International Microbiology (2020) 23:121–126
https://doi.org/10.1007/s10123-019-00100-5

REVIEW

The signaling mechanisms involved in the dimorphic phenomenon of the Basidiomycota fungus *Ustilago maydis*

José Ruiz-Herrera[1] · Fernando Pérez-Rodríguez[1] · John Velez-Haro[1,2]

Received: 17 January 2019 / Revised: 2 September 2019 / Accepted: 12 September 2019 / Published online: 8 January 2020

Abstract

In the present manuscript, we describe the mechanisms involved in the yeast-to-hypha dimorphic transition of the plant pathogenic Basidiomycota fungus *Ustilago maydis*. During its life cycle, *U. maydis* presents two stages: one in the form of haploid saprophytic yeasts that divide by budding and the other that is the product of the mating of sexually compatible yeast cells (sporidia), in the form of mycelial dikaryons that invade the plant host. The occurrence of the involved dimorphic transition is controlled by the two mating *loci a* and *b*. In addition, the dimorphic event can be obtained in vitro by different stimuli: change in the pH of the growth medium, use of different carbon sources, and by nitrogen depletion. The presence of other factors and mechanisms may affect this phenomenon; among these, we may cite the PKA and MAPK signal transduction pathways, polyamines, and factors that affect the structure of the nucleosomes. Some of these factors and conditions may affect all these dimorphic events, or they may be specific for only one or more but not all the processes involved. The conclusion reached by these experiments is that *U. maydis* has constituted a useful model for the analysis of the mechanisms involved in cell differentiation of fungi in general.

Keywords *Ustilago maydis* · Dimorphism · Transduction pathways · Metabolic signals

Introduction

Ustilago maydis is a Basidiomycota fungus belonging to the Ustilaginaceae family of the order Ustilaginales, and a biotrophic pathogen of the *Zea* species that include corn (*Zea mays* L), and what is considered its probable ancestor, teozintle, corresponding to the subspecies *Zea mays* ssp. *parviglumis* and ssp. *mexicana*. Although, because of its low virulence, *U. maydis* does not represent an important problem for the agriculture, it is considered a model for the comprehension of a number of physiological characteristics of fungi. Accordingly, its study has been extremely useful for the analysis of the genes responsible of DNA recombination, mating, gene regulation, dimorphism, and pathogenicity (e.g., Christenssen, 1963; Ruiz-Herrera et al. 2000; Banuett 2002; Brefort et al. 2009; Han et al. 2019). Also, it may be indicated that *U. maydis* has been used in México for human consumption since pre-Columbian times, and is now highly appreciated in the Mexican and international cuisines.

In the present review, we refer to the regulation of the dimorphic behavior of *U. maydis* making special emphasis on the role of the signal pathways and some specific aspects involved in the differentiation and virulence of the fungus.

Dimorphism of *Ustilago maydis* and the role of the MAPK and PKA pathways

As dimorphism, we define the capacity of some fungi to grow in the yeast-like or mycelial forms in response to different stimuli. *U. maydis* is considered a dimorphic fungus because in natural conditions, it can grow as saprophytic haploid yeasts that reproduce by budding or in the form of virulent dikaryotic hyphae that grow apically and have the capacity to infect its natural hosts. The stimulus that induces the yeast-to-hypha transition is mating. *U. maydis* is heterothallic, and its mating is tetrapolar, i.e., mating is controlled by two alleles: *a* and *b*, *a* with two alleles (idiomorphs) and *b* with almost 30

✉ José Ruiz-Herrera
jruiz@ira.cinvestav; jose.ruiz@cinvestav.mx

[1] Departamento de Ingeniería Genética, Unidad Irapuato, Centro de Investigación y de Estudios Avanzados del IPN, Km. 9.4 Carretera Irapuato-León, Irapuato, Gto., Mexico

[2] Instituto Tecnológico de Celaya, Celaya, Gto., Mexico

(Puhalla 1970). The *a* allele encodes a pheromone, and the receptor of the opposite pheromone, and *b*, components involved in mycelial growth, and the initiation and later stages of the pathogenic process. Mating occurs when cells containing different *a* and *b* alleles come close by and form conjugation tubes that grow to each other in response to the pheromone produced by the opposite mating cell, which is sensed by the corresponding pheromone receptor. Finally, both mating hyphal filaments fuse at their tips forming a dikaryotic hypha that forms an appresorium for the invasion of the host plant (see Froeliger and Leong 1991; Trueheart and Herskowitz 1992; Bölker et al. 1992; Lanver et al. 2018).

The yeast-to-hypha dimorphic transition that involves apical growth of the conjugation tubes, mating, and growth of the invading dikaryotic hypha is regulated by the MAPK and PKA signal pathways. Accordingly, after pheromone reception, a signal is transferred to both pathways that finally activate the transcription factor Prf1 that acts also as master regulator of the virulent process in response to signals from the host plant. Transfer of the signal to the PKA pathway involves the Gpa3 subunit, whereas a small G protein is involved in the signal transfer to the MAPK pathway (for reviews, see Klosterman et al. 2007; Brefort et al. 2009; Martínez-Soto and Ruiz-Herrera 2015).

U. maydis is able to grow in the laboratory in synthetic media. Thus, haploid strains grow in the yeast-like form in liquid or solid media containing glucose or sucrose and ammonium or nitrate salts (in fact, *U. maydis* can grow in the absence of an added nitrogen source, because it harbors a nitrogen-fixing bacterium endosymbiont (see Ruiz-Herrera et al. 2015), but this phenomenon is unrelated to the topic analyzed here). Mycelial growth can be induced on these solid media by mixing sexually compatible strains as result of their mating (the so-called Fuz reaction). Nevertheless, several conditions have been found that induce the mycelial growth of the fungus changing the composition of the growth medium. Thus, a relation between nitrogen metabolism and hyphal growth was identified (see Klosterman et al. 2007), and the best example was the observation that nitrogen starvation of diploid strains of *U. maydis* induces their growth as long filaments (Banuett and Herskowits 1994). Also, the nature of the carbon source is important for the type of growth of the fungus. As indicated above, *U. maydis* haploids grow yeast-like in synthetic medium containing a hexose or a disaccharide, but if the carbon source is changed for a fatty acid, haploid strains of *U. maydis* grow in the hyphal form, although its growth rate is reduced significantly (Klose et al. 2004). The authors suggested that it was the hydrophobic surface of the drops of the fatty acids the responsible agent for dimorphic induction. Considering that acetate is the metabolic product of fatty acids, in a further communication, Kretschmer et al. (2018) described that this acid induces reactive species producing cellular death, a reduction in virulence, and mitochondrial stress. But in these experiments, no dimorphic transition was observed. On the other hand, we have recently demonstrated that if ethanol and especially acetate are used as the sole carbon source at neutral pH, they induce the growth of haploid strains of *U. maydis* in the form of very long, thin, and septate hyphae (M. Salazar-Chávez and J. Ruiz-Herrera, preliminary observations; Fig. 1a, b). This result suggests that the change in the carbon metabolism pathways is the yeast-to-mycelium inducing factor. As a hypothesis to explain the drastic difference among these two results, we may suggest difference in oxygenation of the medium and subtle differences in the concentration of the salts in the medium.

As occurring for the dimorphic transition that takes place during mating, the pKA and the pheromone-response MAPK pathways are involved in the dimorphic transition of *U. maydis* in vitro, except that their roles are opposite; thus, the PKA pathway is involved in yeast-like development, whereas the MAPK pathway is involved in mycelial growth. Accordingly, it was observed that *uac1* mutants, that are affected in adenylate cyclase, grew constitutively in the hyphal form (Gold et al. 1994). This phenotype could be reverted to yeast-like growth by addition of cAMP, and by suppression, a technique that led to isolation of several yeast-like strains that were named *ubc* for Ustilago bypass cycle (Barrett et al. 1993; Gold et al. 1994). Their complementation gave rise to recovery of the hyphal phenotype. With these data, the corresponding *ubc* genes were found to encode the several members of the MAPK pathway involved in mating (for a review see Klosterman et al. 2007), demonstrating that this pathway is involved in hyphal growth of *U. maydis*.

Other stimuli involved in dimorphism, pH

Besides the inducers of the yeast-to-mycelium transition described above, we found that pH of the growth medium was also another factor that affected morphogenesis of *U. maydis*. Thus, it was demonstrated that whereas growth of the fungus at neutral or alkaline pH is in the yeast-like form, in acid pH medium, with a maximum of pH 3, *U. maydis* grows in the form of septated mycelium (Ruiz-Herrera et al. 1995a; Fig. 1c, d, respectively). The process was freely reversible from the mycelium to yeast transition, but the opposite was restricted to only a few hours of fungal growth. Interestingly, mutants deficient in *a* or *b* idiomorphs were unaffected in the process, indicating that this occurred by a mechanism independent of the one involved in mating (see above). Also, the dimorphic transition did not involve the mechanism of pH control (Pal/Rim process). Accordingly, in mutants in the gene encoding the transcription factor Pac/Rim101, the dimorphic transition at pH 3 was not affected (Aréchiga-Carvajal and Ruiz-Herrera 2005).

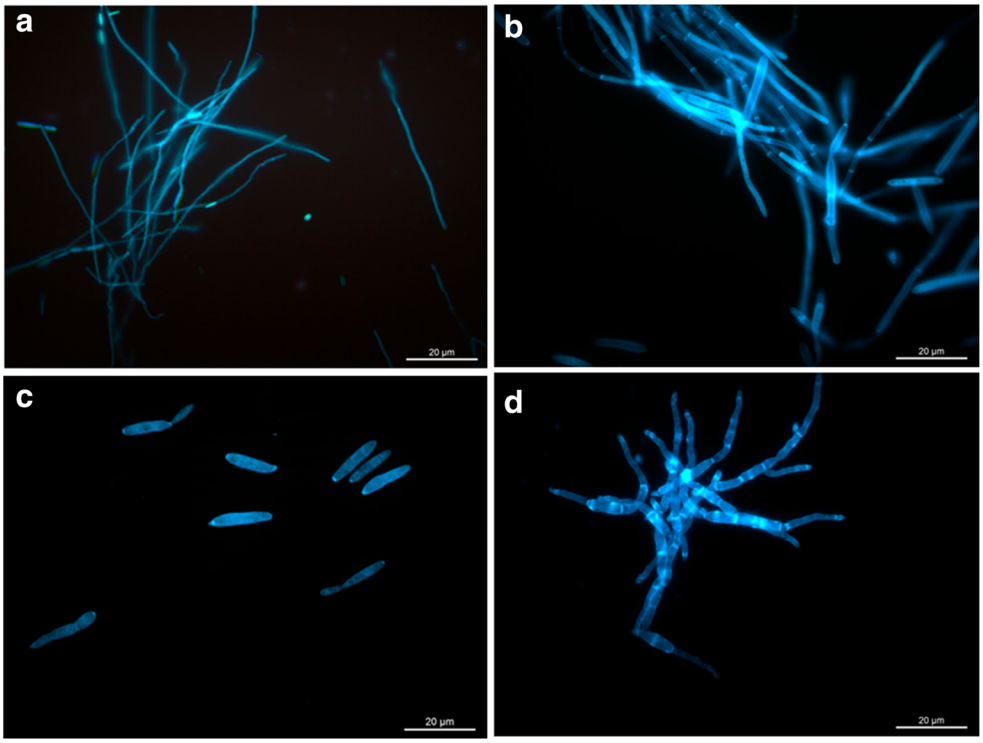

Fig. 1 Photographs that exemplify the dimorphic capacity of *Ustilago maydis*. **a** Hyphal cells obtained by growth at pH 7 in a synthetic medium containing ethanol as carbon source. **b** Morphology of cells grown in pH 7 synthetic medium with acetate. **c** Morphology of yeast cells grown in pH 7 synthetic medium with glucose as carbon source. **d** Hyphal cells grown in the same glucose-containing synthetic medium at pH 3. Calcofluor White staining. Magnification bar in all photographs, 20 µm

On the contrary, the yeast-to-mycelium dimorphic transition induced at pH 3 did not occur in mutants affected in different members of the MAPK pathway, and was inhibited by cAMP addition to the wild type strain. These data are evidence that the MAPK pathway is involved in mycelial growth, and the PKA pathway is involved in yeast-like growth, independently of the stimulus utilized (Martínez-Espinoza et al. 2004; Martínez-Soto and Ruiz-Herrera 2015).

More recently, using microarrays, we performed a transcriptomic analysis of the *U. maydis* yeast-to-mycelium dimorphic transition induced by cell transfer from neutral to acid pH (Martínez-Soto and Ruiz-Herrera 2013). In this study, we used the FB2 wild type strain, and as controls, a constitutive yeast mutant (CL211, Martínez-Espinoza et al. 1997) and a constitutive mycelial strain (*Δgcn5*, González-Prieto et al. 2014). By comparison of the data obtained by means of a Venn diagram, we identified 132 genes specifically involved in dimorphism. Besides those encoding unclassified proteins, the classes best represented were Metabolism and Transport and Cell Communication (24.7% and 15.6% of the total regulated genes).

Other factors involved in dimorphism: histone acetyltransferases, polyamines, DNA methylation, histidine kinases, and homeotic genes

Interestingly, it was observed (Gonzalez-Prieto et al. 2014) that the haploid **gcn5* mutants deficient in the gene encoding the histone acetyltransferase Gcn5 (see above) were avirulent and displayed a constitutive mycelial growth in liquid media independently of the pH. On solid media, contrasting with the wild type whose colonies were smooth and were formed by yeast-like cells, the colonies of the mutants showed a Fuz-like growth due to mycelium formation. The mutant phenotype was not reverted by cAMP addition, but only by transformation with the wild type gene. Mating was not affected in the mutants; they formed conjugation tubes, and induced the sexual partners to do the same, with formation of mating filaments. Taking into consideration that histone acetylation gives rise to DNA relaxation in the nucleosomes, these data indicate that access of different activators of DNA transcription, including transcription factors, negatively regulate the expression of genes involved in hyphal growth induced by mating or by acid pH, and that the Gcn5 histone acetyl transferase is necessary to regulate these mechanisms. This result is suggestive of an epigenetic control of dimorphism and virulence in *U. maydis*.

Further studies designed to investigate the role of Gcn5 involved the transcriptomic analysis of the FB2 *Δgcn5* mutant compared with the one from the wild type strain. This study revealed that a great number of genes, a total of 1176, were regulated by Gcn5. Of these, 547 were up-regulated and 629 were down-regulated (Martínez-Soto et al. 2015). Interestingly, it was found that a number of genes related to pathogenesis were up-regulated. Also, a high number of genes involved in the dimorphic process induced by acid pH were down-regulated by the histone acetyl transferase (82 in total).

Of these, 66 were down-regulated and only 16 were up-regulated. These results confirmed the data and suggestions described above, including also the virulence mechanism. Interestingly, some of the regulated genes formed clusters, an indication that they may be co-regulated. It is important to point out that 21 of these groups correspond to the pathogenesis clusters identified during the sequencing of the *U. maydis* genome (Kämper et al. 2006).

Histone deacetylase Hos2 and Clr3, which carry out the opposite reaction to Gcn5, were found that affect mating, and accordingly virulence, through a reaction involving directly on the mating genes through the cAMP-PKA pathway (Elías-Villalobos et al. 2015).

Putrescine (a diamine), spermidine (a triamine), and spermine (a tetraamine) are the most widely distributed polyamines in all prokaryotic and eukaryotic organisms. Polyamines are essential to carry out a great number of cellular functions, mainly proliferative and differentiation processes (Valdés-Santiago and Ruiz-Herrera 2015; Gevrekci 2017). *U. maydis* contains only putrescine and spermidine, and it was demonstrated that higher concentrations of polyamines than those necessary to sustain vegetative growth are required to support dimorphism induced by acid pH (Guevara-Olvera et al. 1997). Thus, an *odc* mutant (lacking ornithine decarboxylase (ODC), the first enzyme of the biosynthetic pathway of polyamine biosynthesis) grew normally with a 0.4 mM concentration of putrescine, but was unable to grow in the hyphal form at pH 3, requiring 5 mM putrescine or spermidine to carry out the dimorphic reaction in liquid or solid media. Further on, construction of a double mutant in the genes encoding ornithine decarboxylase and polyamine oxidase, *odc/pao* that was unable to form putrescine by the biosynthetic mechanism or by retroconversion from spermidine, showed a similar behavior, indicating that spermidine is the polyamine required for dimorphism (Valdés-Santiago et al. 2010; reviewed in Rocha and Wilson 2019). The formation of conjugation tubes and growth of the dikaryotic mycelium in *odc* mutants, also require high polyamine concentrations (F. Pérez-Rodríguez and J. Ruiz-Herrera in preparation), a further indication that similar mechanisms operate in the dimorphic transition induced by different stimuli.

Also, DNA methylation appears to play a role in the dimorphic transition of *U. maydis*, as well as in other fungi. Accordingly, using a modification of the amplified fragment length polymorphism method, we were able to demonstrate differences in the methylation patterns of DNA obtained from yeast or mycelial cells of *Mucor rouxii*, *Yarrowia lipolytica*, and the subject of the present review, *Ustilago maydis* (Reyna-López et al. 1997). The specificity of these changes in relation to dimorphism was confirmed by similar experiments where the dimorphic transition of *Y. lipolytica* and *M. rouxii*, and in an *odc* mutant for *U. maydis* was inhibited by addition of the ODC inhibitor diamino butanone (DAB; Reyna-López and Ruiz-Herrera 2004).

Regarding the mode of action of polyamines on dimorphism, there are a number of hypotheses, but no clear answer has been yet provided. Since DNA methylation is involved in gene silencing, we suggested a long time ago that polyamines might be involved in avoiding DNA methylation. In support of this hypothesis, we observed that the *Mucor* spp. *CUP* gene was expressed during spore germination only after germ tubes were formed. Addition of DAB inhibited both germ tube formation and gene expression (Cano-Canchola et al. 1992). A more direct evidence of the hypothesis was provided by in vitro experiments. It was observed that activity of cytosine-DNA methylases, but not adenine-DNA methylases, nor the corresponding restriction enzymes, was inhibited by polyamines at physiological concentrations (Ruiz-*Herrera* et al. 1995b).

More recently, in search of homologs of the histidine kinases involved in the two-component system *in U. maydis*, a homolog of the *Tco1* gene *of Cryptococcus neoformans* was identified (Yun et al. 2017). The role of this gene was analyzed by mutation. The authors found that the mutants were affected in the mating process, decreasing the levels of the genes encoding pheromones and pheromone receptors. Accordingly, they were unable to form conjugation and mating tubes, and failed to form filaments on solid medium (Fuz reaction). No analysis of the dimorphic transition in vitro was pursued. In a similar way, it may be indicated that the *bW* and *bE* genes involved in mating contain a homeobox (i.e., they are homeotic genes) and are required for hyphal growth during this process (Schulz et al. 1990), but they are not involved in the dimorphic transition induced in vitro by growth at an acid pH (Ruiz-Herrera et al. 1995a).

Concluding remarks

In the preceding pages, we have analyzed the yeast-to-hypha dimorphic transition of *Ustilago maydis*, analyzing the phenomenon from different points of view.

This process occurs in vivo during mating when the yeast-like cells change their growth pattern to apical, first during the formation of conjugation tubes, and second during the growth of the dikaryotic host-invading hypha. But in the absence of the plant, the dimorphic transition can be induced by a number of stimuli: nitrogen starvation, use of different carbon sources, change in the external pH, and possibly others not analyzed yet. Some aspects of these processes have common elements, for example, the signal transduction pathways, whereas others are different, and some of these systems may be important for pathogenesis, whereas others are not. The analysis of the genes regulated under each condition confirms this duality. Other factors that are involved in one or more of the different dimorphic inducing conditions that have been studied are among others, the alteration of the nucleosome structure due

to DNA methylation or histone or demethylation, and the requirement of polyamines.

The role played by the dimorphic phenomenon, if any, except for the one occurring in vivo, is difficult to discern, and to our knowledge, this aspect has been neglected in the studies on the matter. Nevertheless, the great plasticity of the behavior of *U. maydis* has been extremely important to discern the different mechanisms governing, not only the virulence, but also its behavior.

In conclusion, we can affirm that *U. maydis* has served as a model for studies performed in other fungi, pathogens or not, that will lead to a better knowledge of fungal physiology, behavior, and development. Although there are still many aspects of *U. maydis* that remain unknown, it is evident that our knowledge on this interesting fungus has advanced steadily in the last years.

Acknowledgments The work of the authors cited in the text was partially supported by Consejo Nacional de Ciencia y Tecnología (CONACYT) México. The review and comments improving this manuscript by Emeritus Prof. Angel Domínguez (University of Salamanca) are deeply acknowledged.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

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