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Avoiding alternative electron sinks - regulating the thylakoid electron transport chain to avoid oxidative stress

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Introduction:
The absorption of light energy by chlorophyll in the presence of oxygen inevitably leads to the formation of reactive oxygen species (ROS; see Fig. 1; see Asada, 1999; Foyer and Noctor, 2000 for reviews). Intersystem crossing in the antenna or charge recombination in the reaction centre of photosystem II (PSII) produce triplet excited chlorophyll which sensitises the formation of singlet excited oxygen. Electron flow to oxygen produces superoxide which dismutates to form hydrogen peroxide which in turn can react to form hydroxyl and other radicals. Scavenging any reactive species produced is a high priority for the plant, as reflected in the high investment that plants make in antioxidants and antioxidant enzymes. When plants are exposed to environmental stress, the demands on such scavenging systems are increased and their capacity is, therefore, likely to be a determinant of the ability of plants to tolerate stress. In addition to scavenging ROS, plants also possess mechanisms that allow them to regulate light harvesting and electron transport in ways that minimise the formation of ROS. This paper reviews some of our recent work investigating these regulatory mechanisms.

Avoiding electron transport to oxygen:
The major route for the reduction of oxygen by the thylakoid electron transport chain is thought to be through the reaction of O_2 with reduced iron sulphur clusters on the acceptor side of PSI (A/B/X), or with reduced ferredoxin. The committed step in the

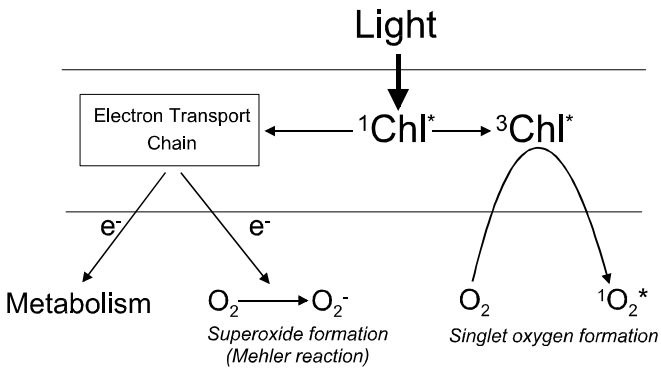

Fig. 1: *Principal routes for the light-induced formation of reactive oxygen species in plant chloroplasts. Excited chlorophyll ($^1Chl^*$) will normally drive electron transport through the thylakoid electron transport chain. If the capacity of metabolism is limited, excess electrons may be donated to oxygen, forming superoxide (**the Mehler reaction**). Excited chlorophyll can also undergo intersystem crossing, forming triplet chlorophyll. This can in turn sensitise the formation of **singlet excited oxygen**. Both superoxide and singlet excited oxygen can initiate a cascade of reactions, forming free radicals that damage the cell. For a review see Asada (1999), Foyer and Noctor (2000)*

reduction of these acceptors is charge separation in the PSI reaction centre. Therefore, regulation of PSI charge separation would be a possible mechanism for avoiding the over-reduction of PSI acceptors and so of O_2 reduction. PSI charge separation could be controlled either by controlling the rate at which excitation energy arrives at P700 or by controlling the rate at which P700 is re-reduced following charge separation. It is the latter mechanism that is discussed here.

The rate limiting step in the electron transport chain is generally thought to be the oxidation of plastoquinol by the cytochrome b_6f complex. *In vitro*, this step is regulated by the pH of the thylakoid lumen, oxidation being inhibited at low pH. This process has been termed "photosynthetic control" by analogy to the "respiratory control" that occurs to limit electron transport in the mitochondrion. Although there is evidence of the potential for such regulation *in vivo*, until recently most studies had failed to demonstrate its occurrence in leaves under normal physiological conditions.

The flux through the electron transport chain can easily be monitored by recording the decay of oxidised P700 following a light to dark transition (Harbinson and Hedley 1989; see Fig. 2). From such data, it is possible to determine a pseudo-first order rate constant for P700 reduction, k, which can be used as an indicator of the conductance of the electron transport chain (but see Sacksteder and Kramer, 2000 for reservations). With changing irradiance, the kinetics of P700 reduction were found to be surprisingly constant (Harbinson and Hedley 1989; Kramer et al. 1999; Laisk and Oja 1994). By contrast, however, Ott et al. (1999) observed that in the plant *Silene dioica* P700 reduction kinetics are sensitive to light, with k decreasing with increasing irradiance (Fig. 3.). This sensitivity was, however, only present at temperatures at or above the growth temperature. At lower temperatures, no irradiance dependence could be detected. This lack of sensitivity to light correlated with an insensitivity to elevated CO_2 (Fig. 4). We interpret this temperature effect as indicating that at low temperatures the capacity of electron transport was limiting for overall photosynthesis, removing the need for any down regulation.

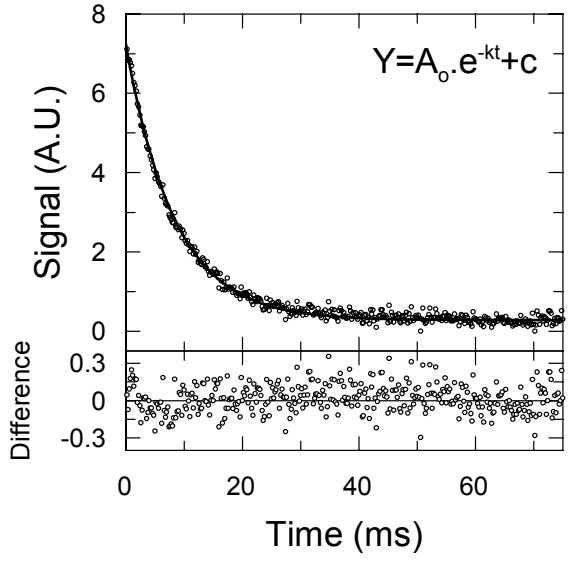

***Fig 2**: Decay kinetics of P700 following a light dark transition. Actinic light is removed by closure of an electronic shutter and P700 redox state monitored as a change in the absorbance at 830 nm, using a Walz fluorometer with a P700 DW emitter detector unit. In order to determine the kinetics of the decay, a mono-exponential curve is fitted, allowing a pseudo-first order rate constant, k, to be estimated.*

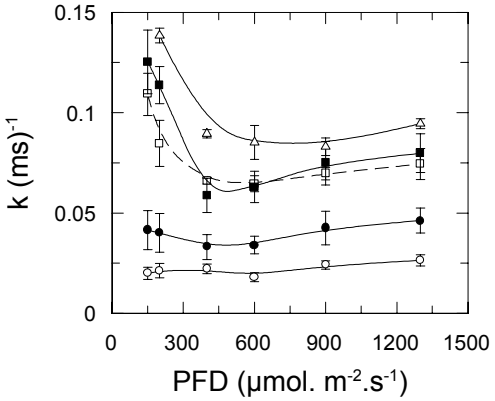

Fig. 3: *Effect of light intensity on the conductance of the thylakoid electron transport chain. Conductance is indicated by k, the rate constant for reduction of P700 upon darkening of a leaf. At temperatures above 20°C, k is sensitive to light. Below 20°C, the temperature sensitivity is damped. Symbols: open circles: 10°C; closed circles: 15°C; open squares: 20°C; closed squares: 25°C, triangles: 30°C. Data from Ott et al. 1999.*

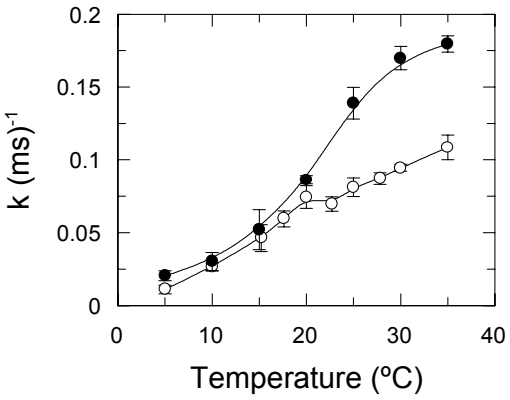

Fig. 4: *Effect of temperature and CO_2 supply on conductance of the electron transport chain. Conductance is indicated by k, the rate constant for reduction of P700 upon darkening of a leaf. At temperatures above 20°C, k is CO_2 sensitive. Below 20°C k is temperature insensitive. Symbols: open: ambient air; closed: elevated CO_2 Data from Ott et al. 1999.*

In addition to observing irradiance sensitivity of k, Ott et al. (1999) demonstrated that down regulation of electron transport could also occur in response to drought (Fig. 5) and to feeding of leaves with sucrose. The latter was intended to mimic conditions where photosynthesis is limited by the accumulation of end-products, as may occur when plants are sink-limited.

Overall, these data demonstrate that the electron transport chain is regulated *in vivo* in a manner that would limit the reduction of oxygen. The question remains as to how this regulation is achieved. Ott et al. (1999) observed a lack of correlation between fast relaxing non photochemical quenching, which they used as an indication of ΔpH, and the value of k. Each of these parameters varied under conditions where the other was constant. This was interpreted as indicating that a factor other than pH was regulating k. They suggested that there maybe some redox sensing mechanism feeding back and inhibiting electron transport.

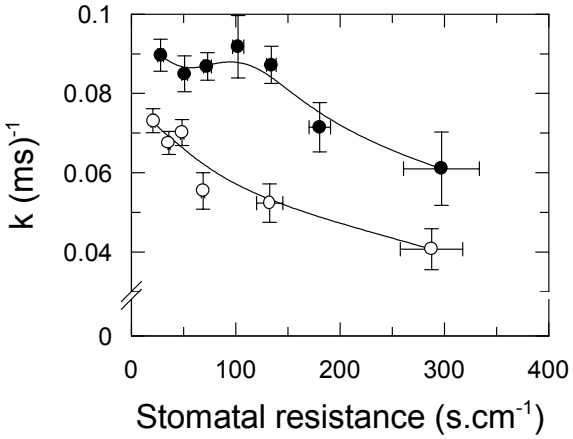

Fig. 5*: Effect of drought on the conductance of the thylakoid electron transport chain. Conductance was measured as the rate constant for reduction of the oxidised Photosysem I electron donor, P700 (k). Plants were droughted over the course of 2 weeks and k and stomatal resistance measured. Open symbols: measurements performed in the presence of ambient air, closed symbols measured in the presence of saturating concentrations of CO_2. Data from Ott et al. 1999.*

Avoiding singlet oxygen formation:
A side effect of restricting the flow of electrons through the electron transport chain is that there will be a reduction in the efficiency with which PSII is able to dissipate absorbed energy. This will tend to lead to an increase in the lifetime of excitation energy in the PSII antenna (reflected as an increase in chlorophyll fluorescence yield) and an increase in the probability of charge recombination in the PSII reaction centre, due to the slowing of Q_A oxidation by Q_B. Both of these events are likely to cause an increase in triplet chlorophyll formation and the latter may be involved in acceptor side photoinhibition of PSII. For these reasons, it has been suggested that electron transport to oxygen may actually be beneficial, helping to dissipate excess absorbed energy (Polle 1996).

It is thought that the process of high energy state quenching (qE) may play an essential role in protecting plants against PSII photoinhibition (see Horton et al. 1996 for a review). This requires the presence of a ΔpH across the thylakoid membrane and is activated in the presence of the xanthophyll zeaxanthin. The question arises, how is the ΔpH required to drive this quenching generated, especially under conditions where linear electron transport is limited? One suggestion is that electron transport to oxygen in the Mehler reaction may be generating this (see Polle 1996 for a discussion).

Clarke and Johnson (2001) examined the extent to which electron transport through PSII and PSI are sensitive to oxygen concentration in barley across a range of temperatures. Measurements were made in the presence of saturating CO_2 in order to suppress photorespiration. PSII electron transport was found to be sensitive to oxygen only at higher temperatures (15°C and above; see Fig. 6). At low temperatures, there was no sensitivity to oxygen, indicating that no Mehler reaction occurred. At temperatures where PSII electron transport was O_2 sensitive, reducing O_2 partial pressure was observed to have little or no effect on non photochemical quenching of chlorophyll fluorescence (Fig. 7). Indeed, there was if anything a tendency for NPQ to increase under conditions of low oxygen. These data were taken to indicate that any electron transport to oxygen was not contributing to the ΔpH that regulated qE.

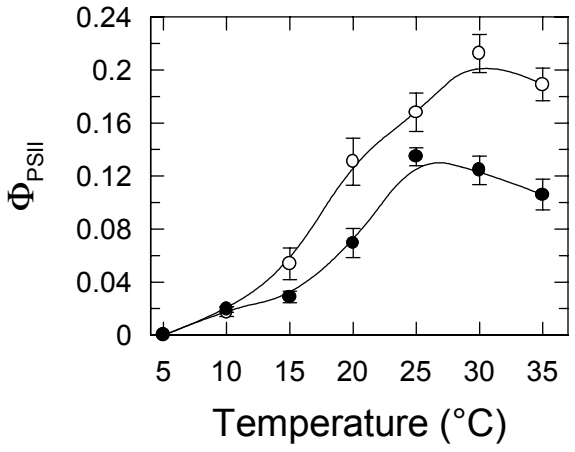

Fig 6: *Temperature dependence of the efficiency of Photosystem II (Φ_{PSII}) measured at 1600 µmol.m^{-2}.s^{-1} in the presence of saturating CO_2 and either 21% (open symbols) or 2% (closed symbols) O_2. Where values measured in the presence of 2% O_2 are lower than those in the presence of 21%, this indicates that electron transport to oxygen is occurring*

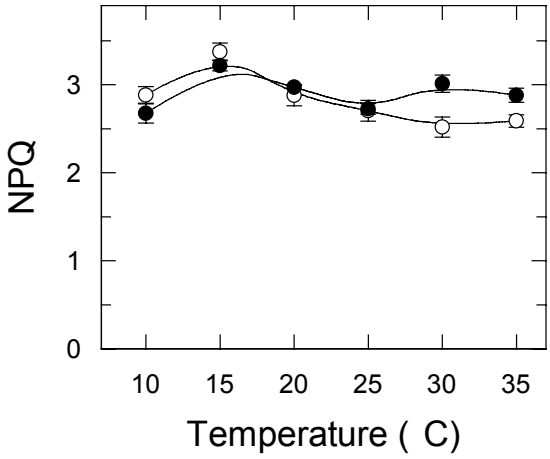

Fig 7: *Temperature dependence of non photochemical quenching of chlorophyll fluorescence (NPQ) measured at 1600 µmol.m^{-2}.s^{-1} in the presence of saturating CO_2 and either 21% (open symbols) or 2% (closed symbols) O_2 (Data from Clarke and Johnson, 2001)*

An alternative mechanism to generate ΔpH under conditions where linear electron transport is not possible, would be through cyclic electron transport. A number of workers have suggested that cyclic electron transport may play a protective role under conditions of stress, with, for example, the kinetics of P700 reduction being accelerated after exposure of a leaf to high light (Cornic et al. 2000). There has, however, been little direct evidence for cyclic electron transport. Typically, measurements of PSI and PSII efficiency have been shown a linear correlation between the two parameters, passing through the origin. Such relationships have been interpreted as evidence against cyclic electron transport. Clarke and Johnson (2001) examined the temperature sensitivity of both PSI and PSII electron transport. PSII electron transport was seen to rise with temperature with a Q10 of approximately 3 (i.e. a 3-fold rise for a 10°C rise in temperature). This compares to PSI, which rose with a Q10 of approximately 2. This was interpreted as indicating that cyclic electron transport was enhanced at low temperatures. Measurements of PSI and PSII electron transport in response to changing CO_2 concentrations also lead to the same conclusion. If PSI electron transport is measured using the kinetics of P700 reduction (see Sacksteder and Kramer 1998) this is seen to be surprisingly constant with CO_2, whereas PSII electron transport falls as CO_2 concentration decreases (Fig . 8).

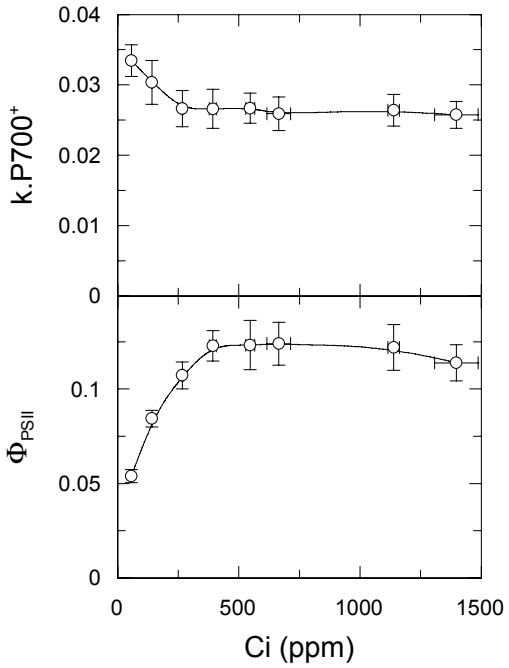

Fig. 8: *Relationship between internal CO_2 concentration and k.P700$^+$, a measure of PSI turnover (top) and Φ_{PSII} a measure of PSII turnover (bottom) measured in barley.*

Conclusion:

Based on the evidence presented by a number of authors (Harbinson and Hedley 1989; Harbinson 1994; Laisk and Oja 1994, 1995; Genty and Harbinson 1996; Ott et al. 1999; Kramer et al. 1999; Clarke and Johnson 2001) is seems clear that the potential exists for the thylakoid electron transport chain to be regulated and that this regulation operates under conditions where the capacity for carbon fixation falls below that of electron transport. The mechanism of this regulation remains to be elucidated but may be sensing the redox poise of the stroma (Ott et al., 1999). There is some evidence that under conditions where linear electron transport is down-regulated, there may be a relative increase in cyclic electron transport around PSI, which generates a ΔpH necessary for high energy state quenching. Together these mechanisms help protect the plant from oxidative damage during exposure to environmental stress.

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