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Review

Sending mixed signals: Redundancy vs. uniqueness of signaling components in the plant pathogen, *Ustilago maydis*

María D. García-Pedrajas [a], Marina Nadal [b], Michael Bölker [c], Scott E. Gold [b], Michael H. Perlin [d,*]

[a] *Estación Experimental 'La Mayora', CSIC, Algarrobo-Costa, Málaga, Spain*
[b] *Department of Plant Pathology, University of Georgia, Athens, GA, USA*
[c] *Department of Biology, Philipps-Universität, Marburg, Germany*
[d] *Department of Biology, Program on Disease Evolution, University of Louisville, Louisville, KY 40292, USA*

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ABSTRACT

The ability to respond to a changing environment separates successful organisms from their competitors. Thus, signal transduction is a crucial aspect of an organism's growth, development, differentiation, and reproduction. Nowhere is this more evident than in the co-evolution of obligate pathogens with their host organisms. The genome sequence of *Ustilago maydis*, the pathogen of maize, has provided a powerful tool in the assessment and characterization of signaling pathways for this organism. Inspection of the sequence reveals that while *U. maydis* has a streamlined gene content, it appears to contain a full repertoire of the standard signaling cascades present in other fungi. A full range of paralogues are present to provide redundancy of function on the one hand while, on the other, distinct strategies for survival. This review explores signaling based on the conserved mitogen-activated protein (MAP) kinase and cAMP-dependent protein kinase A (PKA) pathways as well as ancillary functions, with emphasis on the unique aspects of the *U. maydis* approach to utilizing this architecture.

1. Introduction

The sequencing, ongoing annotation, and eventually, closure, of the *Ustilago maydis* genome (http://mips.gsf.de/genre/proj/ustilago/; http://www.broad.mit.edu/annotation/genome/ustilago_maydis/Home.html; Kämper et al., 2006) has provided the *Ustilago* research community with opportunities to make comparative genomic analyses and observations on the complete inventory of predicted genes, in addition to their corresponding pathways. Specifically, we now have the ability to take stock of the signaling pathways and their components that have been characterized in other organisms. We can now ask whether the necessary components are present in *U. maydis* and assess the likelihood that their participation in signaling parallels the roles of their counterparts in other organisms. For many of the components of signaling pathways present in other fungi, an examination of the *U. maydis* genome reveals a plethora of homologs. Some of these multiple proteins represent overlapping functions; simultaneous disruption of the homologs is lethal. In other cases, this redundancy is associated with some overlap in function but accompanied by specialized roles for the respective proteins. An illustration of this apparent redundancy appears at several levels of the highly conserved mitogen-activated protein kinase (MAPK) and cAMP-dependent protein kinase A (PKA) pathways. As an example, among signal transducers (Fig. 1) there are many small G-protein and GTP-binding proteins, including Rho/Cdc42/Rac (6), Ras and Ras-like proteins (25–30), and heterotrimeric G-protein subunits (4 Gα, 1 Gβ, 1 Gγ).

In this article, we review characterization of various signaling pathways in *U. maydis*, most of which are associated with virulence to some degree. For initial orientation we relate genomic and experimental observations in the *Saccharomyces cerevisiae* paradigm (Fig. 1). The analysis we present here is derived from a combination of genome sequence inspection and direct experimental evidence. An additional summary figure of genes with known functions that illustrates the interplay between the MAPK and cAMP-dependent PKA pathways has been published recently (Klosterman et al., 2007, Fig. 1.3) and will not be duplicated here.

2. Signaling pathways

2.1. MAP kinase pathways

The mitogen-activated protein kinase (MAP kinase) pathways are a highly conserved set of pathways that transduce a variety of external signals, leading to changes in gene expression affecting a wide array of cellular processes: growth, differentiation, apoptosis, and inflammation. In fungi in general, and in *U. maydis* in particular, these pathways are used during mating to allow cells to

* Corresponding author. Fax: +1 502 852 0725.
E-mail address: mhperl01@gwise.louisville.edu (M.H. Perlin).

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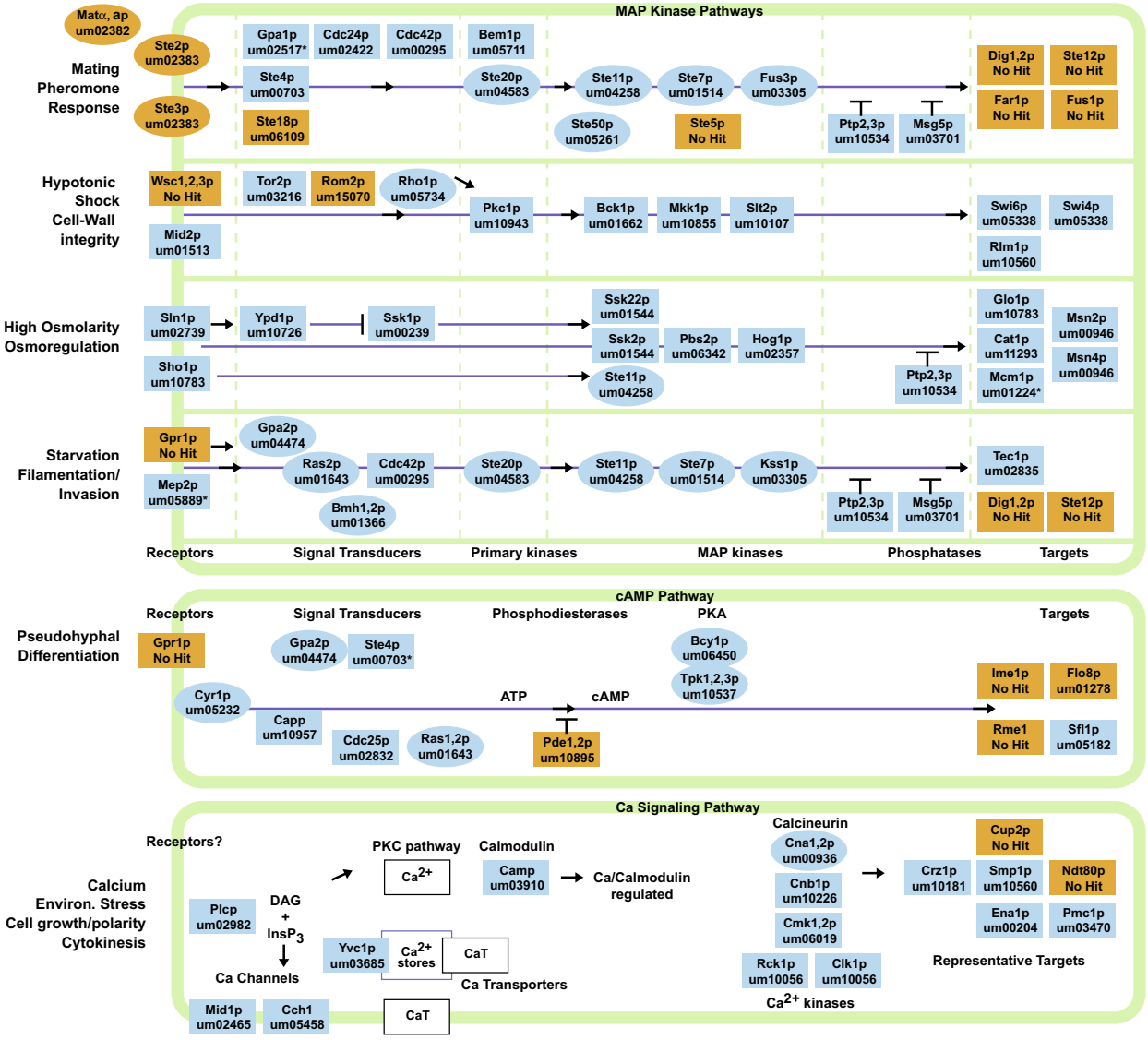

Fig. 1. Comparison of major signaling pathways between *S. cerevisiae* and *U. maydis*. There is conservation of the central proteins in the various signaling pathways. Receptors and downstream targets are less conserved. *S. cerevisiae* proteins used in homology searches are named. The annotation (um) number below the yeast protein name indicates a corresponding hit from the MUMDB or the Broad Institute databases. Blue and orange colored shapes indicate similarities of the yeast and *U. maydis* proteins of *e*-value $<1 \times 10^{-10}$ and $>1 \times 10^{-10}$, respectively. Ovals indicate *U. maydis* homologs shown to play a role in pathogenicity. An asterisk next to an annotation number means that a role in pathogenicity was tested but not found. The placement of proteins within pathways generally follows the yeast paradigm but is based on an analogous figure for *Magnaporthe grisea* (Dean et al., 2005) reproduced with permission. However, the *U. maydis* orthologs may function in alternative pathways. Additionally, while the *M. grisea* figure lists the ammonium transporter Mep2p as a receptor in the starvation pathway signaling through the MAPK cascade, there is also evidence suggesting a connection to the Pseudohyphal differentiation pathway, either directly or indirectly through the cAMP-dependent PKA pathway (Lorenz and Heitman, 1998; Smith et al., 2003; Van Nuland et al., 2006).

respond to pheromone, and are often also involved in responses to environmental stimuli, such as nutrient availability and changes in pH. A discussion of the components of this pathway and ancillary participants in this signaling in *U. maydis* follows.

2.1.1. MAP kinases

Experimental evidence demonstrated that *U. maydis* contains homologs to all three members of the MAPK module that transmit the pheromone signal in *S. cerevisiae*. During mating in yeast a MAP kinase kinase kinase (Ste11p) phosphorylates and activates a MAP kinase kinase (Ste7p) and Ste7p in turn phosphorylates and activates the MAP kinase Fus3p. The genes encoding Ubc4/Kpp4 (um04258), Fuz7/Ubc5 (um01514) and Ubc3/Kpp2 (um03305), homologs of Ste11p, Ste7p and Fus3p, respectively, were identified in a screen for suppressors of the constitutively filamentous phenotype of the *uac1*$^-$ mutant (Mayorga and Gold, 1999; Andrews et al., 2000) and by sequence similarity with the yeast genes (Banuett and Herskowitz, 1994; Müller et al., 1999). The roles of these proteins in mating are similar to those of their *S. cerevisiae* counterparts.

A MAP kinase cascade sharing components with the mating module is also involved in the pseudohyphal switch that diploid cells of *S. cerevisiae* undergo in response to nitrogen limitation (Gimeno et al., 1992; Roberts and Fink, 1994). Thus, in addition to Fus3p, Ste7p phosphorylates and activates another MAP kinase, Kss1p. Genetic analysis indicates that although Fus3p and Kss1p can partially substitute for each other, Fus3p acts primarily in the mating pathway leading to haploid cell fusion, whereas Kss1p acts primarily in invasive or pseudohyphal growth (Bardwell et al., 1996). In *U. maydis* there is also evidence of a second MAP kinase