# Signal transduction in response to excess light: getting out of the chloroplast

## Philip Mullineaux* and Stanislaw Karpinski†

Plants are continually in danger of absorbing more light energy than they can use productively for their metabolism. Acclimation to environmental conditions therefore includes the development of mechanisms for dissipating or avoiding the accumulation of such excess excitation energy. Acclimation could be controlled by many signal transduction pathways that would be initiated by the perception of excess excitation energy both inside and outside the chloroplast. Recent studies in related areas provide models of how these signalling pathways could operate in acclimation to excess light. Components of photosynthetic electron transport chains, reactive oxygen species, redox-responsive protein kinases, thiol-regulated enzymes, chlorophyll precursors and chloroplast-envelope electron transport chains all have roles in these models.

### Addresses
*Department of Disease and Stress Biology, John Innes Centre, Colney, Norwich NR4 7UH, UK;
e-mail: phil.mullineaux@bbsrc.ac.uk
†Department of Botany, Stockholm University, Lilla Frescati v.5, Frescati , SE-106 91, Stockholm, Sweden

### Abbreviations

| | |
|---|---|
| ABA | abscisic acid |
| APX1 | ASCORBATE PEROXIDASE1 |
| EEE | excess excitation energy |
| FMN | flavin mononucleotide |
| JA | jasmonic acid |
| LHC | light-harvesting complex |
| LHCP | LHC protein |
| NADPH | reduced form of nicotinamide adenine dinucleotide phosphate |
| NPH1 | NONPHOTOTROPHIC HYPOCOTYL 1 |
| NPL1 | NPH1-like1 |
| PET | photosynthetic electron transport |
| PSII | photosystem II |
| PsbS | gene encoding the PSII subunit S protein |
| ROS | reactive oxygen species |
| TAK1 | THYLAKOID-ASSOCIATED KINASE1 |

## Introduction

The amount of light energy encountered by plants in excess of that which they need for photosynthetic productivity is termed excess excitation energy (EEE) [1•,2–4]. The amount of EEE that plants experience may also be dictated by additional environmental and developmental factors that cause the amount of light energy required for cellular processes to vary. Disease, nutrient and water limitation, and rapid changes in temperature can promote EEE even at light intensities that would not pose a problem under benign conditions.

The possibility of generating EEE is ever-present for land plants, which cannot move away from adverse environmental conditions. Failure to dissipate or avoid accumulating EEE leads to photooxidative damage to the photosynthetic apparatus, which is often manifested as bleaching, chlorosis or bronzing of leaves [1•,2–5]. Many mechanisms have evolved that serve to dissipate EEE and act as 'safety valves' to ensure that the harvesting of light energy does not inadvertently lead to cellular damage [1•]. Acclimation to a range of adverse environmental conditions might include increasing the number and efficiency of dissipatory mechanisms and developing physiological, biochemical and structural changes that avoid the accumulation of EEE.

Immediate responses to the conditions that promote EEE must initiate signalling pathways that lead to whole-plant acclimation. In this review, we draw on a wider literature to suggest ways in which such signalling pathways might be initiated and function.

## Dissipation and avoidance of EEE

Several excellent reviews have been written recently that describe the protective mechanisms of dissipation and avoidance of EEE [1•,2,3,6,7]. Briefly, dissipation of EEE in plants is achieved by a combination of so-called non-photochemical and photochemical quenching processes.

Non-photochemical quenching processes include the transfer of triplet-state chlorophyll excitation energy to carotenoids that, in turn, dissipate the excess energy as heat during their return to a non-excited ground state [1•,3,6]. In Arabidopsis, a chlorophyll-pigment-binding protein, photosystem II (PSII) subunit S protein (which is encoded by the nuclear gene PsbS), has no effect on photosynthetic efficiency but is absolutely required for non-photochemical quenching [8••].

Photochemical quenching of EEE is the collective term for processes that increase the consumption of electrons by the deployment of additional metabolic sinks. These include the reduction of $O_2$ by electrons fed directly from PSII or PSI (i.e. the Mehler reaction), increased rates of photorespiratory and chlororespiratory metabolism, and perhaps increased C, N and S metabolism [2,7,9–12]. An increase in the production of reactive oxygen species (ROS) is an inevitable by-product of the reduction of $O_2$ in many of these processes. This increases further the dissipatory value of such reactions, as both electrons and energy are required to maintain an extensive network of antioxidants that prevent the accumulation of ROS [7]. Although potentially damaging, ROS also play a part in other protective responses, such as the reversible photoinhibition of PSII

activity [13], and induce acclimation to conditions that promote EEE both locally and systemically [5].

Acclimation to conditions that promote EEE not only includes an increase in the number and activity of dissipatory processes, but also may involve protective strategies that avoid the absorption of excitation energy. These include the movement of chloroplasts away from high light sources [14••], a decrease in the number of photosynthetic reaction centres per unit leaf area [15], leaf curling [16], an increase in the thickness of cuticular wax or other EEE-protective screens [17] and changes in leaf and whole-plant morphology [2]. The dynamic balance of ROS and antioxidant systems has been suggested to play a crucial role in determining how quickly plants react and acclimate to changes in their environment [5,18]. This suggests that the regulatory and signal transduction pathways involved in establishing whole-plant acclimation must contain individual components that are sensitive to this ROS/antioxidant balance.

### Signal transduction pathways that respond to EEE
An emerging literature clearly indicates that signalling pathways are integrated into a regulatory network, such that many pathways may share common routes or interact with one another [19–21]. We could predict, therefore, that a major part of an EEE-responsive signalling pathway(s) may well join such a regulatory network in order to effect acclimation at the whole-plant level. The remainder of this review focuses on those features of EEE signalling responses that pose the following questions: How are the conditions that promote EEE perceived and transduced into a signal, especially but not exclusively within the chloroplast? And how does a chloroplast-derived signal exit the chloroplast to join a cellular regulatory network?

### The perception of EEE and the initiation of signal transduction
The photosynthetic apparatus is a prime candidate for the perception of EEE. In principle, any increase in the activity of dissipatory processes could initiate signalling pathways. In response to EEE, increases in electron transport rates and consequent redox changes in photosynthetic electron transport (PET) components would be almost instantaneous. The regulation of both nuclear and chloroplast genes that encode components of photosynthesis and antioxidant metabolism have been associated with redox changes in PET [5,22,23•,24]. Examples of such nuclear-encoded genes include *Cab* (which encodes a chlorophyll a/b binding protein), *Lhc* (the gene encoding the light-harvesting complex protein [LHCP]; [23•]), *RbcS* (which encodes the small unit of ribulose-1,5-bisphosphate carboxylase/oxygenase; [23•]), *ASCORBATE PEROXIDASE1* (*APX1*) and *APX2* [5,22]. Chloroplast-encoded genes that have been associated with redox changes in PET include *psbA* (the gene encoding the D1 protein of the PSII reaction centre) and *psaAB* (which encodes the PSI reaction centre protein; [24]). Redox changes in the
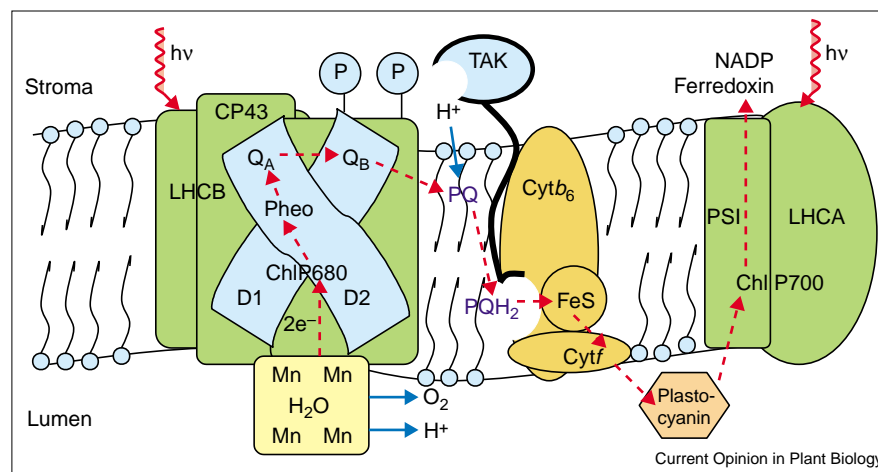
vicinity of $Q_A$ (the primary quinone electron acceptor), $Q_B$ (the secondary quinone electron acceptor) or plastoquinone (Figure 1) have been suggested to be key starting points for signalling [5,22,23•,24,25]. Equally, it is likely that changes in the redox state of PET components could be coincident with, rather than determine, changes in gene expression. For example, increased rates of PET could lead to the channelling of electrons into other routes, such as the chlororespiratory pathway, which in turn could initiate a signalling pathway. Alternatively, changes in pH across thylakoid membranes upon an increase in EEE could be a rapid and powerful signal initiator [3].

The exposure of plants to wavelengths of light that favour absorption from PSII over that from PSI triggers the reversible phosphorylation of the LHCP in PSII, which then migrates from PSII towards PSI. This process is termed 'state transition', and is thought to ensure a balance in the light harvesting and, consequently, the functioning of the two photosynthetic reaction centres [24,26••]. THYLAKOID-ASSOCIATED KINASE1 (TAK1) is central to this process [26••]. The simplest model of how the phosphorylation of LHCP is linked to redox changes in PET components is a postulated direct interaction between the cytochrome $b_6$/f complex and TAK1 (Figure 1); TAK1 then disengages to carry out the phosphorylation of thylakoid proteins including LHCP [26••]. This type of signal-transduction event may well prove to be a model for the type of events that initiate changes in nuclear gene expression in response to EEE.

Changes in the concentrations or rates of production of ROS, from the Mehler reaction or conceivably from chlororespiration, could be additional initiators of signalling pathways inside the chloroplast. Increases in foliar $H_2O_2$ concentrations have been shown to be important for the induction of *APX2* expression in excess-light-stressed *Arabidopsis* leaves, but the sub-cellular origin of this $H_2O_2$ was unknown [5]. Conversely, the infiltration of the thiol antioxidant glutathione (γ-glutamylcysteinyl glycine) into *Arabidopsis* leaves [27], or increasing the glutathione biosynthetic capacity of the chloroplasts of transgenic tobacco, increased the sensitivity of the leaves to excess light. Changes in chlorophyll *a* fluorescence parameters in such leaves implied a marked increase in EEE that led to damage to their photosynthetic apparatus. In both studies, this was associated with a failure to increase antioxidant-scavenging capacity in response to EEE-induced oxidative stress [22,28]. Both treatments could have changed the chloroplast redox status by upsetting the balance between ROS and antioxidant levels. This implies that signalling by chloroplast-derived ROS and/or the prevailing redox state of the chloroplast could be important determinants regulating the expression of nuclear-encoded genes [27,28,29••]. The propagation of such signals inside the chloroplast may have parallels to the regulation of the translation of chloroplast-encoded mRNAs and the activity of Calvin-cycle enzymes by protein disulphide

## Figure 1

Schematic description of the photosynthetic apparatus and the transport of electrons, abstracted from water, by the different PET chain components. Many of these components may be initiators of signalling in response to excess excitation energy and are discussed in the text. Chl, chlorophyll; CP43, chloroplast protein 43; D1, D1 protein; FeS, iron-sulphur centre; Pheo, pheophytin; PQ, oxidised plastoquinone; $PQH_2$, reduced plastoquinone; $Q_A$, primary quinone electron acceptor;, $Q_B$, secondary quinone electron acceptor.



isomerase (PDI) and thioredoxin, respectively [30,31]. Both of these regulatory systems function by carrying out thiol-disulphide exchange reactions on specific redox-sensitive cysteine residues in target proteins and are dependent on the activity of PET to provide reducing equivalents. Their sensitivity to conditions that promote EEE [30,32••] show that these processes are highly responsive to chloroplast redox state.

To our knowledge, neither non-photochemical quenching mechanisms nor highly energised photosynthetic pigments have been directly implicated in any of the signalling mechanisms initiated by EEE. However, *NPH1-like1* (*NPL1*) from *Arabidopsis*, which governs the relocation of chloroplasts within the cell in response to high light intensity, encodes an extraplastidial serine/threonine protein kinase that contains a covalently bound flavin mononucleotide (FMN) moiety that is a blue-light chromophore [14••]. NPL1 is closely related to the NONPHOTOTROPHIC HYPOCOTYL1 (NPH1) class of protein kinases that are involved in other blue-light-mediated responses in plants, such as phototropism [14••,33]. It is postulated that the FMN moiety of both NPH1 and NPL1 confers on them redox sensitivity that leads to their activation [14••,33].

### The transmission of signals across the chloroplast envelope

It should be noted that many of the hormones or signalling molecules associated with stress, such as jasmonic acid (JA) and abscisic acid (ABA), are synthesised wholly or in part within the chloroplast [34,35]. The involvement of EEE in both abiotic and biotic stresses clearly shows that such molecules could, in effect, signal for EEE. For example, in drought-stressed plants, the recently described mediation of ABA-controlled stomatal closure by $H_2O_2$ [36••] is a clear indication of the way in which such signalling molecules could interact with the products of EEE. To our knowledge, the routes that these molecules might use to exit the chloroplast are not known.

$H_2O_2$ is thought to freely diffuse as easily as water across biological membranes [5,37]. Chloroplast-derived $H_2O_2$ could, therefore, directly influence the functions of extra-plastidial signalling components. The potential for $H_2O_2$ to act as an intracellular mobile signalling molecule is demonstrated by its role in the systemic responses of plants to excess light [5], pathogens [38] and physical damage [39].

Oxidative damage to the thylakoid membranes brought about by EEE could lead to the release of oxygenated fatty acid derivatives, including JA, known collectively as oxylipins [39,40••]. The appearance of these compounds in plant tissues is associated with physical damage and disease [39,40••], but there is no reason why such compounds could not be formed under conditions that promote EEE in the chloroplast [19]. Such compounds may be membrane-permeable and, in *Arabidopsis*, have been shown to induce expression of *GLUTATHIONE-S-TRANSFERASE1* (*GST1*), a gene whose expression responds to a wide range of conditions, including those that promote EEE [19,40••].

In *Chlamydomonas*, chlorophyll precursors have been implicated in chloroplast-to-nucleus signalling during dark-to-light transitions [41••]. Exogenous supply of these precursors to cultures mediated the light-induction of the expression of heat-shock genes in mutants otherwise incapable of this response to light [41••]. Kropat *et al*. [41••] hypothesised, therefore, that in higher plants as well as in algae, the release or export of chlorophyll precursors from the chloroplast is an active process that can occur under certain stress conditions and that can initiate defence responses.

Rates of photorespiration increase substantially in response to EEE [9,37,42]. The consequent increase in photorespiratory metabolism in the peroxisome causes increased rates of $H_2O_2$ formation, brought about by the oxidation of glycollate [37]. Increased levels of photorespiratory $H_2O_2$ have been shown to elicit cellular antioxidant defences in

transgenic tobacco plants that are deficient in catalase [37], but whether this occurs in wildtype plants remains to be established. Nevertheless, photorespiratory metabolites must be candidates for a means of getting an EEE-responsive signal out of the chloroplast.

The different scenarios described above all depend upon the passage of some molecule out of the chloroplast to propagate a signal leading to nuclear gene expression. Spectroscopic studies have shown, however, that the chloroplast envelope contains a number of constituents that are involved in the transfer of electrons and therefore could provide another exit for a chloroplast-derived signal. These include iron-sulphur proteins, semiquinones, flavins and α-tocopherol [43]. It is possible to draw a scheme in which an electron transport chain starts with NADPH in the stroma, spans the chloroplast envelope and ends with $O_2$ as the terminal electron acceptor on the chloroplast outer surface [43]. From the stoichiometry of electron transfer from NADPH, there is a clear implication that ROS could be formed outside the chloroplast and could, therefore, act to propagate any signal emanating from it. Exposure to excess light, for example, would at least transiently increase PET rates, which could stimulate the activity of an envelope-spanning electron transport chain.

## Joining the mainstream

In many of the schemes outlined above, $H_2O_2$ or other ROS are the end-products of signalling pathways that emanate directly or indirectly from the chloroplast. There are a number of ways in which one can envisage how such a signal might be propagated further. ROS could directly interact with redox-sensitive transcription factors paralleling OxyR and Sox/RS in *E. coli* or I-κB:NF-κB in animals [44,45]. The activation of transcription factors would then lead to change in gene expression.

Intracellular $Ca^{2+}$ fluxes and the action of signalling molecules, such as phosphoinositides and JA, have been shown to respond to oxidative stress and have been suggested as a means whereby cross-talk between pathways is achieved [21,46–48]. The activation of extensive calcium-dependent protein kinase cascades could provide a mechanism whereby a chloroplast-derived ROS signal would merge into a regulatory network. The indirect activation of a mitogen-activated protein kinase cascade in $H_2O_2$-treated *Arabidopsis* protoplasts, which induced the expression of genes involved in defence against oxidative stress and suppressed genes that are involved in plant growth [49••], is a clear indication of how such networks might operate.

## Conclusions

We have described briefly some of the means whereby EEE can initiate signalling leading to acclimation to changing environmental circumstances. Given the flexibility of plants' responses to environmental change and the importance of protection against EEE, it would not be surprising to find that all of these possible signalling routes exist, reflecting the simultaneous operation of many different protective mechanisms.

There is a need to understand how wide a range of adverse environmental factors provoke the same changes in cellular metabolism, reflecting the changed expression of a common or overlapping set of genes. The holistic view of cellular metabolism that metabolite, protein and transcript profiling technology could bring to this problem may prove to be seminal. It is to be hoped that a rational and prediction-based understanding of how plants thrive in highly variable and often adverse environments will emerge from such systems analysis.

## References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:

  •  of special interest
  •• of outstanding interest

1.  Niyogi KK: **Safety valves for photosynthesis.** *Curr Opin Plant Biol*
•   2000, **3**:445-460.
A concise and accessible overview of the mechanisms for dissipating excess excitation energy in photosynthesis.

2.  Huner NPA, Öquist G, Sarhan F: **Energy balance and acclimation to light and cold.** *Trends Plant Sci* 1998, **3**:224-230.

3.  Horton P, Ruban AV, Walters RG: **Regulation of light harvesting in green plants.** *Annu Rev Plant Physiol Plant Mol Biol* 1996, **47**:655-684.

4.  Havaux M, Bonfils J-P, Lutz C, Niyogi KK: **Photodamage of the photosynthetic apparatus and its dependence on the leaf developmental stage in the *npq1 Arabidopsis* mutant deficient in the xanthophyll cycle enzyme violaxanthin de-epoxidase.** *Plant Physiol* 2000, **124**:273-284.

5.  Karpinski S, Reynolds H, Karpinska B, Wingsle G, Creissen G, Mullineaux PM: **Systemic signalling and acclimation in response to excess excitation energy in *Arabidopsis*.** *Science* 1999, **284**:654-657.

6.  Muller P, Li X-P, Niyogi KK: **Non-photochemical quenching. A response to excess light energy.** *Plant Physiol* 2001, **125**:1558-1566.

7.  Asada K: **The water–water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons.** *Annu Rev Plant Physiol Plant Mol Biol* 1999, **50**:601-639.

8.  Li X-P, Björkmann O, Shih C, Grossman AR, Rosenquist M,
••  Jansson S, Niyogi KK: **A pigment-binding protein essential for regulation of photosynthetic light harvesting.** *Nature* 2000, **403**:391-395.
The isolation and characterisation of the *Arabidopsis npq4-1* mutant shows that the PsbS protein plays a central role in the dissipation of excess light energy. PsbS is a member of a family of LHC proteins but is not involved in light harvesting. This work clearly shows that co-evolution of dissipatory and light-harvesting machinery has occurred. It is an excellent example of a combined genetic, biochemical and biophysical approach to solving an important problem.

9.  Wingler A, Lea PJ, Quick PW, Leegood RC: **Photorespiration: metabolic pathways and their role in stress protection.** *Philos Trans R Soc Lond Biol* 2000, **355**:1517-1529.

10. Nixon PJ: **Chlororespiration.** *Philos Trans R Soc Lond Biol* 2000, **355**:1541-1547.

11. Fryer MJ, Andrews JR, Oxborough K, Blowers DA, Baker NR: **Relationship between CO$_2$ assimilation, photosynthetic electron transport, and active O$_2$ metabolism in leaves of maize in the field during periods of low temperature.** *Plant Physiol* 1998, **116**:571-580.

12. Streb P, Feierabend J: **Significance of antioxidants and electron sinks for the cold-hardening-induced resistance of winter rye leaves to photo-oxidative stress.** *Plant Cell Environ* 1999, **22**:1225-1237.

13. Russell AW, Critchley C, Robinson SA, Franklin LA, Seaton GGR, Chow W-S, Anderson J, Osmond CB: **Photosystem II regulation and dynamics of the chloroplast D1 protein in *Arabidopsis* leaves during photosynthesis and photoinhibition.** *Plant Physiol* 1995, **107**:943-952.

14. Kagawa T, Sakai T, Suetsugo N, Oikawa K, Ishiguro S, Kato T,
•• Tabata S, Okada K, Wada M: ***Arabidopsis* NPL1: a phototropin homolog controlling the chloroplast high-light avoidance response.** *Science* 2001, **291**:2138-2141.
This work highlights the molecular basis for the regulation of at least one mechanism for chloroplast movement in response to high light. NPL1 is a member of the NPH1 class of serine-threonine protein kinases [33]. This class of kinase proteins contain a FMN-binding domain, which is found within a conserved so-called LOV (light, oxygen and voltage) domain and which is also found in a range of prokaryotic proteins. The flavin moiety acts as a blue-light chromophore and confers redox sensitivity upon NPL1. NPL1, like the closely related NPH1 [33], may phosphorylate plasma-membrane-located protein(s).

15. Walters RG, Rogers JJM, Shephard F, Horton P: **Acclimation of *Arabidopsis thaliana* to the light environment: the role of photoreceptors.** *Planta* 2000, **209**:517-527.

16. Neuner G, Braun, V, Buchner O, Taschler D: **Leaf rosette closure in the alpine rock species *Saxifraga paniculata* Mill.: significance for survival of drought and heat under high irradiation.** *Plant Cell Environ* 1999, **22**:1539-1548.

17. Barnes JD, Percy KE, Paul ND, Jones P, McLaughlin CK, Mullineaux PM, Creissen G, Wellburn AR: **The influence of UV-B radiation on the physicochemical nature of tobacco (*Nicotiana tabacum* L.) leaf surfaces.** *J Exp Bot* 1996, **47**:99-109.

18. Noctor G, Veljovic-Jovanovic S, Foyer CH: **Peroxide processing in photosynthesis: antioxidant coupling and redox signalling.** *Philos Trans R Soc Lond Biol* 2000, **355**:1465-1475.

19. Mullineaux P, Ball L, Escobar C, Karpinska B, Creissen G, Karpinski S: **Are diverse signalling pathways integrated in the regulation of *Arabidopsis* antioxidant defence gene expression in response to excess excitation energy?** *Philos Trans R Soc Lond Biol* 2000, **355**:1531-1540.

20. Bowler C, Fluhr R: **The role of calcium and activated oxygens as signals for controlling cross-tolerance.** *Trends Plant Sci* 2000, **5**:241-246.

21. Overmeyer K, Tuominen H, Kettunen R, Betz C, Langebartels C, Sandermann H Jr, Kangasjärvi J: **Ozone-sensitive *Arabidopsis rcd1* mutant reveals opposite roles for ethylene and jasmonate signaling pathways in regulating superoxide-dependent cell death.** *Plant Cell* 2000, **12**:1849-1862.

22. Karpinski S, Escobar C, Karpinska B, Creissen G, Mullineaux PM: **Photosynthetic electron transport regulates the expression of cytosolic ascorbate peroxidase genes in *Arabidopsis* during excess light stress.** *Plant Cell* 1997, **9**:627-640.

23. Oswald O, Martin T, Dominy PJ, Graham IA: **Plastid redox state**
• **and sugars: interactive regulators of nuclear-encoded photosynthetic gene expression.** *Proc Natl Acad Sci USA* 2000, **98**:2047-2052.
The regulation of the expression of genes associated with photosynthesis is strongly associated with carbon status. If sugars are present above a certain concentration, then the expression of photosynthetic genes is downregulated. Upon sugar starvation, such genes are upregulated. Unexpectedly, this regulation is achieved via redox changes that are associated with PET rather than by a more direct sugar-sensing mechanism. The features of this system are similar to those described for excess light-induced *APX* gene expression [22].

24. Pfannschmidt T, Nilsson A, Allen JF: **Photosynthetic control of chloroplast gene expression.** *Nature* 1999, **397**:625-628.

25. Escoubas J, Lomas M, LaRoche J, Falkowski PG: **Light intensity regulation of *cab* gene transcription is signaled by the redox state of the plastoquinone pool.** *Proc Natl Acad Sci USA* 1995, **92**:10237-10241.

26. Snyders S, Kohorn BD: **Disruption of thylakoid-associated kinase 1**
•• **leads to alteration of light harvesting in *Arabidopsis*.** *J Biol Chem* 2001, **276**:32169-32176.
How plants adjust to varying light quality by changing the balance of light absorption through PSII and PSI is crucial to their survival and is termed state transition. This molecular genetic study shows that TAKs are essential for state transitions in *Arabidopsis*. State transitions are achieved by the phosphorylation of LHCPs, causing their movement from PSII to PSI. The proposed association of the cytochrome b$_6$/f component of the PET chain with TAK1 provides a direct link between PET redox state and the functioning of TAK1, a regulatory protein.

27. May MJ, Vernoux T, Leaver C, Van Montagu M, Inze D: **Glutathione homeostasis in plants: implications for environmental sensing and plant development.** *J Exp Bot* 1998, **49**:649-667.

28. Creissen GP, Firmin J, Fryer M, Kular B, Leyland N, Reynolds H, Pastori G, Wellburn F, Baker N, Wellburn A, Mullineaux PM: **Elevated glutathione biosynthetic capacity in the chloroplasts of transgenic tobacco plants paradoxically causes increased oxidative stress.** *Plant Cell* 1999, **11**:1277-1291.

29. Vernoux T, Wilson RC, Seeley KA, Reichheld JP, Muroy S, Brown S,
•• Maughan SC, Cobbett CS, Van Montagu M, Inze D *et al.*: **The *ROOT MERISTEMLESS 1/CADMIUM SENSITIVE 2* gene defines a glutathione-dependent pathway involved in initiation and maintenance of cell division during postembryonic root development.** *Plant Cell* 2000, **12**:517-527.
This clear demonstration of the role that the cellular antioxidant glutathione plays in root meristem development, via its influence on the cell cycle, has far-reaching consequences (see also [27]). Glutathione is a major cellular redox determinant and is sensitive to changes brought about by EEE. The authors of this paper provide a framework to describe how such environmental effects could influence the processes governing plant growth and development.

30. Kim J, Mayfield SP: **Protein disulfide isomerase as a regulator of chloroplast translational activation.** *Science* 1997, **278**:1954-1957.

31. Dai S, Schwendtmayer C, Schurmann P, Ramaswamy S, Eklund H: **Redox signaling in chloroplasts: cleavage of disulfides by an iron-sulfur cluster.** *Science* 2000, **287**:655-658.

32. Hutchison RS, Groom Q, Ort DR: **Differential effects of**
•• **chilling-induced photooxidation on the redox-regulation of photosynthetic enzymes.** *Biochemistry* 2000, **39**:6679-6688.
The authors of this paper describe a possible biochemical basis for 'light-plus-chilling' sensitivity in crop species such as tomato. The thioredoxin-mediated regulation of some, but not all, Calvin cycle enzymes is chilling sensitive. The interaction between thioredoxin *f* and its target enzymes largely explains the chilling-induced inhibition of photosynthesis in tomato leaves and their subsequent photooxidative stress.

33. Huala E, Oeller PW, Liscum E, Han I-S, Larsen E, Briggs WR: ***Arabidopsis* NPH1: a protein kinase with a putative redox-sensing domain.** *Science* 1997, **278**:2120-2123.

34. Qin XQ, Zeevaart JAD: **The 9-cis-epoxycarotenoid cleavage reaction is the key regulatory step of abscisic acid biosynthesis in water-stressed bean.** *Proc Natl Acad Sci USA* 1999, **96**:15354-15361.

35. Mueller MJ: **Enzymes involved in jasmonic acid biosynthesis.** *Physiol Plant* 1997, **100**:653-663.

36. Pei Z-M, Murata Y, Benning G, Thomine S, Klusener B, Allen GJ,
•• Grill E, Schroeder JI: **Calcium channels activated by hydrogen peroxide mediate abscisic acid signalling in guard cells.** *Nature* 2000, **406**:731-734.
ABA is shown to cause the production of ROS in guard cells, which brings about stomatal closure in response to drought. ROS do this by inducing the activation of Ca$^{2+}$-permeable channels in the plasma membrane. The activation of Ca$^{2+}$ channels is a common response to many abiotic and biotic stresses [20,46], and is associated with effectors such hormones and elicitors. This work shows how ROS may integrate different signals into a common cellular response and that at least one ABA-sensitive mutant is defective in ROS perception.

37. Willekens H, Chamnongpol S, Davey M, Schraudner M, Langebartels C, Van Montagu M, Inze D, Van Camp W: **Catalase is a sink for H$_2$O$_2$ and is indispensable for stress defence in C$_3$ plants.** *EMBO J* 1997, **16**:4806-4816.

38. Bolwell GP: **Role of active oxygen species and NO in plant defence responses.** *Curr Opin Plant Biol* 1999, **2**:287-294.

39. Orozco-Cardenas ML, Narvaez-Vasquez J, Ryan CA: **Hydrogen peroxide acts as a secondary messenger for the induction of defense genes in tomato plants in response to wounding, systemin and methyl jasmonate.** *Plant Cell* 2001, **13**:179-191.

40. Vollenweider S, Weber H, Stolz S, Chetelet A, Farmer EE: **Fatty acid**
•• **ketodiences and fatty acid ketotrienes: Michael addition acceptors that accumulate in wounded and diseased** *Arabidopsis* **leaves.** *Plant J* 2000, **24**:467-476.
The cocktail of oxygenated fatty acids and their breakdown derivatives, collectively called oxylipins, are potent signalling molecules. The authors of this paper use a detailed chemical analysis to conclude that the $\alpha,\beta$-unsaturated carbonyl groups of ketodienes are the key features of oxylipins that induce stress-defence gene expression. Low molecular weight compounds such as acrolein and butan-2-one could mimic this induction. These compounds can also induce lesions in leaves similar to those encountered as a result of the hypersensitive response that is associated with gene-for-gene plant–pathogen interactions. These data provide a rational basis to explain how this wide range of compounds can have a similar or the same signalling role.

41. Kropat J, Oster U, Rudiger W, Beck CF: **Chloroplast signalling in**
•• **the light induction of nuclear** *HSP70* **genes requires the accumulation of chlorophyll precursors and their accessibility to cytoplasm/nucleus.** *Plant J* 2000, **24**:523-531.
The authors clearly illustrate the potential of chlorophyll biosynthetic precursors to act as chloroplast-to-nucleus signalling molecules, at least in *Chlamydomonas*.

42. Kozaki A, Takeba G: **Photorespiration protects C3 plants from photooxidation.** *Nature* 1996, **384**:557-560.

43. Jäger-Vottero P, Dorne A-J, Jordanov J, Douce R, Joyard J: **Redox chains in chloroplast envelope membranes: spectroscopic evidence for the presence of electron carriers, including iron-sulfur centers.** *Proc Natl Acad Sci USA* 1997, **94**:1597-1602.

44. Åslund F, Beckwith J: **Bridge over troubled waters: sensing stress by disulfide bond formation.** *Cell* 1999, **96**:751-753.

45. Jamieson DJ, Storz G: **Transcriptional regulators of oxidative stress responses.** In *Oxidative Stress and the Molecular Biology of Antioxidant Defenses.* Edited by Scandalios J. New York: Cold Spring Harbor Laboratory Press; 91-115.

46. Knight H, Knight MR: **Abiotic stress signalling pathways: specificity and cross-talk.** *Trends Plant Sci* 2001, **6**:262-267.

47. DrØbak BK, Watkins PAC: **Inositol (1,4,5) trisphosphate production in plant cells: an early response to salinity and hyperosmotic stress.** *FEBS Lett* 2000, **481**:240-244.

48. Rao MV, Lee H-I, Creelman RA, Mullet JE, Davis KR: **Jasmonic acid signaling modulates ozone-induced hypersensitive cell death.** *Plant Cell* 2000, **12**:1633-1646.

49. Kovtun Y, Chiu W-L, Tena G, Sheen J: **Functional analysis of**
•• **oxidative stress-activated mitogen-activated protein kinase cascade in plants.** *Proc Natl Acad Sci USA* 2000, **97**:2940-2945.
The authors describe the indirect activation of a mitogen-activated protein kinase cascade, which induces the expression of genes that are responsive to oxidative stress and suppresses those associated with growth. Their elegant approach, involving the co-introduction of promoter::luciferase gene fusions with constructs that overexpress the different components of the kinase cascade into *Arabidopsis* protoplasts, which are then treated with $H_2O_2$. This is the first convincing demonstration that a ROS does indeed activate this type of regulatory system in plants.