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# The Sugar Sensor, Trehalose-6-phosphate Synthase (Tps1), Regulates Primary and Secondary Metabolism During Infection by the Rice Blast Fungus: Will *Magnaporthe oryzae*'s "sweet tooth" become its "Achilles' heel"?

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## Abstract

Rice blast disease is considered one of the most serious diseases of cultivated rice and is mediated by the causal agent, *Magnaporthe oryzae*. During infection, dome-shaped fungal cells, called appressoria, form on the surface of the leaf and generate turgor through the accumulation of glycerol. This enormous pressure is directed down onto a thin penetration hypha emerging from the base of the cell, forcing it through the surface of the rice leaf and allowing fungal colonization of the plant interior. The non-reducing disaccharide, trehalose, is present in conidia of *M. oryzae* and is mobilized during appressorium formation. The first step in trehalose biosynthesis involves trehalose-6-phosphate synthase (Tps1), and deletion of the *TPS1* gene in *M. oryzae* abolishes its ability to cause disease. This loss of pathogenicity was thought to be due to the role trehalose might play in turgor generation in the appressorium, or from the loss of the trehalose intermediate, trehalose-6-phosphate, a known signaling molecule in other organisms. However, subsequent analysis determined that, in *M. oryzae*, it is the Tps1 protein itself that is a central regulator of plant infection. Here, we discuss how the role of trehalose metabolism in *M. oryzae* development was determined to differ from other eukaryotes and show how, independent of its biosynthetic role, Tps1 functions as a sugar sensor to integrate carbon and nitrogen metabolism and regulate a subset of primary and secondary metabolic pathways, such as the oxidative pentose phosphate pathway and pigment formation, respectively, during plant colonization. This is a critical role that allows the fungus to adapt to the nutritional and redox conditions encountered in the plant cell and establish disease.

**Keywords:** rice blast; *Magnaporthe oryzae*, trehalose-6-phosphate synthase; glucose-6-phosphate; NADPH-dependent genetic switch; secondary metabolism

## Introduction

The non-reducing disaccharide, trehalose (*α*-D-glucopyranosyl-*α*-D-glucopyranoside) is found in a wide range of organisms, including bacteria, plants, invertebrates and fungi (Kaasen et al. 1994; Muller et al. 2001; Nwaka and Holzer 1998; Wyatt and Kale 1957). While commonly occurring as a storage compound, the trehalose molecule can also play a purely mechanical role in the protection of cells against numerous environmental stresses such as prolonged periods of desiccation (Crowe et al. 1998; Singer and Lindquist 1998). In addition, the metabolism of trehalose has been implicated in the regulation of diverse cellular processes in plants and fungi, such as growth and development in *Arabidopsis thaliana* and maize (Eastmond et al. 2002; Satoh-Nagasawa et al. 2006), the control of glycolysis in yeast (Hohmann et al. 1993; Thevelein and Hohmann 1995) and the establishment of disease by the devastating fungal pathogen of rice, *Magnaporthe oryzae* (Foster et al. 2003; Wilson et al. 2007). Focusing on this

intriguing regulatory role, how might trehalose metabolism affect such disparate cellular activities? To address this question, this review discusses our current understanding of trehalose metabolism with an emphasis on its role in regulating a subset of primary and secondary metabolic pathways in *M. oryzae* during rice plant infection.

## Trehalose metabolism in plants and yeast

The most common biosynthetic route to synthesize trehalose starts with trehalose-6-phosphate synthase (OtsA in bacteria or TPS in eukaryotes). TPS catalyzes the production of the intermediate trehalose-6-phosphate (T6P) from glucose -6-phosphate (G6P) and UDP-glucose (Foster et al. 2003; Vandesteene et al. 2010). T6P is subsequently dephosphorylated by trehalose phosphate phosphatase (TPP) to form trehalose (Figure 1). In *Saccharomyces cerevisiae*, trehalose biosynthesis also requires two regulatory subunits, TPS3 and TSL (Bell et al. 1998; Paul et al. 2008; Reinders et al. 1997), while *M. oryzae* carries only a single



**Figure 1.** Trehalose biosynthetic pathway.

TPS3 homologue (Wilson et al. 2007). During trehalose catabolism, trehalases hydrolyse trehalose into two glucose units.

In plants, trehalose is present in trace amounts and yet *AtTPS1*, the *A. thaliana* TPS1 homolog, is essential for embryo maturation (Eastmond et al. 2002). The embryos of  $\Delta AtTps1$  deletion strains demonstrated a series of pleiotropic effects, such as a reduction in the cell division rate, cell wall thickening and a delay in the early seed development that impacts the early cotyledon stage (Eastmond et al. 2002; Gomez et al. 2006). Moreover,  $\Delta AtTps1$  embryos, compared to wild type, accumulated sucrose and starch granules and demonstrated a reduction of genes involved in starch and sucrose degradation and an increase of genes involved in lipid mobilization and gluconeogenesis (Gomez et al. 2006). Subsequent work demonstrated regulation of these processes was mediated by the trehalose intermediate T6P, thus ascribing a signaling role to this molecule (Schluepmann et al. 2003).

T6P also plays a signaling role in yeast. In *S. cerevisiae*, alteration in trehalose production affects carbon catabolite repression, glycogen accumulation and sporulation (De Silva-Udawatta and Cannon, 2001; Thevelein and Hohmann 1995).  $\Delta tps1$  mutant strains of yeast are unable to utilize glucose as a sole carbon source (Van Aelst et al. 1993) because T6P inhibits hexokinase activity (Blazquez et al. 1993). The loss of T6P in  $\Delta tps1$  strains results in the unregulated influx of glucose into glycolysis which, because the early steps in glycolysis are ATP-consuming, ensures a catastrophic depletion of ATP and free inorganic phosphate (Blazquez et al. 1993; Thevelein and Hohmann 1995) and the accumulation of phosphorylated glycolytic intermediates – a phenomenon termed glycolytic misregulation.

### The role of trehalose metabolism during infection by the rice blast fungus *Magnaporthe oryzae*

The filamentous fungus, *Magnaporthe oryzae*, is the causal agent of the devastating rice blast disease. This pathogen is the most serious disease of cultivated rice and is considered a grave threat to global food security due to its annual destruction of 10–30% of the world rice crop – enough rice to feed more than 60 million people – resulting in losses of approximately US \$6 billion (Pennisi 2010; Wilson and Talbot 2009; Zeigler et al. 1994). Because traditional breeding strategies have largely struggled to contain this disease, researchers have turned to molecular analysis to determine what cellular processes are required

by the fungus to cause disease in an attempt to uncover new and effective targets for mitigation strategies. Thus, in recent years, *M. oryzae* has been developed as an excellent model organism for studying molecular plant pathogen interactions. Both the pathogen and its host have sequenced genomes (Dean et al. 2005; Yu et al. 2002) and unlike many plant pathogenic fungi, the fungus can be cultured away from the host on defined media. This allows the facilitation of detailed biochemical and molecular analysis, and genetic manipulation by transformation, to unlock the secrets of infection by this fungus.

Rice blast infection begins when a three-celled asexual spore of *M. oryzae* lands on the surface of a rice leaf and germinates. While the germ tube is growing across the surface of the leaf, the fungus is actively monitoring its environment. If it is provided with a nutrient-free, hydrophobic surface, the germ tube begins to swell and form a specialized dome-shaped structure, called the appressoria (Wilson and Talbot 2009). Appressorium development is dependent on functioning MAP kinase and G-protein signaling pathways (Xu and Hamer, 1996), and both a cell cycle event and autophagic cell death of the asexual spore (Kershaw and Talbot 2009; Saunders et al. 2010; Veneault-Fourrey and Talbot 2007). Cutinases secreted by the fungus are also required for appressoria development (Skamnioti and Gurr 2007). Once the appressorium has matured, turgor pressure generated through the accumulation of up to 3 M glycerol (de Jong et al. 1997) acts on a penetration peg emerging at the base of the cell, causing it to breach the leaf surface. The fungus enters and grows within the plant tissue surrounded by the invaginated plant plasma membrane, moving from cell to cell through plasmodesmata and obtaining nutrients via bulbous branched hyphal cells (Kankanala et al. 2007). Small cys-rich proteins with likely roles in biotrophic invasion are secreted by *M. oryzae* at this time (Mosquera et al. 2009). During this biotrophic stage of growth, plant cells remain viable as the fungus moves through them, but later, necrotic lesions form on the surface of the leaf from which newly formed fungal spores are dispersed into the environment, allowing the life cycle to continue.

An active area of research has been to understand what storage compounds present in the conidium could contribute to turgor generation in the appressorium. Foster et al. (2003) focused on the role trehalose might play in generating turgor, and showed that this sugar is mobilized during appressorium formation. Through functional analysis of trehalose anabolism and catabolism in *M. oryzae*, they determined that trehalose breakdown is important for the efficient development of the fungus in plant tissue, while trehalose synthesis by *Tps1* is absolutely required for pathogenesis. Using homologous recombination to delete the *TPS1* gene, they showed that the resulting  $\Delta tps1$  strains could not produce trehalose, were severely attenuated for virulence and, like the yeast  $\Delta tps1$  strains, displayed glycolytic misregulation through

an inability to grow on glucose-containing media (Foster et al. 2003). Interestingly, *Δtps1* strains were reduced in sporulation but able to form appressoria.

### New role for Tps1 as an integrator of carbon and nitrogen metabolism in *Magnaporthe oryzae*

Like *S. cerevisiae*, *M. oryzae Δtps1* mutants appeared to exhibit glycolytic misregulation as evidenced by an inability to grow on glucose-containing media and the accumulation of glycolytic intermediates (Foster et al. 2003; Wilson et al. 2007). However, in dissecting the relationship between glycolytic misregulation and plant disease, and the mechanisms by which trehalose contributes to disease, several lines of evidence emerged that suggested this was not necessarily the case. Unlike in yeast, T6P was not shown to have an effect on *M. oryzae* hexokinase activity (Wilson et al. 2007). ATP levels were elevated (rather than depleted) in *M. oryzae Δtps1* mutant strains (Wilson et al. 2007) and, not seen for yeast *Δtps1* mutants, growth of *M. oryzae Δtps1* mutant strains on glucose-containing media was restored by adding amino acids (Foster et al. 2003). In investigating this growth suppressing role of amino acids in the growth media, it was subsequently discovered that the amino acids act not as suppressors of glycolytic misregulation, as first thought, but rather as alternative sources of nitrogen. In a profound departure from the situation in yeast, rather than being sensitive to glucose, *M. oryzae Δtps1* mutant strains were shown to be unable to utilize the nitrate, present in Cove's minimal growth media, as a nitrogen source (Wilson et al. 2007). When amino acids were added to the media or when other sole nitrogen sources were used, the *Δtps1* strains were able to utilize the alternative nitrogen source and grow regardless of carbon source. This was true of all the amino acids, except cysteine, and crucially was also true of nitrite. The metabolism of nitrite differs from that of nitrate by one enzymatic step, nitrate reductase. Therefore, the ability of *Δtps1* strains to grow on nitrite- but not nitrate-containing media (with glucose) resulted in the conclusion that *Δtps1* strains are nitrate non-utilizing (Wilson et al. 2007). The next question to be asked was what, therefore, is the connection between carbon (i.e. trehalose) and nitrogen (i.e. nitrate) metabolism? The answer was glucose-6-phosphate (G6P).

G6P is the substrate of Tps1, but also of glucose-6-phosphate dehydrogenase (G6PDH). G6PDH uses G6P to generate NADPH in the oxidative pentose phosphate pathway, and NADPH provides the reducing power for many enzymatic reactions, including the reduction of nitrate to nitrite by nitrate reductase (NR). Tps1 was shown to regulate NADPH production in the pentose phosphate pathway through control of G6PDH, and *Δtps1* strains have reduced NADPH levels during growth on nitrate-containing media due to decreased G6PDH activity. This Tps1-dependent regulation of G6PDH by Tps1 occurs in

response to G6P sensing, as demonstrated by the introduction of mutations into the G6P binding site of Tps1 that destroy the catalytic site. However, while all the mutations resulted in strains unable to make trehalose or T6P, mutations that allowed G6P access to the binding site resulted in strains that were able to cause disease (and one of which was also restored for growth on nitrate), while mutations that occluded G6P from the active site completely were non-pathogenic. The conclusions drawn were that trehalose and T6P are not required for pathogenicity, thereby discounting a significant role for trehalose in turgor generation and T6P signaling in virulence. Rather, it is the sensing of G6P by Tps1 that results in the activation of G6PDH in the pentose phosphate pathway and concomitant production of NADPH resulting in nitrate utilization. In addition, Tps1 was also shown to regulate glycogen utilization and pathogenicity by controlling the expression of genes encoding two known *M. oryzae* virulence factors, the *MPG1* hydrophobin gene and *PTH11* encoding a G-protein-coupled receptor-encoding involved in surface sensing. (DeZwaan et al. 1999; Soanes et al. 2002; Talbot 2003; Wilson et al. 2007). Therefore, independent of its biosynthetic role, Tps1 has a signaling function in controlling a number of disparate cellular processes – through the integration of carbon and nitrogen metabolism in response to G6P – that are essential to the establishment of disease.

### Tps1 regulates gene expression via the modulation of NADPH

How is Tps1, ostensibly a biosynthetic enzyme, able to regulate gene expression in response to G6P sensing and cause disease? The answer lies in understanding how genes are regulated in *M. oryzae* in response to available nitrogen source. Principally, in addition to post-translational regulation of G6PDH activity by Tps1 during growth on nitrate, there is also Tps1-dependent transcriptional control of genes required for nitrate utilization, with expression of both the NR (*NIA1*) and nitrite reductase (*NII1*) structural genes down-regulated in *Δtps1* strains compared to wild type (Wilson et al. 2007). In the soil saprophyte, *Aspergillus nidulans*, expression of the *NIA1* homologue, *niaD*, is dependent on the wide domain transcription factor, AreA, and the AreA repressor, NmrA (Andrianopoulos et al. 1998; Todd et al. 2005). During growth on nitrate, AreA, a GATA family member, activates *niaD* transcription by binding GATA sites in the *niaD* promoter (Wilson and Arst 1998). During growth on the preferred nitrogen source ammonium, NmrA physically interacts with AreA to mask its DNA-binding domain and prevent binding to its cognate promoter sites, thereby regulating AreA-dependent gene expression, including *niaD*, in response to available nitrogen source. Interestingly, the *M. oryzae nmrA* homologue, *NMR1*, was constitutively expressed in *Δtps1* mutant strains

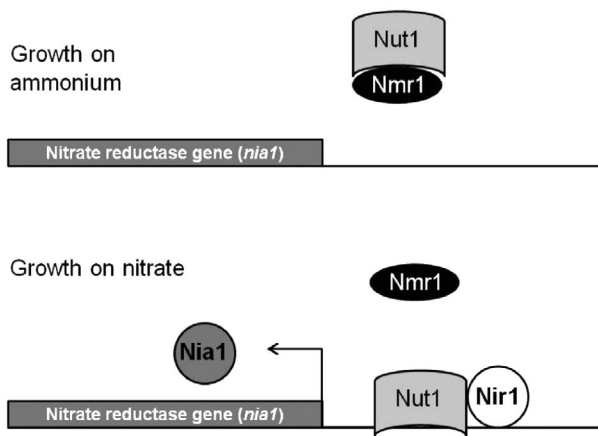


regardless of nitrogen source (Wilson et al. 2007). Downloaded by Because *M. oryzae* carries a GATA transcription factor, Nut1, with functional homology to AreA (Froeliger and Carpenter 1996), it was determined through gene expression and yeast two-hybrid analysis that, in wild type in the presence of ammonium, Nmr1 binds Nut1 to inhibit DNA binding, while under nitrate growth conditions, Nmr1 dissociates from Nut1, allowing it to activate the transcription of *NIA1* (Wilson et al. 2010; Wilson et al. 2007) (Figure 2). In  $\Delta tps1$  mutant strains, therefore, Nmr1 is constitutively present and likely bound to Nut1 under all nitrogen conditions, preventing *NIA1* gene expression and locking the fungus into an ammonium-responsive status regardless of nitrogen source. How, then, is nitrate utilization related to pathogenicity? Gene deletions involving *NIA1*, *NUT1* and *NIR1* (a pathway specific activator of *NIA1*) resulted in *M. oryzae* mutant strains unable to utilize nitrate (Froeliger and Carpenter 1996; Lau and Hamer 1996; Wilson et al. 2010), but fully pathogenic on rice leaves. Therefore, the ability to utilize nitrate is not required for rice blast disease.

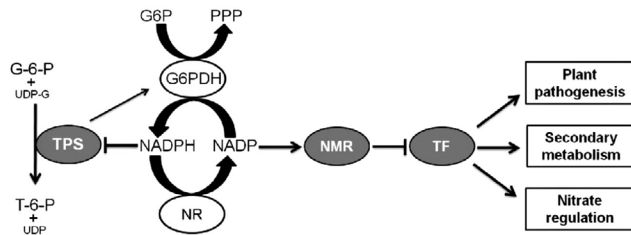
Although Nut1 and nitrate utilization is not required for pathogenicity, it seemed likely that constitutive Nmr1 activity resulting in Nut1 inhibition in  $\Delta tps1$  strains might also regulate other GATA transcription factors necessary for infection. The genome of *M. oryzae* carries a total of three *NMR* orthologues, *NMR1*, *NMR2* and *NMR3*, and at least 10 GATA transcription factors (Dean et al. 2005). The *NMR* orthologues were first disrupted in  $\Delta tps1$  strains and all the resulting mutants, surprisingly, restored pathogenicity to  $\Delta tps1$  strains (Wilson et al. 2010). This suggests the Nmr proteins negatively regulate other GATA factors necessary for pathogenicity and are themselves negatively regulated by Tps1 in the pathway: Tps1 – Nmr – GATA transcription factor(s) → Pathogenicity (where – indicates negative and → indicates positive regulation). To identify what these GATA factors might be, yeast two-hybrid

experiments were conducted that showed the Nmr repressor proteins physically interacted with at least two other GATA factors in addition to Nut1: Asd4 and Pas1 (Wilson et al. 2010). Functional characterization of Asd4 and Pas1 showed that  $\Delta asd4$  deletion strains were reduced in sporulation while  $\Delta pas1$  strains hypersporulated. Tellingly,  $\Delta asd4$  strains were also unable to make functional appressoria; demonstrating regulation of infection-related morphogenesis by Tps1 occurs via Nmr control of at least one GATA transcription factor. What genes does Tps1 regulate via the Nmr control of GATA transcription factors? Gene expression analysis by Wilson et al (2010) determined six genes encoding NADPH-requiring enzymes (including NR) and two genes encoding known virulence factors (*MPG1* and the polyketide *ALB1* involved in melanin production in the appressorium (Chumley and Valent 1990) are regulated by Tps1 and the Nmr proteins.

How does Tps1 regulate Nmr activity? Yeast two hybrid analysis determined there was no physical interaction between Tps1 and the Nmrs, so any regulation had to be indirect. A clue came from investigating the relationship between Tps1 and G6PDH activity, where it was discovered that overexpressing the *G6PDH* gene in  $\Delta tps1$  strains increased G6PDH activity by  $260.4 \pm 5\%$  and partially restored virulence in a susceptible rice cultivar, suggesting that G6PDH activity, and presumably NADPH production, is important during appressorium-mediated plant infection. Tps1 was also shown to competitively bind NADPH such that, at elevated concentrations, NADPH could displace the native substrates from the binding site, thereby preventing G6P sensing and inactivating the Tps1-signaling pathway. NADPH is formed from NADP in the pentose phosphate pathway, and sequence analysis revealed the *M. oryzae* Nmr proteins, like the *A. nidulans* NmrA homolog, possess a Rossmann-fold motif required for NADP binding (Lamb et al. 2004; Wilson et al. 2010). Mutating the Rossmann fold by introducing a Thr to Val change at position 13 abolished the function of *M. oryzae* Nmr1, suggesting it requires NADP for binding. In *A. nidulans*, mutating the equivalent Thr-14 of NmrA to Val diminished the affinity of NmrA for NADP *in vitro*, but the NmrA protein can still bind AreA (although the affect of this mutation on the NmrA/ AreA interaction *in vivo* is not known (Lamb et al. 2004)). However, consistent with the observations in *M. oryzae*, the corresponding T14V sequence change in an NmrA homologue from *Dictyostelium discoideum*, PadA, did not complement the *padA*- mutant phenotype *in vivo* (Nunez-Corcuera et al. 2008), suggesting the conserved threonine residue in the Rossmann fold of this Nmr homologue is also required for function *in vivo*. Because, as noted above, a number of genes regulated by Tps1 encode NADPH-requiring enzymes, taken together, these observations support the NADPH-signaling pathway shown in Figure 3. In response to G6P sensing by Tps1, NADPH is produced from NADP by G6PDH. NADPH



**Figure 2.** How Nmr1 interacts with the regulator of nitrogen metabolism, Nut1, during growth on preferred (ammonium) and less preferred (nitrate) nitrogen sources.



**Figure 3.** Model describing a novel mechanism of gene regulation by Tps1 in response to G6P. Adapted from Wilson et al. (2010). PPP = pentose phosphate pathway; TF = GATA transcription factors; Nmr represents Nmr1, Nmr2 and Nmr3.

production is linked to both NADPH consumption and available G6P by a negative-feedback mechanism involving the competitive displacement of G6P and UDPglucose substrates from the Tps1 active site by NADPH, presumably preventing G6P sensing and inhibiting G6PDH activity. When NADPH-requiring enzymes, such as NR, consume NADPH, more NADPH will be produced if G6P can re-enter the Tps1 active site. However, if cellular G6P levels are low and not detected by Tps1, NADP will accumulate, resulting in NADP-dependent activation of the Nmr proteins and repression of gene expression, including those encoding NADPH-requiring enzymes that consume available NADPH. This model describes a sensitive mechanism for regulating gene expression in response to fluctuating NADPH/NADP levels and available G6P. The importance of this switch to rice blast likely lies in allowing the fungus to rapidly detect and adapt to the transition from the nutrient-free surface of the leaf to the nutrient-rich interior of the host. The appressorium develops only under the nutrient-free conditions of the leaf surface, when NADP levels are expected to be high and the Nmr proteins are actively repressing. This is also the constitutive situation in  $\Delta tps1$  strains, which form appressoria and can penetrate onion epidermal tissue (Wilson et al. 2010). Once wildtype appressoria have forced the penetration peg through the surface of the leaf, the influx of nutrition in the form of G6P is sensed by Tps1 and results in G6PDH activation, increased NADPH production at the expense of NADP, and the alleviation of Nmr gene repression. The subsequent and rapid genetic reprogramming that ensues allows the fungus to elaborate infectious hyphae *in planta*, suppress or evade host defenses, and establish disease. Therefore, unlike in plants and yeast, the role of Tps1 in *M. oryzae* is to orientate fungal development with regards to the interior of the host plant.

### Evidence for Tps1-dependent regulation of secondary metabolism

Melanin is a secondary metabolite that plays an essential structural role in strengthening the appressorium and allowing turgor generation (Chumley and Valent

1990). The Tps1-dependent expression of *ALB1*, encoding a polyketide synthase involved in melanin biosynthesis, intriguingly suggests that, in addition to regulating primary metabolic pathways (i.e. the pentose phosphate pathway), Tps1 might also control certain secondary metabolism pathways. In nature, fungi produce an enormous array of natural products (Calvo et al. 2002), and plant pathogenic fungi in particular produce diverse secondary metabolites that aid in pathogenicity (Wolpert et al. 2002). However, the capacity of most plant pathogens to produce secondary metabolites pales into comparison with that predicted for *M. oryzae*, whose genome is greatly expanded for the number of genes associated with secondary metabolism compared to other fungi. In the *M. oryzae* genome, 23 genes are predicted to encode polyketide synthases (PKS), six genes encode non-ribosomal peptide synthases (NRPS) and eight genes encode PKS-NRPS hybrid enzymes (Dean et al. 2005). While the secondary metabolites produced by this large repertoire of enzymes, and the role they play in virulence, is largely unknown, one of the hybrid PKS-NRPS proteins is encoded by the avirulence gene *ACE1* (Bohnert et al. 2004; Collemare et al. 2008). *M. oryzae* isolates carrying the functional AVR gene *ACE1* are unable to infect rice cultivars carrying the corresponding R gene *Pi33*. However, mutation of the putative catalytic site of the  $\beta$ -ketoacyl synthase domain of Ace1 abolishes recognition of the fungus by resistant rice, suggesting that effector-triggered immunity in rice can be caused not by host recognition of the Ace1 protein, but by recognition of the secondary metabolite it produces (Bohnert et al. 2004). It is conceivable, considering the large numbers of genes likely involved in secondary metabolism, that *M. oryzae* produces a battery of compounds to suppress plant defenses and perturb host metabolism to the benefit of the invading pathogen. Considering Tps1 controls the expression of genes required *in planta* for establishing disease, if secondary metabolites are involved in suppressing the host defense, are any of the genes involved under Tps1 control?

Tps1 has been shown to regulate a number of genes encoding NADPH-requiring enzymes (Wilson et al. 2010). Two of those genes, the aldo/keto reductase *ALD1* (MGG\_15113.6) and the short chain dehydrogenase *SDY1* (MGG\_10910.6) (Wilson et al. 2010) are spatially separated in the genome by only one gene, MGG\_10909.6, encoding a hypothetical protein (Dean et al. 2005). The expression of MGG\_10909.6, like *ALD1* and *SDY1*, is also under Tps1 control and is expressed 8-fold higher in wild type compared to  $\Delta tps1$  strains, after growing the fungus on nitrate-containing media following a shift from complete media (Fernandez and Wilson, unpublished results). This suggests these genes are co-regulated in a Tps1-dependent manner. Examining the expression of the gene immediately upstream of *ALD1*, MGG\_10907 (encoding a hypothetical protein), showed it was 10-fold more highly expressed in wild

type strains than  $\Delta tps1$  strains during growth on nitrate media (Fernandez and Wilson, unpublished results). Then, the expression of the three genes lying downstream of *SDY1* was analyzed: MGG\_10911 encoding a hypothetical protein (22-fold more highly expressed in wild type strains than  $\Delta tps1$  strains); MGG\_10912 encoding a putative polyketide synthase (14-fold more highly expressed in wild type strains than  $\Delta tps1$  strains); and MGG\_10913 encoding a second short chain dehydrogenase (14-fold more highly expressed in wild type strains than  $\Delta tps1$  strains) (Fernandez and Wilson, unpublished results). The genes, located immediately up- and downstream of this small seven gene Tps1-dependent cluster, do not appear to be regulated differently in wild type compared to  $\Delta tps1$  strains. Therefore, the expression of these seven clustered genes are co-regulated in a Tps1-dependent manner and include at least one PKS, suggesting this gene cluster might be involved in secondary metabolite production. Considering *ALD1* and *SDY1* gene expression is also dependent on Nmr function (Wilson et al. 2010), it seems likely that this cluster is regulated by Tps1 and the Nmr proteins in response to G6P sensing and NADPH levels. The significance of this result is two-fold; firstly, the regulation of at least two PKS gene (*ALB1* and MGG\_10912) and a small gene cluster supports a role for Tps1 in regulating at least a subset of secondary metabolic pathways; secondly, it raises the novel possibility that, downstream of Tps1 and the Nmr proteins, clustered gene regulation is linked to NADPH metabolism. Such a role for Tps1 in secondary metabolism is logical considering Tps1 is required for the fungus to adapt to the host interior, and one way it needs to achieve this is through the suppression of the host defenses, perhaps through the use of secondary metabolites. It must be pointed out, however, that the AVR gene *ACE1* is not subject to Tps1-dependent expression regulation (Fernandez and Wilson, unpublished results). Future work will involve using Tps1 as a tool to identify which secondary metabolites are altered in abundance in  $\Delta tps1$  strains compared to wild type, which Tps1-dependent genes are associated with their production, and what role the resulting product has on establishing rice blast disease. In addition, Tps1 could serve as a model for how integrators of metabolism might function in other fungi to regulate secondary metabolism. Identifying and understanding such integrators would be particularly important for mitigating against important agricultural diseases caused by fungal secondary metabolites, such as aflatoxin contamination in corn, produced by *Aspergillus* spp. and the most carcinogenic natural product known (Wilson et al. 2002), and deoxynivalenol (DON), the mycotoxin produced in wheat and barley grain infected by *Fusarium* head blight or scab (Bushnell et al. 2010).

## Summary

Trehalose metabolism plays a role in a diverse number of physiological processes in a diverse range of organisms. In the devastating rice pathogen *M. oryzae*, the trehalose biosynthetic enzyme Tps1 was shown to be essential for pathogenicity but subsequent dissection of the  $\Delta tps1$  phenotype ruled out glycolytic misregulation and trehalose biosynthesis as determinants of virulence. Rather, the Tps1 protein was shown to function as a sensor of its native substrate G6P to regulate infection-related gene expression and control primary and secondary metabolic pathways during disease progression.

We believe the extensive role described here for *M. oryzae* Tps1 in many cellular processes – involving enzyme activation, control of metabolic flux and transcriptional regulation – is unprecedented for a biosynthetic protein in fungi and leads one to ask how such a signaling function for this Tps1 homologue could arise? Is it specific to the hemibiotroph lifestyle of plant pathogens like *M. oryzae*, to plant pathogens in general, or does it operate in other fungi and plants? In the case of fungi, Tps1 homologues have been identified and disrupted in other fungi, such as *TPSA* in *A. nidulans* (Fillinger et al. 2001), but whether the resulting mutant strains demonstrate glycolytic misregulation and an inability to grow on glucose media, or are unable to grow on nitrate regardless of the carbon source in the media, is an important distinction that needs to be elucidated. Downstream of Tps1, *Nut1*/*AreA* and *Nmr* homologues have been shown in other plant pathogens to be involved in fungal pathogenicity and secondary metabolite production (Lopez-Berges et al. 2010; Wagner et al. 2010). However, whether *Nmr* and *AreA* activity in these fungi is connected to a metabolic integrator such as Tps1, and whether G6P sensing by the integrator (or integrators) is transmitted via an NADPH/NADP signal transduction pathway, is currently unknown. In *M. oryzae*, Tps1-dependent regulation of primary metabolism in response to G6P and NADPH levels likely allows the fungus to adapt rapidly to fluctuating nutritional and redox conditions found within the plant cell, while controlling the production of secondary metabolites could help the fungus suppress or evade the host response. Therefore, Tps1 presents itself both as a tool for identifying new secondary metabolic pathways required for fungal virulence, and as a target for novel rice blast mitigation strategies. Future research should ensure this sugar sensor, essential for pathogenicity, becomes the Achilles' heel of the otherwise formidable rice blast fungus.

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