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Chlorophyll fluorescence of *Chlamydomonas reinhardtii*: insights into the complexities

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Reviewer 1

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*Only major points from review and responses included.

Reviewer 1

I am intrigued by the results reported in figure 6, which suggest that upon acclimation to high light, qE is increased but also qT is greater. This is based on the analysis of Fm in light and dark, which is very useful, although one cannot unambiguously distinguish between qE and other forms of NPQ. Would it be possible to test this possibility by repeating the experiment in the presence of nigericin (as for example in Allorent et al 2016 PNAS, 113: 14864-14869).

Author

Thank you for your time in reviewing my submission, and for the suggestions to improve it. As shown in Allorent et al. 2013; 2016, high light-induced quenching is prevented by nigericin, but qT still occurs (cells start in state 2). The data has been added as Supplemental and referred to on page 7. In low light cultures there is very limited qE so the effect of nigericin is small, and before quenching analyses cells are less in state 2 due to low chloro-respiration induced by briefly dark treating HL-acclimated cells.

Reviewer 1

'A lowered pH activates violaxanthin de-epoxidase (VDE), which uses ascorbic acid as substrate to convert violaxanthin to zeaxanthin, so that excitation energy can be transferred from chl. to zeaxanthin and dissipated as heat (Fig. 1; No. 2).' This is one hypothesis: Horton/Ruban and colleagues have proposed a conformational change mechanism for quenching (Pascal et al 2005, Nature 436: 134-137), which does not require energy transfer from Chl to Zea. Fleming and co-workers are instead more in

favour of the radical cation hypothesis, which also considers a different mechanism than energy transfer from Chl to Zea (Holt et al 2005, Science 307: 433-436).

Author

I have been less specific about the role of zeaxanthin and changed the text to "…to convert violaxanthin to zeaxanthin, which then may potentially directly quench or indirectly (e.g. affecting LHC conformational change) contribute to energy dissipation as heat."

Reviewer 1

'In C. reinhardtii up to 80% of LHCII can rapidly disassociate from PSII (Ünlü et al., 2014; Nawrocki et al, 2016)'. The amount of this migration is still debated: it is much less in Nawrocki et al, and intermediate in Nagy et al 2014, PNAS 111: 5042-5047.

Author

The text was modified to "a major fraction"