2018-08

SUMO enters the ring: the emerging role of SUMOylation in Magnaporthe oryzae pathogenicity

Littlejohn, George

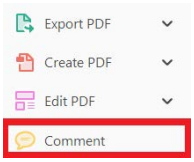
10.1111/nph.15336
New Phytologist
Wiley

WILEY

USING e-ANNOTATION TOOLS FOR ELECTRONIC PROOF CORRECTION

Required software to e-Annotate PDFs: Adobe Acrobat Professional or Adobe Reader (version 11 or above). (Note that this document uses screenshots from Adobe Reader DC.)
The latest version of Acrobat Reader can be downloaded for free at: http://get.adobe.com/reader/

Once you have Acrobat Reader open on your computer, click on the Comment tab (right-hand panel or under the Tools menu).



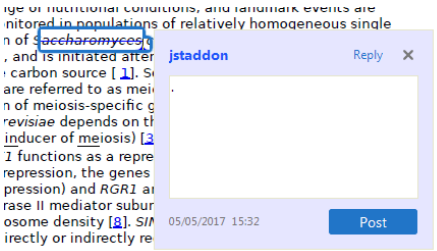
This will open up a ribbon panel at the top of the document. Using a tool will place a comment in the right-hand panel. The tools you will use for annotating your proof are shown below:

1. Replace (Ins) Tool – for replacing text.

Strikes a line through text and opens up a text box where replacement text can be entered.

How to use it:

- Highlight a word or sentence.
- Click on .
- Type the replacement text into the blue box that appears.

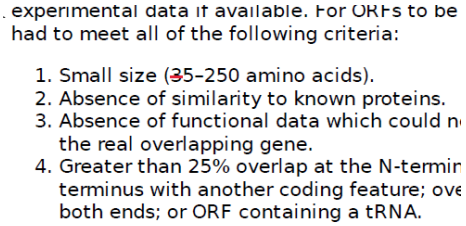


2. Strikethrough (Del) Tool – for deleting text.

Strikes a red line through text that is to be deleted.

How to use it:

- Highlight a word or sentence.
- Click on .
- The text will be struck out in red.

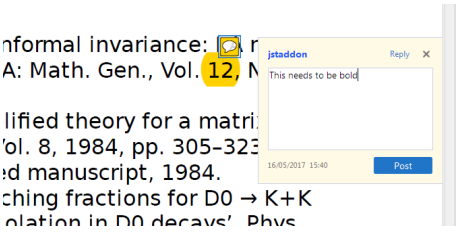


3. Commenting Tool – for highlighting a section to be changed to bold or italic or for general comments.

Use these 2 tools to highlight the text where a comment is then made.

How to use it:

- Click on .
- Click and drag over the text you need to highlight for the comment you will add.
- Click on .
- Click close to the text you just highlighted.
- Type any instructions regarding the text to be altered into the box that appears.

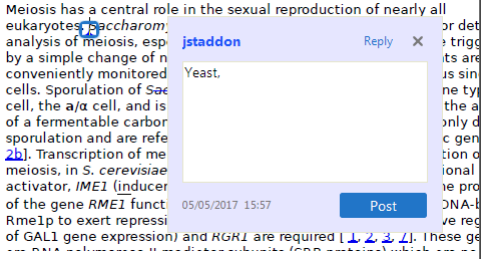


4. Insert Tool – for inserting missing text at specific points in the text.

Marks an insertion point in the text and opens up a text box where comments can be entered.

How to use it:

- Click on .
- Click at the point in the proof where the comment should be inserted.
- Type the comment into the box that appears.



USING e-ANNOTATION TOOLS FOR ELECTRONIC PROOF CORRECTION

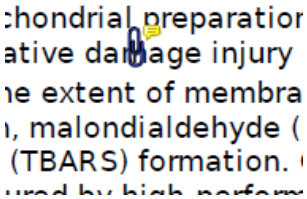
5. Attach File Tool – for inserting large amounts of text or replacement figures.

Inserts an icon linking to the attached file in the appropriate place in the text.

How to use it:

- Click on  .
- Click on the proof to where you'd like the attached file to be linked.
- Select the file to be attached from your computer or network.
- Select the colour and type of icon that will appear in the proof. Click OK.

The attachment appears in the right-hand panel.

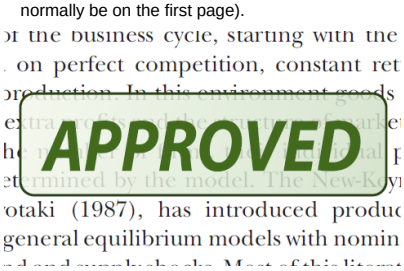


6. Add stamp Tool – for approving a proof if no corrections are required.

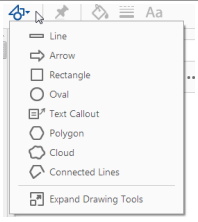
Inserts a selected stamp onto an appropriate place in the proof.

How to use it:

- Click on  .
- Select the stamp you want to use. (The Approved stamp is usually available directly in the menu that appears. Others are shown under *Dynamic*, *Sign Here*, *Standard Business*).
- Fill in any details and then click on the proof where you'd like the stamp to appear. (Where a proof is to be approved as it is, this would normally be on the first page).

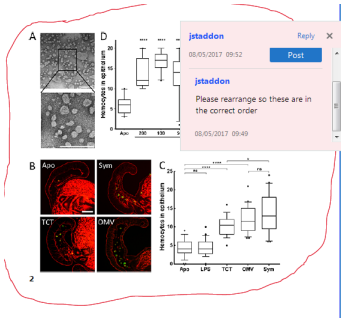


Drawing tools available on comment ribbon



7. Drawing Markups Tools – for drawing shapes, lines, and freeform annotations on proofs and commenting on these marks.

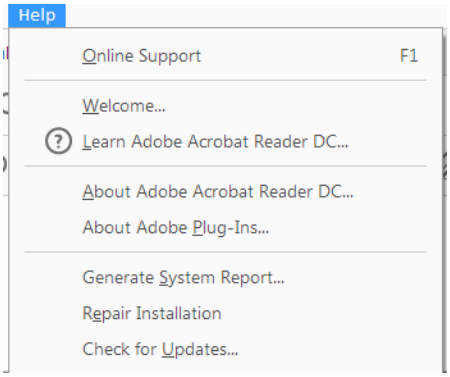
Allows shapes, lines, and freeform annotations to be drawn on proofs and for comments to be made on these marks.



How to use it:

- Click on one of the shapes in the Drawing Markups section.
- Click on the proof at the relevant point and draw the selected shape with the cursor.
- To add a comment to the drawn shape, right-click on shape and select *Open Pop-up Note*.
- Type any text in the red box that appears.

For further information on how to annotate proofs, click on the Help menu to reveal a list of further options:



WILEY

Author Query Form

Journal: NPH

Article: 15336/2018-27183

Dear Author,

During the copyediting of your manuscript the following queries arose.

Please refer to the query reference callout numbers in the page proofs and respond to each by marking the necessary comments using the PDF annotation tools.

Please remember illegible or unclear comments and corrections may delay publication.

Many thanks for your assistance.

Query reference	Query	Remarks
1	**AUTHOR: Please verify that the linked ORCID identifier is correct for author "George R. Littlejohn".**	
2	**AUTHOR: Please confirm that given names (blue) and surnames/family names (vermilion) have been identified correctly.**	
3	**WILEY: Please insert the page range, this issue.**	
4	**WILEY: Please insert the page range, this issue.**	
5	**AUTHOR: Please provide volume and page range for reference Lim et al. (2018).**	
6	**WILEY: Please insert the page range, this issue.**	

Commentary

SUMO enters the ring: the emerging role of SUMOylation in *Magnaporthe oryzae* pathogenicity

Rice blast is a devastating disease found wherever rice is successfully cultivated. It causes grain yield losses of 10 to 30% per annum, which is sufficient to feed 60 million people, and makes it the most serious fungal threat to global rice production (Skamnioti & Gurr, 2009). There is, therefore, a long-recognized and significant socioeconomic and moral imperative to tackle rice blast disease (Talbot, 2003). The resultant effort to understand the basic biology of rice blast infection, particularly over the last quarter of a century, has led to rice and the causative agent of rice blast, the filamentous ascomycete *Magnaporthe oryzae,* being recognized as a model pathosystem (Ebbole, 2007). Many elegant examples of *M. oryzae* and rice cell biology have informed our understanding of plant–pathogen interactions. Particularly influential insights have come in disentangling appressorium-mediated fungal penetration of plants (Dagdas *et al.*, 2012; Ryder *et al.*, 2013) and how maintenance of the biotrophic phase of infection is achieved (Kankanala *et al.*, 2007; Khang *et al.*, 2010; Giraldo *et al.*, 2013; Gupta *et al.*, 2015). Recent studies have begun to follow infection beyond invasion of the first cell and are characterizing whole tissue colonization (Jones *et al.*, 2016; Sakulkoo *et al.*, 2018).

'Through the experiments presented in their paper, Liu et al. *show SUMOylation to be necessary for biological processes that are fundamental to the success of* M. oryzae *in almost all life cycle stages.'*

The role of post-translational protein modifications has been fundamental to understanding many of the cell biological processes involved in *M. oryzae* pathogenicity. For example, *M. oryzae* has been shown to be sensitive to changes in reactive oxygen species (ROS) and therefore subject to changes in oxidation state of cellular proteins, which alter their functions in the cell (Ryder *et al.*, 2013). *Magnaporthe oryzae*, like other eukaryotes, is also known to target proteins for degradation via the modifier, ubiquitin (Shi *et al.*, 2016). In addition, protein phosphorylation of target proteins has been shown to be essential in establishment and maintenance of infections (Oses-Ruiz *et al.*, 2017; Sakulkoo *et al.*, 2018). To date, though, there has been a paucity of studies showing other forms of protein modifications resulting in activation or fine-tuning of function of *M. oryzae* proteins during infection.

In their article in this issue of *New Phytologist*, Liu *et al.* (pp. 000–000) show for the first time the global importance of SUMOylation of *M. oryzae* proteins in development and pathogenicity through appressorium-mediated infection. Unlike ubiquitin, Small Ubiquitin-like MOdifier (SUMO) does not normally target proteins for degradation, but rather modifies their function (Melchior, 2000), and like other protein modifications, such as phosphorylation, methylation or acetylation, SUMOylation is reversible and found across eukaryotic lineages. Liu *et al.* use a combination of proteomic, gene expression, genetic, microscopic and gross phenotypic analyses to comprehensively show SUMOylation to be involved in several processes already known to be important in appressorium-mediated pathogenicity (Khang *et al.*, 2010; Saunders *et al.*, 2010; Dagdas *et al.*, 2012; Oses-Ruiz *et al.*, 2017; Sakulkoo *et al.*, 2018).

Of the 940 SUMOylated targets shown by affinity purification and predicted by bioinformatics analyses, Liu *et al.* identify SUMOylated proteins with established functions in autophagy, signal transduction, primary metabolism, stress response, including redox related proteins, and several developmental processes, including four septins and cell cycle-related proteins. Indeed, so wide-ranging are the suggested targets of SUMOylation in *M. oryzae*, that KEGG pathway analysis also suggests that mitogen-activated protein kinase (MAPK) signalling is also SUMO-regulated. The set of predicted targets, and a well-chosen set of experiments, using deletion mutants generated in genes of the SUMOylation pathway, lead to striking observations that place SUMOylation in a fundamental position in *M. oryzae* development and pathogenicity. Through the experiments presented in their paper, Liu *et al.* show SUMOylation to be necessary for biological processes that are fundamental to the success of *M. oryzae* in almost all life cycle stages. Cell cycle-related phenomena such as septation in hyphae and conidia and distribution of nuclei in the pre-penetration conidium, germ tube and appressorium are shown to be altered in SUMOylation mutants. Penetration and pathogenicity are also shown to be affected in SUMOylation mutants, with a wide range of mechanisms known to be important for pathogenicity tested by Liu *et al.*, and demonstrated to be reliant on SUMOylation. The SUMO mutant, *Δsmt3*, exhibits a reduction in pathogenicity and mislocalization of septins compared to wildtype. Liu *et al.* generated *M. oryzae* strains for each of the four identified SUMOylated septin proteins (*SEP 3*, *4*, *5* and *6*), which had their putative SUMOylation sites mutated and the mutant proteins translationally fused with GFP. These strains show

This article is a Commentary on Liu *et al.*, **219**: 000–000.

Journal Code N P H
Manuscript No. 15336 / 2018-27183
WILEY
No. of pages: 2 Dispatch: 21.6.18
PE: Vigneshwari CE: Raja
Color:

the malformation of a septin ring at the appressorium pore, previously shown to be essential in appressorium-mediated penetration of the plant surface (Dagdas *et al.*, 2012), and pathogenicity reduced to the level of deletion mutants for the genes in question. This evidence provides one of the two main findings of the paper. Liu *et al.* also convincingly show, although not yet in the molecular detail of the septin observations, that SUMOylation mutants fail to deliver (via distinct mechanisms; Giraldo *et al.*, 2013) both cytoplasmic and apoplastic effector proteins to the host cell. This is undoubtedly a key observation, which suggests a possible explanation for the demonstrated inability of SUMOylation mutants to suppress host ROS accumulation in early infection via direct or indirect action of *M. oryzae* effectors. In addition to the published structural role of Slp1 in allowing *M. oryzae* to avoid host ROS burst (Mentlak *et al.*, 2012), there is also evidence for direct suppression of host ROS burst via effector action in *Pseudomonas syringae* infections of *Arabidopsis thaliana* (de Torres-Zabala *et al.*, 2015), suggesting ROS accumulation may be a widely conserved target of effectors during early infection.

The timeliness of the paper by Liu *et al.* is clear as there is currently, in early online print, a similar, but less wide-ranging study by Lim *et al.* (2018), which has produced a near-identical set of mutants in the SUMOylation machinery and has replicated some of the gene expression and morphological results found by Liu *et al.* It is not at all surprising that SUMO should be so heavily involved with many different processes important in *M. oryzae* biology as it is a common post-translational control mechanism in eukaryotes. However, both these mutually confirmatory studies taken together do serve to bring SUMO swiftly and firmly into the canonical model of understanding *M. oryzae* infection.

Final considerations

The implications of the work presented by Liu *et al.* are, like their paper, wide-ranging. This impressive study will doubtless be influential in the cell biology of *M. oryzae* infection, but may also lead to insights that may be used to combat rice blast disease, as SUMOylation now presents itself as a potential target for designing chemical or genetic control strategies for *M. oryzae*.

Acknowledgements

Thanks to Ciaran Griffin for helpful comments.

ORCID

George R. Littlejohn http://orcid.org/0000-0002-8768-2598

George R. Littlejohn

School of Biological and Marine Sciences, University of Plymouth, Portland Square Building, Drake Circus, Plymouth, PL4 8AA, UK (tel +44 (0)1752 584487; email george.littlejohn@plymouth.ac.uk)

References

Dagdas YF, Yoshino K, Dagdas G, Ryder LS, Bielska E, Steinberg G, Talbot NJ. 2012. Septin-mediated plant cell invasion by the rice blast fungus, *Magnaporthe oryzae*. *Science* **336**: 1590–1595.

Ebbole DJ. 2007. *Magnaporthe* as a model for understanding host–pathogen interactions. *Annual Review of Phytopathology* **45**: 437–456.

Giraldo MC, Dagdas YF, Gupta YK, Mentlak TA, Yi M, Martinez-Rocha AL, Saitoh H, Terauchi R, Talbot NJ, Valent B. 2013. Two distinct secretion systems facilitate tissue invasion by the rice blast fungus *Magnaporthe oryzae*. *Nature Communications* **4**: 1996.

Gupta YK, Dagdas YF, Martinez-Rocha AL, Kershaw MJ, Littlejohn GR, Ryder LS, Sklenar J, Menke F, Talbot NJ. 2015. Septin-dependent assembly of the exocyst is essential for plant infection by *Magnaporthe oryzae*. *Plant Cell* **27**: 3277–3289.

Jones K, Kim DW, Park JS, Khang CH. 2016. Live-cell fluorescence imaging to investigate the dynamics of plant cell death during infection by the rice blast fungus *Magnaporthe oryzae*. *BMC Plant Biology* **16**: 69.

Kankanala P, Czymmek K, Valent B. 2007. Roles for rice membrane dynamics and plasmodesmata during biotrophic invasion by the blast fungus. *Plant Cell* **19**: 706–724.

Khang CH, Berruyer R, Giraldo MC, Kankanala P, Park SY, Czymmek K, Kang S, Valent B. 2010. Translocation of *Magnaporthe oryzae* effectors into rice cells and their subsequent cell-to-cell movement. *Plant Cell* **22**: 1388–1403.

Lim Y, Kim K, Lee Y. 2018. SUMOylation is required for fungal development and pathogenicity in the rice blast fungus *Magnaporthe oryzae*. *Molecular Plant Pathology*. doi: 10.1111/mpp.12687

Liu C, Li Z, Xing J, Yang J, Wang Z, Zhang H, Chen D, Peng YL, Chen XL. 2018. Global analysis of sumoylation function reveals novel insights into development and appressorium-mediated infection of the rice blast fungus. *New Phytologist* **219**.

Melchior F. 2000. SUMO-nonclassical ubiquitin. *Annual Review of Cell and Developmental Biology* **16**: 591–626.

Mentlak TA, Kombrink A, Shinya T, Ryder LS, Otomo I, Saitoh H, Terauchi R, Nishizawa Y, Shibuya N, Thomma BPHJ *et al.* 2012. Effector-mediated suppression of chitin-triggered immunity by *Magnaporthe oryzae* is necessary for rice blast disease. *The Plant Cell* **24**: 322–335.

Oses-Ruiz M, Sakulkoo W, Littlejohn GR, Martin- Urdiroz M, Talbot NJ. 2017. Two independent S-phase checkpoints regulate appressorium-mediated plant infection by the rice blast fungus *Magnaporthe oryzae*. *Proceedings of the National Academy of Sciences, USA* **114**: E237–E244.

Ryder LS, Dagdas YF, Mentlak TA, Kershaw MJ, Thornton CR, Schuster M, Chen J, Wang Z, Talbot NJ. 2013. NADPH oxidases regulate septin-mediated cytoskeletal remodeling during plant infection by the rice blast fungus. *Proceedings of the National Academy of Sciences, USA* **110**: 3179–3184.

Sakulkoo W, Osés-Ruiz M, Garcia EO, Soanes DM, Littlejohn GR, Hacker C, Correia A, Valent B, Talbot NJ. 2018. A single fungal MAP kinase controls plant cell-to-cell invasion by the rice blast fungus. *Science* **359**: 1399–1403.

Saunders DGO, Aves SJ, Talbot NJ. 2010. Cell cycle–mediated regulation of plant infection by the rice blast fungus. *The Plant Cell* **22**: 497–507.

Shi HB, Chen GQ, Chen YP, Dong B, Lu JP, Liu XH, Lin FC. 2016. MoRad6-mediated ubiquitination pathways are essential for development and pathogenicity in *Magnaporthe oryzae*. *Environmental Microbiology* **18**: 4170–4187.

Skamnioti P, Gurr SJ. 2009. Against the grain: safeguarding rice from rice blast disease. *Trends in Biotechnology* **27**: 141–150.

Talbot NJ. 2003. On the trail of a cereal killer: exploring the biology of *Magnaporthe grisea*. *Annual Review of Microbiology* **57**: 177–202.

de Torres-Zabala M, Littlejohn GR, Jayaraman S, Studholme D, Bailey T, Lawson T, Tillich M, Licht D, Bölter B, Delfino L *et al.* 2015. Chloroplasts play a central role in plant defence and are targeted by pathogen effectors. *Nature Plants* **1**: 15074.

Key words: appressorium-mediated infection, fungal infection, *Magnaporthe oryzae*, pathogenicity, post-translational protein modification, rice blast disease, small ubiquitin-like modifier (SUMO), SUMOylation.