TPS complex-mediated regulation of trehalose 6-phosphate homeostasis is critical for development and pathogenesis in Magnaporthe oryzae

Xin Chen, Yakubu Abubakar, Chengdong Yang, Xiaxia Wang, Pengfei Miao, Mei Lin, Yuetong Wen, Qiuqiu Wu, Haoming Zhong, Yuping Fan, Meiru Zhang, Zonghua Wang, Jie Zhou, and Wenhui Zheng

Corresponding Author(s): Wenhui Zheng, State Key Laboratory for Ecological Pest Control of Fujian and Taiwan Crops

Review Timeline:

Submission Date: April 14, 2021
Editorial Decision: June 1, 2021
Revision Received: August 30, 2021
Accepted: September 8, 2021

Editor: Ileana Cristea

Reviewer(s): Disclosure of reviewer identity is with reference to reviewer comments included in decision letter(s). The following individuals involved in review of your submission have agreed to reveal their identity: Zhengguang Zhang (Reviewer #1); Rongyu Li (Reviewer #2)

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

DOI: https://doi.org/10.1128/mSystems.00462-21
June 1, 2021

Dr. Wenhui Zheng
State Key Laboratory for Ecological Pest Control of Fujian and Taiwan Crops
Fuzhou, Fujian 350002
China

Re: mSystems00462-21 (TPS complex-mediated regulation of trehalose 6-phosphate homeostasis is critical for development and pathogenesis in Magnaporthe oryzae)

Dear Dr. Wenhui Zheng:

Thank you for submitting your manuscript to mSystems. We have completed our review and I am pleased to inform you that, in principle, we expect to accept it for publication in mSystems. However, acceptance will not be final until you have adequately addressed the reviewer comments.

The reviewers suggested a number of additions and changes that will further strengthen the manuscript. In particular, one of the reviewers indicated that additional evidence may be necessary to demonstrate the T6P is induced during fungus development. We invite the submission of a revised manuscript that addresses the reviewers comments.

Thank you for the privilege of reviewing your work. Below you will find instructions from the mSystems editorial office and comments generated during the review.

Preparing Revision Guidelines
To submit your modified manuscript, log onto the eJP submission site at https://msystems.msubmit.net/cgi-bin/main.plex. Go to Author Tasks and click the appropriate manuscript title to begin the revision process. The information that you entered when you first submitted the paper will be displayed. Please update the information as necessary. Here are a few examples of required updates that authors must address:

- Point-by-point responses to the issues raised by the reviewers in a file named "Response to Reviewers," NOT IN YOUR COVER LETTER.
- Upload a compare copy of the manuscript (without figures) as a "Marked-Up Manuscript" file.
- Each figure must be uploaded as a separate file, and any multipanel figures must be assembled into one file.
- Manuscript: A .DOC version of the revised manuscript
- Figures: Editable, high-resolution, individual figure files are required at revision, TIFF or EPS files are preferred

For complete guidelines on revision requirements, please see the Instructions to Authors at https://msystems.asm.org/sites/default/files/additional-assets/mSys-ITA.pdf. Submissions of a paper that does not conform to mSystems guidelines will delay acceptance of your manuscript.

Corresponding authors may join or renew ASM membership to obtain discounts on publication fees. Need to upgrade your membership level? Please contact Customer Service at
Reviewers comments:

Reviewer #1 (Comments for the Author):

The manuscript describes the balance of T6P accumulation in the rice blast disease which caused the defects in the fungal invasion structure and virulence. The author found a self-mutation of its MoTPS3 caused the recovery on the deletion of MoTps2. The author design various methods to verify the hypothesis that the organisms maintain a safe T6P level and cope with its cytotoxicity effects by the self-mutation. The manuscript was well-written, however, in my point, the ms lack some key evidences for the conclusion which led to the misleading to the readers.

The most important tissue is when the T6P enhanced? In line 86-87, When trehalose is no more required (such as in the absence of 86 stress)? However, the author showed no evidence here that during the development of the fungus the T6P was induced. As this evidence missing, the author lacks the results that the Tps3 mutation in vivo. I do not agree to the conclusion in the MS that the self-mutation of TPs3 is required for the virulence.

Another major gap is that, how the tps3 mutation happened? NO evidence here. Are these sites active at the basic level even not in the deletion of MoTps2? The mutation of Tps3 is only dependent to Tps2 or once immature of the appressorium? After reading carefully throughout the Ms, I still can not follow that why the mutation occurred. I think the author should provided more evidence here.

Reviewer #2 (Comments for the Author):

Chen et al. investigated the mechanism by which the physiologic level of trehalose-6-phosphate (T6P) is regulated in the rice blast fungus M. oryzae. Despite excellent presentation, few
Grammatical errors need to be fixed in the manuscript.

L128-132: You rather observed (not characterized) a spontaneous mutation that partially restores the defects. Also, this sentence is too long, making it difficult to grab the message. I suggest you break it into two simpler sentences.

L142: define aa (as it appears here for the very first time)

L149: '.........in the Motps2 mutant' please recast as '.........in the Motps2 mutant background'

L151: '.........to their compare their growth rates' please check and fix the error.

L236: '.........appropriate location of CHS genes is essential for cell wall homeostasis' (please provide a reference for this).

L272-273: under what condition(s) does the mutant produce spontaneous suppressor strain? This is necessary because I cannot see the strain emerging in other culture plates as in Fig. 1 A.

L595: Delete one 'Afterwards'.

The materials and methods section does not explain how the various forms of mutation that exist in the spontaneous strain were identified.
Response to Reviewer #1

The manuscript describes the balance of T6P accumulation in the rice blast disease which caused the defects in the fungal invasion structure and virulence. The author found a self-mutation of its MoTPS3 caused the recovery on the deletion of MoTps2. The author design various methods to verify the hypothesis that the organisms maintain a safe T6P level and cope with its cytotoxicity effects by the self-mutation. The manuscript was well-written, however, in my point, the ms lack some key evidences for the conclusion which led to the misleading to the readers.

Response

We thank the reviewer for the constructive criticism and time spent to analyze this manuscript. Our responses and explanations in relation to the comments are provided below. We hope that the changes we have made resolve all your concerns about the article. We are more than happy to make any further changes that will improve the paper and/or facilitate successful publication.

The most important tissue is when the T6P enhanced? In line 86-87, When trehalose is no more required (such as in the absence of 86 stress)? However, the author showed no evidence here that during the development of the fungus the T6P was induced. As this evidence missing, the author lacks the results that the Tps3 mutation in vivo. I do not agree to the conclusion in the MS that the self-mutation of TPs3 is required for the virulence.

Response

Thanks for your positive comments. Coupled with previous studies and our results, the accumulated T6P can only be found in the ΔMotps2 mutant (~2000-fold) from the fungal mycelia. Both ΔMotps1 and ΔMotps3 mutants are impaired of T6P production. In Fig. 4 e and Fig.6 a, our results also indicated that the intracellular T6P maintains a relatively low level in the mycelium. The ΔMotps2 mutant has severe defects in conidiation and appressorium formation. We could not obtain enough conidia and appressorial tissues for further GC-MS analysis. In Fusarium graminearum, loss of FgTps2 also leads to highly accumulated T6P level in both mycelia and conidia. It is possible that the conidia and appressoria of the ΔMotps2 mutant also accumulated high level of T6P and thus leads to the observed severe defects. MoTps1 is involved in the biosynthesis of T6P. To increase the intracellular level of T6P, we generated ΔMotps1-OE strains by overexpressing the trehalose 6-phosphate synthase MoTPSI in the wild type (Fig.R1 A). Although over-expression of MoTPSI leads to defects in growth, conidiation and virulence, the ΔMotps1-OE strain has no significant effect on T6P production (Fig.R1 B). Since trehalose biosynthesis is induced when exposed to environmental stresses, we further monitored the expression level of the TPS complex subunits through qRT-PCR assays. We noticed that the expression levels of MoTPSI and MoTPS3 increased by ~1.8-fold under oxidative stress (Fig.R1 C). In the ΔMotps1-OE strains, the expression level of the MoTPSI was
up-regulated by ~10-fold, while there was only little increase in the T6P level. These results also pushed us to investigate how *M. oryzae* physiologically overcomes the stress due to T6P accumulation. Interestingly, we noticed that the ΔMotps2 mutant undergoes spontaneous mutation in its *MoTPS3* gene to take care of the accumulated T6P. Further results indicated that the spontaneous mutants have low level of intracellular T6P. This finding indicates an important role of the MoTps3 in modulating T6P level. Based on these results, we suggested that *M. oryzae* maintains a low level of T6P under normal condition, and the T6P level could increase under stress condition.

![Fig.R1](image)

**Fig.R1** Over-expression of *MoTPS1* has no significant effect on the production of trehalose and trehalose 6-phosphate

(A) Over-expression of *MoTPS1* leads to defects in growth, conidiation and virulence. (B) The Δ*Motps1-OE* strain has no significant effects on trehalose and T6P production. (C) Expression levels of the TPS complex subunits under treatment with H$_2$O$_2$.

Trehalose is a non-reducing disaccharide that protects proteins and cellular membranes from inactivation or denaturation caused by a variety of stress conditions. In response to specific environmental stresses, activities of the TPS complex subunits are increased and thus induce trehalose biosynthesis. With the recovery of stress, trehalose biosynthesis is no more required and trehalose needs to be degraded. Degradation of trehalose by trehalases provides the energy necessary for stress recovery. We are sorry that the sentence in L86-87 makes you confused and this sentence has been revised as “Under stress recovery condition, trehalose biosynthesis is no more required” in the revised manuscript.

In 2007, Wilson RA *et.al* indicated that Δ*Motps3* mutant is unable to cause rice blast disease
(Wilson, Jenkinson et al. 2007), this can be found in the introduction part in L101-103. Based on this finding, we did not bother to generate $\Delta Motps3$ mutant. Since our study mainly focuses on the effects of mutation in MoTPS3 on T6P level, we generated the $\Delta Motps3$-$\Delta Motps2$ double deletion mutant, and further assays indicated that deletion of MoTPS3 in the $\Delta Motps2$ mutant background partially restores $\Delta Motps2$ mutant defects in growth, conidiation and virulence. In addition, the disease lesions caused by the $\Delta Motps3$-$\Delta Motps2$ double deletion mutant were significantly smaller than those caused by the wild type (Fig.4, Table 2). Based on these results, we concluded that self-mutation in MoTPS3 down-lowers T6P level and thus partially rescues $\Delta Motps2$ mutant defects.

(D) Guy11, $\Delta tps1$ and $\Delta tps3$ strains were inoculated onto rice seedlings to analyse pathogenicity. Spores were inoculated at a rate of $5 \times 10^4$ spores/ml

Wilson, R. A., J. M. Jenkinson, R. P. Gibson, J. A. Littlechild, Z. Y. Wang and N. J. Talbot (2007). “Tps1 regulates the pentose phosphate pathway, nitrogen metabolism and fungal virulence.” EMBO J 26(15): 3673-3685.

Another major gap is that, how the tps3 mutation happened? NO evidence here.

Response

This is actually the very first work that unveils the occurrence of this mutation due to persistent T6P accumulation in M. oryzae. As such, we decided to dissect in details how this spontaneous mutation occurs and the mechanism by which T6P induces the mutation in our future studies. However, we discussed some hypotheses that could possibly help to uncover this mechanism to aid further work. Spontaneous mutation is ultimate source of genetic variation and a fundamental component of evolution. Spontaneous mutation rate tends to rise when they are under antibiotic treatment, starvation, or other stresses. These genetic variations in turn provide beneficial
evolutionary changes for adaption (Flynn, Chain et al. 2017, Liu and Zhang 2019, Ho, Macrae et al. 2020). Genes encoding the proteins involved in trehalose biosynthesis are mechanistically linked to metabolism, cell wall homeostasis, stress responses, and virulence (Thammahong, Puttikamonkul et al. 2017). Our results indicated that the ΔMotps2 mutant displayed high sensitivity to cell wall stress (Fig.3) and rapamycin (unpublished data). Moreover, the accumulated T6P in ΔMotps2 mutant disrupt metabolite homeostasis (Fig.6). These results suggested that the accumulated T6P in ΔMotps2 mutant disrupts cellular homeostasis and could serve as a stress agent that induces the genetic variation. In M. oryzae, MoTps1 is responsible for the biosynthesis of T6P, and MoTps3 physically interacts with MoTps1 (Fig.5b). It is possible that the accumulated T6P in ΔMotps2 mutant can be sensed by MoTps1-MoTps3 and therefore induces the mutation in MoTps3.

In 2014, Song et al. indicated in Fusarium graminearum that the trehalose 6-phosphatase phosphatase FgTps2 is required for fungal development, virulence and mycotoxin production (Song, Li et al. 2014). Loss of FgTps2 also leads to severe intracellular accumulation of T6P. To better understand how the accumulated T6P induces spontaneous mutation (and to satisfactorily address the reviewer’s concern), we generated ΔFgtps2 mutant in F. graminearum. As shown in Fig. R2, the ΔFgtps2 mutant also produced spontaneous mutation strains when cultured on SYM medium, similar to what we demonstrated in M. oryzae. A previous study suggested in F. graminearum that the observed defects of ΔFgtps1-ΔFgtps2 mutant were less severe when compared to those in ΔFgtps2 mutant (Song, Li et al. 2014) (Table R1). In addition, both trehalose and T6P production were abolished in the ΔFgtps1-ΔFgtps2 mutant. These results indicated that the accumulated T6P could also act as a spontaneous mutation inducer in response to intracellular T6P accumulation and FgTPS1 or FgTPS3 could act as the potential mutation targets in F. graminearum.

![Fig.R2 Spontaneous mutation of ΔFgtps2 mutant in Fusarium graminearum. The fast-growing sectors were marked by red arrows.](image-url)
Table R1 Conidiation, spore character, chitin content, chitin synthase activity and hyphal cell wall of the wild-type strain 5035, three null mutants and two complementation strains (Song, Li et al. 2014)

| Strain              | Conidiation (10⁶ ml⁻¹) | No. of septa | Hyphal width (μm) | Chitin content (μg mL⁻¹) | Chitin synthase activity (nmol h⁻¹ mg⁻¹) | Cell wall width (nm) |
|---------------------|------------------------|-------------|-------------------|--------------------------|----------------------------------------|-----------------------|
| 5035                | 9.83 ± 1.40a           |             | 8.08 ± 1.45a      | 3.97 ± 1.56              | 0.93 ± 0.11b                           | 4.91 ± 0.14a          |
| Δmotps1             | 8.01 ± 1.45a           |             | 3.96 ± 1.40a      | 3.97 ± 1.56              | 0.93 ± 0.11b                           | 4.91 ± 0.14a          |
| TPS3C               | 9.70 ± 1.56            |             | 3.97 ± 1.56       | 3.97 ± 1.56              | 0.93 ± 0.11b                           | 4.91 ± 0.14a          |
| Δmotps2             | 9.70 ± 1.56            |             | 3.97 ± 1.56       | 3.97 ± 1.56              | 0.93 ± 0.11b                           | 4.91 ± 0.14a          |
| Δmotps2           | 10.02 ± 1.45a          |             | 3.97 ± 1.56       | 3.97 ± 1.56              | 0.93 ± 0.11b                           | 4.91 ± 0.14a          |
| Δmotps3Δmotps2      | 9.70 ± 1.56            |             | 3.97 ± 1.56       | 3.97 ± 1.56              | 0.93 ± 0.11b                           | 4.91 ± 0.14a          |

Data in these table are represented as average ± standard error. Different letters represent a significant difference at P < 0.01.

Flynn, J. M., F. J. Chain, D. J. Schoen and M. E. Cristescu (2017). "Spontaneous Mutation Accumulation in Daphnia pulex in Selection-Free vs. Competitive Environments." Mol Biol Evol 34(1): 160-173.

Ho, E. K. H., F. Macrae, L. C. Latta, P. McLlroy, D. Ebert, P. D. Fields, M. J. Benner and S. Schrack (2020). "High and Highly Variable Spontaneous Mutation Rates in Daphnia." Mol Biol Evol 37(11): 3258-3266.

Liu, H. and J. Zhang (2019). "Yeast Spontaneous Mutation Rate and Spectrum Vary with Environment." Curr Biol 29(10): 1584-1591 e1583.

Song, X. S., H. P. Li, J. B. Zhang, B. Song, T. Huang, X. M. Du, A. D. Gong, Y. K. Liu, Y. N. Feng, R. S. Agboola and Y. C. Liao (2014). "Trehalose 6-phosphate phosphatase is required for development, virulence and mycotoxin biosynthesis apart from trehalose biosynthesis in Fusarium graminearum." Fungal Genet Biol 63: 24-41.

Thammahong, A., S. Puttikamonkul, J. R. Perfect, R. G. Brennan and R. A. Cramer (2017). "Central Role of the Trehalose Biosynthesis Pathway in the Pathogenesis of Human Fungal Infections: Opportunities and Challenges for Therapeutic Development." Microbiol Mol Biol Rev 81(2): e00053-16(2).

Are these sites active at the basic level even not in the deletion of MoTps2? The mutation of Tps3 is only dependent to Tps2 or once immature of the appressorium? After reading carefully throughout the Ms, I still cannot follow that why the mutation occurred. I think the author should provide more evidence here.

Response
As shown in Fig.R3, the spontaneous mutation sites identified in MoTPS3 are well conserved in different species. Our results also indicated that the spontaneous mutation strains ΔMotps2-m mutant has similar phenotypes when compared to the ΔMotps2 -ΔMotps3 mutant (Fig.4). It is possible that these spontaneous mutations could be induced under other stress conditions. This is also a topic of further studies. As for the existing evidences, the spontaneous mutation is observed when...
*MoTPS2* gene is deleted and this partially ameliorates the defects of the *tps2* mutant. This therefore simply suggests that using MoTps2 as a drug target to combat the rice blast infection could be compromised by this spontaneous mutation of *MoTPS3*. Whether there are other conditions that can lead to this mutation apart from the deletion of *MoTPS2* will make part of an interesting subject of our next research as this will shade more light on the feasibility of targeting the TPS complex for antifungal drug discovery.
Fig. R3 Amino acids alignment of Tps3 homologues in different species. The base deletion mutation site was marked by red cycle and the base insertion site was marked by blue arrow.
Response to Reviewer #2

We would like to thank the editorial board and the reviewers for their constructive comments concerning this article (control no. mSystems00462-21 R0). These comments are all valuable and helpful for improving our article. All the authors have seriously discussed about all these comments. We therefore modified the manuscript accordingly to accommodate the reviewer’s observations and meet the requirements of the journal. Point-by-point responses to the reviewer’s comments are listed below.

L128-132: You rather observed (not characterized) a spontaneous mutation that partially restores the defects. Also, this sentence is too long, making it difficult to grab the message. I suggest you break it into two simpler sentences.

Response
Thanks a lot for your valuable suggestion to improve the quality of our manuscript. The sentence has been revised as “In addition, the TPS complex possesses a spontaneous ‘correction’ way to modulate T6P homeostasis by spontaneous mutation of the regulatory subunit MoTps3. This spontaneous correction function further suppresses the MoTps1 activity to down-regulate T6P, resulting in partial restoration of the ΔMotps2 mutant defects in growth, conidiation and pathogenicity.”

L142: define aa (as it appears here for the very first time)

Response
Thanks for your careful checks. We have defined “aa” in L142 in the revised manuscript.

L149: ’...........in the Motps2 mutant’ please recast as ’............in the Motps2 mutant background’

Response
Thanks for your suggestion, the “in the ΔMotps2 mutant” has been changed to “in the ΔMotps2 mutant background” in L149.

L151: ’...........to their compare their growth rates’ please check and fix the error.

Response
We are sorry for this grammatical error. The sentence has been revised as “The wild type (70-15), mutant (ΔMotps2) and complemented strain (ΔMotps2-com) were then cultured on CM, MM and RBM culture media for 7 days to compare their growth rates.” The revision is shown in L149-151.

L236: ‘...........appropriate location of CHS genes is essential for cell wall homeostasis’ (please provide a reference for this).
Response
Thanks for this important comment; we have inserted the related references in L236. The sentence was revised as “Since the appropriate localization of CHS genes is essential for cell wall homeostasis (29, 39)”. The references are listed as follow:
29. Riquelme M. 2013. Tip growth in filamentous fungi: a road trip to the apex. Annu Rev Microbiol 67:587-609.
39. Thammahong A, Caffrey-Card AK, Dhingra S, Obar JJ, Cramer RA. 2017. Aspergillus fumigatus Trehalose-Regulatory Subunit Homolog Moonlights To Mediate Cell Wall Homeostasis through Modulation of Chitin Synthase Activity. mBio 8: e00056-17.

L272-273: under what condition(s) does the mutant produce spontaneous suppressor strain? This is necessary because I cannot see the strain emerging in other culture plates as in Fig. 1 A.

Response
As shown in the Fig.S7, the ΔMotps2 mutant produced spontaneous suppressor strains when we revived ΔMotps2 mutant from filter papers on SYM medium. Based on our experience, the ΔMotps2 mutant produced spontaneous suppressor strains randomly. This happened when we performed the growth assays, cell wall stress assays, osmotic assays, etc. In our manuscript, the first part of our results section is mainly about the biological function of the MoTps2 (Fig.1, Fig.2 and Fig.3), while Fig.4, Fig.5 and Fig.6 focus on the identification of the mutation sites in the spontaneous suppressor strain and how the mutations of MoTPS3 restore the ΔMotps2 mutant’s defects.

L595: Delete one 'Afterwards'.

Response
Thanks for your correction; we have deleted one “afterwards” in L595. We are sorry for this mistake.

The materials and methods section does not explain how the various forms of mutation that exist in the spontaneous strain were identified.

Response
Thanks for your valuable suggestion. How various forms of mutation in the spontaneous strain were identified was added in the materials and methods part. This can be seen in L553-560 in the revised manuscript.
September 8, 2021

Dr. Wenhui Zheng  
State Key Laboratory for Ecological Pest Control of Fujian and Taiwan Crops  
Fuzhou, Fujian 350002  
China

Re: mSystems00462-21R1 (TPS complex-mediated regulation of trehalose 6-phosphate homeostasis is critical for development and pathogenesis in Magnaporthe oryzae)

Dear Dr. Wenhui Zheng:

Congratulations! Your manuscript has been accepted for publication in mSystems. Thank you for your careful consideration of the reviewers’ comments.

As your manuscript has been accepted, I am forwarding it to the ASM Journals Department for publication. For your reference, ASM Journals’ address is given below. Before it can be scheduled for publication, your manuscript will be checked by the mSystems senior production editor, Ellie Ghatineh, to make sure that all elements meet the technical requirements for publication. She will contact you if anything needs to be revised before copyediting and production can begin. Otherwise, you will be notified when your proofs are ready to be viewed.

As an open-access publication, mSystems receives no financial support from paid subscriptions and depends on authors’ prompt payment of publication fees as soon as their articles are accepted.

**Publication Fees:**
You will be contacted separately about payment when the proofs are issued; please follow the instructions in that e-mail. Arrangements for payment must be made before your article is published. For a complete list of **Publication Fees**, including supplemental material costs, please visit our [website](#).

Corresponding authors may [join or renew ASM membership](#) to obtain discounts on publication fees. Need to upgrade your membership level? Please contact Customer Service at [Service@asmusa.org](mailto:Service@asmusa.org).

For **mSystems research articles**, you are welcome to submit a short author video for your recently accepted paper. Videos are normally 1 minute long and are a great opportunity for junior authors to get greater exposure. Importantly, this video will not hold up the publication of your paper, and you can submit it at any time.

Details of the video are:

- Minimum resolution of 1280 x 720
- .mov or .mp4 video format
- Provide video in the highest quality possible, but do not exceed 1080p
- Provide a still/profile picture that is 640 (w) x 720 (h) max
- Provide the script that was used
We recognize that the video files can become quite large, and so to avoid quality loss ASM suggests sending the video file via https://www.wetransfer.com/. When you have a final version of the video and the still ready to share, please send it to Ellie Ghatineh at eghatineh@asmusa.org.

Thank you for submitting your paper to mSystems.

Sincerely,

Ileana Cristea
Editor, mSystems

Journals Department
American Society for Microbiology
1752 N St., NW
Washington, DC 20036
E-mail: peerreview@asmusa.org
Phone: 1-202-942-9338

Fig. S3: Accept
Fig. S2: Accept
Fig.S7: Accept
Fig. S5: Accept
Fig. S1: Accept
Table S2: Accept
Fig. S4: Accept
Table S1: Accept
Fig. s6: Accept
Table S3: Accept