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Letters

Controversy remains: regulation of pH gradient across the thylakoid membrane

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In a recent review [1], David Kramer, Thomas Avenson and Gerald Edwards discussed a largely overlooked problem in photosynthesis – how the pH gradient (ΔpH) across the thylakoid membrane is regulated. ΔpH serves a dual role in chloroplasts, synthesizing ATP and regulating light harvesting, by inducing non-photochemical quenching (NPQ). As the authors point out, this results in a conflict of interest – conditions that require a slowing of linear electron transport (CO_2 fixation inhibited) also require the generation of a larger ΔpH to lower the efficiency of light capture.

The authors considered two mechanisms that might regulate ΔpH: (i) alternative routes of electron transport that generate ΔpH without ATP consumption, notably cyclic electron transport (CET) around the photosystem I reaction center; (ii) modulation of the conductivity of the chloroplast ATPase. The first model has been widely discussed for many years [2], although, as the authors point out, controversy remains. The second model is based on recent data from David Kramer's laboratory [3]. Kramer and colleagues seek to dismiss a major role for CET in regulating light harvesting. Given recent prominent publications by Yuri Munekage and colleagues [4,5], describing a mutant unable to perform NPQ and apparently deficient in CET, this argument might confuse non-specialist readers and requires further discussion.

Kramer and colleagues argue that to regulate ΔpH, CET would have to occur at high rates (up to 6 times linear electron flux) and that published evidence does not support the occurrence of such rates under steady-state conditions. The figures cited arise from an interpretation of the relationship between linear flow and NPQ [3]. At low CO_2 levels, there is a low rate of linear electron transport owing to the inhibition of the Calvin–Benson cycle, but the extent of NPQ is greater than at high CO_2 levels. For a given level of NPQ, linear flow is about a sixth of that under high CO_2 levels. The argument then follows that for CET to compensate for this drop, it would have to be at a rate that is five to six times greater than the residual linear flow. However, this fails to take into account that linear flow to CO_2 is not a net generator of ΔpH – ATP consumption by CO_2 fixation is greater than the generation expected through linear flow alone. At low CO_2 levels, ATP consumption will drop in parallel with linear electron transport, so dissipation of ΔpH will be considerably reduced. CET generates ΔpH without being directly coupled to its consumption. Therefore, a modest increase in CET would be sufficient to generate a high ΔpH.

Analysis from my laboratory suggests that CET increases at low CO_2 levels, generating ΔpH to regulate light harvesting [6–8]. This conclusion is valid using both the decay of $P700^+$ as a measure of PSI turnover (the method questioned by David Kramer and colleagues [1]) and also using the 'active pool' of P700, as proposed by Christof Klughammer and Ulrich Schreiber [9]. It is consistent with a significant number of observations from other groups [10,11]. David Kramer and colleagues notably glossed over the *pgr5* mutant, identified for its inability to perform NPQ, which has been characterized as deficient in CET [5]. This phenotype remains to be tested under physiological conditions but is likely to provide a definitive test for the role of CET.

The model put forward for regulation of ATPase conductivity [1,3] is an interesting one, but the published evidence is open to alternative interpretations. The

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evidence for this model relies on measurements of the decay of the potential gradient across the thylakoid membrane following a light–dark transition, which David Kramer and co-workers use as a measure of flux through the ATPase [3]. Given the drop in ATP consumption by the Calvin–Benson cycle, one would expect this to decline at low CO_2, without needing to invoke any regulation of conductivity. Curiously, the rate of decay of the potential gradient at low CO_2 is less inhibited than would be predicted from the loss of CO_2 fixation (see Ref. 3, inset Fig. 3). This would suggest that the leak (slip) of protons through the membrane increases at low CO_2. This is a concept that has been debated in the literature as a possible protection against excess acidification of the lumen [12] but which has not been tested *in vivo*. Such a protective mechanism would provide a limit to the maximum acidification of the thylakoid lumen and would require continuous engagement of the cyclic pathway under conditions of low CO_2. The maximum rate of CET that we observe is, as stated by Kramer and colleagues, twice the linear rate at low CO_2 or approximately two-thirds the maximum linear rate [7]. We suggest that this is sufficient to support the ΔpH required to maintain NPQ.

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Letters Response

Response to Johnson: Controversy remains: regulation of pH gradient across the thylakoid membrane

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In our review [1] we addressed the mechanisms with which the chloroplast achieves the flexibility needed for both energy conversion and photoprotection under rapidly changing environmental conditions. Giles Johnson brings up two interesting points. First, he suggests that because linear electron flow (LEF) translocates fewer protons to the lumen than are needed to sustain the ATP:NADPH output balance needed for the Calvin–Benson cycle, this process should not produce a net proton motive force (*pmf*). He argues that a large proton flux from cyclic electron flow around photosystem I (CEF1) is required to supply sufficient *pmf* to initiate 'energy-dependent' exciton quenching (q_E), particularly under low CO_2 conditions.

As we indicated in our review [1], CEF1 might play an important role to cover a predicted deficit in ATP from LEF. However, we have also shown that the increase in q_E observed at low CO_2 is attributable mainly to a decrease in the conductance of the ATP synthase to proton efflux from the lumen (g_H^+), which results in an increase in *pmf* (and q_E) even at low proton flux. Our data further indicate a proton flux proportional to LEF (i.e. no large changes in CEF1 contributions) even at essentially zero levels of CO_2, where photorespiration (and not proton leakage, as Johnson suggests) can account for the residual sinks for photosynthetic energy.

In theory, could there be no net generation of ΔpH with operation of LEF alone, as Johnson suggests? This would only be the case if *pmf* generated by LEF reached full equilibrium with the energy stored in the ATP/($ADP+P_i$) couple (ΔG_{ATP}), and if ΔG_{ATP} remained constant. However, both the effective rate constant of the ATP synthase (e.g. Ref. [2]) and direct measurements of *pmf* decay

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