and mating systems are more likely than others to tip the scales in favour of cooperation. In particular, both polygyny [12] and overlap between generations [13] tend to boost the evolution of altruistic behaviour. Indeed, these traits even allow for altruistic acts directed towards random group members, without a need for kin recognition. These results suggest that the lifestyle of the greater horseshoe bat may be especially conducive to cooperative behaviour based on kin selection. There is only one problem: there is, thus far, no evidence that greater horseshoe bats engage in such behaviour despite many years of research on this species.

In other bats, several cooperative behaviours have been described. They range from feeding starved colony members [14], to nursing foreign pups [15], to information transfer about food [16] and suitable roosts [17]. Remarkably, most of these cooperative behaviours are apparently not preferentially directed towards kin. Blood regurgitation in vampire bats is even one of the few possible cases of reciprocal altruism in wild animals. Moreover, in the few bat species for which relevant data are available, relatedness does not explain spatial associations of individual females within colonies [18]. In greater horseshoe bats, however, no cooperative behaviours have been described and the only kin-directed behaviour shown — daughters sharing foraging areas with their mothers [19] — cannot be used to explain benefits due to increased relatedness within matrilines. Therefore, our current knowledge of bat sociobiology does not suggest that kin selection is the most important factor for stabilizing bat colonies [20]. In fact, if entire colonies function as social units, higher relatedness within matrilines and hence greater genetic differentiation between matrilines, as observed in greater horseshoe bats [2], may even disrupt social groups when cooperation would be directed towards kin only.

To determine the importance of mate sharing between relatives for kin selected cooperation and group stability we have to look at mate choice in animals where females live in groups consisting of several matrilines and that show cooperation. The current lack of evidence for cooperation among horseshoe bats does ask for a closer look at the social behaviour of this species. However, it should be kept in mind that even in taxa with high levels of relatedness among group members, such as in clonal aphids or haplo-diploid insects, cooperation is the exception rather than the rule [4].

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Photosynthesis: The Processing of Redox Signals in Chloroplasts

Recent work identifies two kinases required for phosphorylation of proteins of chloroplast thylakoid membranes. One kinase, STN7, is required for phosphorylation of light-harvesting complex II; another, STN8, is required for phosphorylation of photosystem II. How do these kinases interact, what do they do, and what are they for?

John F. Allen

Phosphorylation of chloroplast proteins was first reported in 1977, and shown to be light-dependent [1]. The implications of this landmark discovery are still being worked out. Now that mutants are available for a protein kinase whose activity was first demonstrated by John Bennett [1], one story can be retold with increased confidence. But there are clearly multiple chloroplast kinases, with multiple substrates and multiple effects. This was forecast [2], but only recently has a second protein kinase been identified, putting the phenomenon of chloroplast protein phosphorylation on course for more complete biochemical

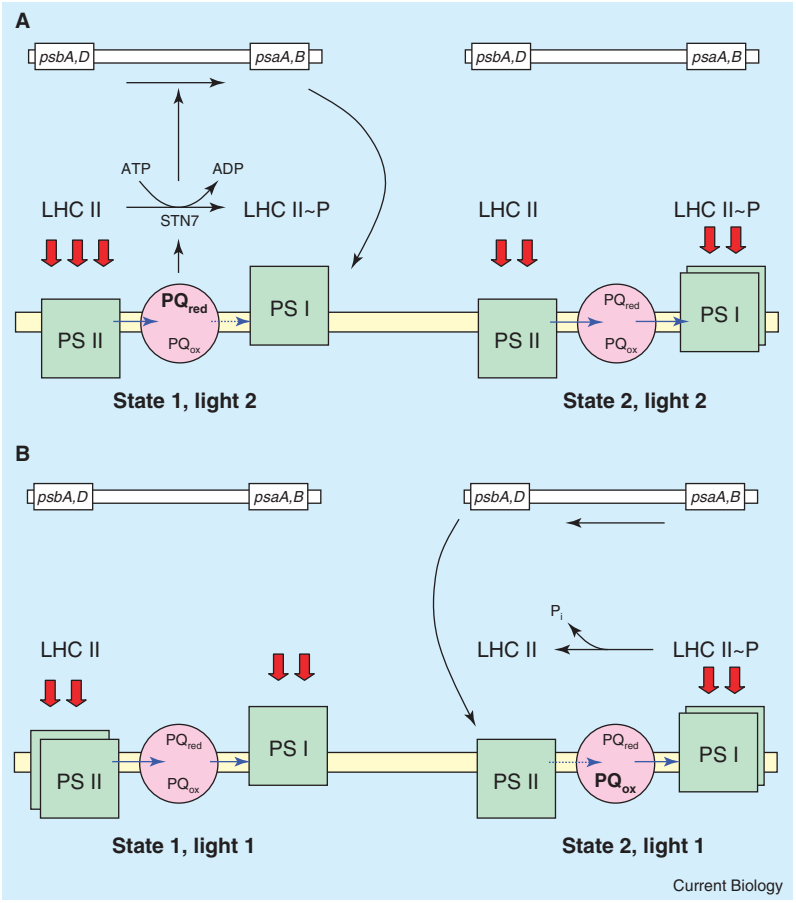

Figure 1. State transitions and control of gene expression in chloroplasts.

Proposed action of the LHC II kinase and transcriptional regulator STN7 during state transitions and adjustment of photosystem stoichiometry by means of transcriptional control of *psaA,B* (photosystem I) and *psbA,D* (photosystem II) reaction centre genes. If plastoquinone (PQ) becomes reduced (A, left) there are two consequences — activation of the STN7 LHC II kinase, and a relative increase in photosystem I (PS I) gene transcription. If plastoquinone becomes oxidised (B, right) then LHC II becomes dephosphorylated, and there is a relative increase in photosystem II (PS II) gene transcription.

investigation. There are provisional reports of further chloroplast kinases and phosphatases: two is certainly not enough. The final number of chloroplast kinases can only be guessed, so the story is likely to become more complex. Here I will argue that we are now catching glimpses of what will be resolved, eventually, as a redox signal-processing unit, a self-adjusting device which incorporates the chloroplast genetic apparatus, in addition to a network of post-translational modifications of pre-existing proteins.

To start with what can still be said: one of the chloroplast protein phosphorylation reactions, acting on apoproteins of the pigment-containing light-harvesting complex II (LHC II), is activated when plastoquinone, an electron carrier, is reduced. This redox-controlled reaction now has a well-characterised role in the regulation of photosynthesis — it serves to distribute absorbed excitation energy optimally between chloroplast photosystems I and II [3]. This means that plants and algae are able to make the best use of the available light energy by continuously monitoring the relative rates of electron transport through the reaction centres of the two photosystems.

For maximal efficiency and safety of operation, the reaction centres should remain balanced in their rates of light energy conversion and light-driven electron transport. If they become unbalanced — for example by a change in spectral composition of light which favours one photosystem over the other — then plastoquinone changes its redox state. It does this simply because plastoquinone accepts electrons from photosystem II, and donates them to photosystem I. When the photosystems are balanced, the rate of electron flow into plastoquinone will equal the rate of electron flow out. When photosytem II runs momentarily faster than photosystem I, plastoquinone becomes reduced, the LHC II kinase is activated, and phosphorylated LHC II, with its light-absorbing chlorophylls, moves from photosystem II to photosystem I, making good the imbalance of light harvesting that delivered surplus energy to photosystem II, where it was wasted, as fluorescence or heat.

Conversely, if photosystem I receives more than its usable share of available light energy, plastoquinone becomes oxidised, the kinase becomes inactive, and a phospho-LHC II phosphatase acts to restore unphosphorylated LHC II, with its chlorophyll molecules, to photosystem II. In essence, this biochemical explanation of 'state transitions' — the adaptive, complementary alteration of the light-harvesting capacity of the photosystems — was mapped out in the 1980s [2]. Besides characterisation of the LHC II kinase and phosphatase, there are at least two important directions for the future. What, actually, happens to LHC II as a result of its phosphorylation? Why, and how, does reaction centre chloroplast gene transcription provide a parallel means of balancing the two photosystems in response to the redox signal from plastoquinone?

Other than LHC II, which serves both photosystems, protein substrates of chloroplast thylakoid membranes seem to be mostly components of photosystem II, including the 'D1' product of the chloroplast *psbA* gene, at the core of the photosystem II reaction centre. Another conspicuous phosphoprotein was described by Bennett [1] as simply "the 9 kDa phosphoprotein". This turns out to

be the product of the chloroplast gene *psbH* and a component of the photosystem II reaction centre. The functional effects of these and other photosystem II phosphorylation reactions are less well understood than that of LHC II, which is encoded in the cell nucleus and imported into chloroplasts as a precursor. The recent identification of protein kinases required for these distinct phosphorylation reactions [4–6] seems to rule out a previous suggestion for PsbA phosphorylation, while supporting the state transition status quo for LHC II.

To dissect LHC II kinase activity, Depège *et al.* [4] used a combination of molecular genetics and chlorophyll fluorescence imaging to isolate potentially relevant mutants of the green alga *Chlamydomonas*, and then to identify and complement the gene. Previous investigations of LHC II kinase activity were biochemically based, and failed to turn up the enzyme. The authors focussed on a mutant missing the slow decrease in chlorophyll fluorescence that is the hallmark of the transition to 'state 2' [3]. This mutant led them to the putative LHC II kinase STT7, and they then moved to the enzyme's *Arabidopsis* homologue, STN7 [5].

In *Arabidopsis*, it was shown that STN7 is required for LHC II phosphorylation and for the fluorescence emission changes that report on the redistribution of LHC II to photosystem I at the expense of photosystem II. The requirement for LHC II phosphorylation for the transition to 'state 2' was confirmed by replacing an amino acid equivalent to residues known in other kinases to be required for binding ATP. Furthermore, the growth of *STN7* mutants was shown to be retarded under conditions of changing, low light intensity, consistent with the presumed physiological role of the state transitions whose mechanism depends upon reversible phosphorylation of LHC II.

Bonardi *et al*. [6] confirmed the chief conclusion of Bellafiore *et al*. [5] — that the protein kinase STN7 is required for the state 2 transition in *Arabidopsis*. They went on to show that STN7 is required for changes in photosystem stoichiometry, which require a plastoquinone redox control of reaction centre gene transcription [7]. Why these two processes depend upon a common protein kinase is an intriguing question. Certainly, LHC II phosphorylation and regulation of transcription both respond to the same redox signal, and within a time scale of a few minutes [8] (Figure 1).

Bonardi *et al*. [6] also showed that a related kinase, STN8, is required for phosphorylation of the photosystem II reaction centre, but not for state transitions, nor for photosystem stoichiometry adjustment. When PsbA phosphorylation is largely eliminated in *STN8* mutants, the rapid repair of PsbA after photoinhibition carries on without apparent change, which seems to rule out one previous suggestion for the physiological role of PsbA phosphorylation. It is likely that other protein kinases and phosphoprotein phosphatases, as yet unreported, will fit into a network of redox signal transduction within chloroplasts, connecting their photosynthetic competence with expression of chloroplast genes [9,10].

It has been shown that LHC II phosphorylation is suppressed *in vivo* at high light intensities, even when plastoquinone is reduced [11]. This does not contradict the established role for plastoquinone-controlled LHC II phosphorylation in state transitions, as these are observed at limiting light intensities and increase the quantum yield of photosynthesis, useful only when light is limiting. Inactivation of LHC II kinase at high light may result from thioredoxin being in its reduced state because of a restriction of electron flow from ferredoxin and NADPH, on the acceptor side of photosystem I [11].

Where does phosphorylation of PsbA and other photosystem II reaction centre proteins fit in? Biochemical experiments separating LHC II phosphorylation from photosystem II phosphorylation indicate that photosystem II electron transfer is promoted, at high light intensities, by photosystem II phosphorylation [12]. While the various thylakoid protein kinase reactions are redox-dependent, the phosphoprotein phosphatase reactions are redox-independent. The phospho-LHC II phosphatase catalyses a much faster reaction than the phospho-photosystem II phosphatases [13], making state transitions freely reversible.

Clearly there are multiple inputs to a chloroplast processing unit, and any single element (such as STN7 or thioredoxin) has multiple outputs (Figure 1). In general, conditions for LHC II and other phosphorylation events, and their effects, may be connected by Boolean logical operators. For example, plastoquinone (PQ) and thioredoxin (TR) seem to provide opposing regulatory inputs into the LHC II kinase reaction, and the conditions might be written as follows

IF $\{[PQ_{red}]$ AND $[TR_{ox}]\}$ THEN $\{STN7_{active}\}$

IF $\{STN7_{active}\}$ THEN $\{[LHC\ II_{phosphorylated}]$ AND $[psaAB_{transcribed}]$ AND $[psbA_{repressed}]\}$

One way of combining inputs and outputs would be for protein kinases or phosphatases themselves to be regulated by phosphorylation [2]. Bacterial sensors and response regulators are sometimes complex, with multiple input domains, and the two components may be combined in a single polypeptide, which functions as part of a regulatory phosphorelay network. It would not be surprising, given the chloroplast's bacterial origins, if histidine and aspartate phosphorylations have been retained as part of an integrated redox signal-processing system that coordinates post-translational modifications with control of gene expression at multiple stages [14].

The end result of these various levels of control seems to be to maintain plastoquinone in a state of redox poise, with a balanced proportion of reduced and oxidised forms. This may have the effect of minimizing potentially damaging, single electron transfers to oxygen [15]. A poised plastoquinone pool is also a precondition for efficient operation

of photosynthetic electron transport. Electrons are fed back from ferredoxin to plastoquinone at varying frequencies, in a photosystem I-mediated cyclic electron transport pathway.

Another crucial cycle is the proton-motive Q-cycle [16] that involves plastoquinone, plastosemiquinone, and plastoquinol, all occupying two distinct binding sites in the cytochrome b_6f complex [17,18]. The semiquinone, with its single, unpaired electron, may be a fleeting intermediate. Under ideal conditions, this free radical may even be avoided completely, by virtue of concerted two-electron reduction of plastoquinone at the Q_i site, and oxidation of plastoquinol at the Q_o site [19]. Nevertheless, this central component mechanism of energy conversion has some highly dangerous chemistry to contain.

Perhaps the core function of the chloroplast's redox central processing unit is to maintain these 'ideal' conditions — allowing a safe proton-motive electron transport through the Q-cycle despite environmental and metabolic shifts in energy input and output. The multiple levels of control, from transcription to post-translational modification, all seem to serve to maintain quinone redox poise. This is just as likely to be an essential and far-reaching feature of mitochondria [20], where the Q-cycle operates in oxidative phosphorylation, using ubiquinone in place of plastoquinone.

The incorporation of a quasi-autonomous genetic system into chloroplast and mitochondrial processing units allows us to view these organelles as 'intelligent' energy-converting devices that detect and respond to environmental changes. For this self-adjusting behaviour, chloroplasts and mitochondria probably use conserved elements of the processing units of the bacteria from which they originated. Instead of 'retrograde' signalling to the nucleus, we should perhaps consider the mechanisms and effects of a true dialogue between these cytoplasmic organelles and the larger processing units of the eukaryotic cell nucleus and cytosol.

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Drosophila Memory: Dopamine Signals Punishment?

Dopamine-containing neurons are widespread in the fly brain and have been implicated in negatively reinforced memory. Current technology allows the investigator to watch dopaminergic neurons in action in the brain of a learning fly.

Alex C. Keene and Scott Waddell

The reward of suffering is experience — Aeschylus

Understanding the molecular and cellular basis of memory is a goal of modern neuroscience. The question can be addressed on many different levels including: What genes are involved? What is the relevant neural circuitry? And, how does that circuitry change when an animal learns? *Drosophila* is a fantastic model system for a rigorous, multi-level analysis of memory. In a recent issue of *Current Biology*, Reimensperger *et al*. [1] report the use of a genetically encoded calcium sensor to image activity of a