.

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