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Minireview

Plastoquinone redox control of chloroplast thylakoid protein phosphorylation and distribution of excitation energy between photosystems: discovery, background, implications

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Abstract

Chloroplast thylakoid protein phosphorylation was discovered, and the most conspicuous phosphoproteins identified, by John Bennett at Warwick University. His initial findings were published in 1977. The phosphoproteins included apoproteins of chloroplast light harvesting complex II. Thylakoid protein phosphorylation was shown to influence distribution of excitation energy between Photosystems I and II in 1979, during a visit by Bennett to the laboratory of Charles J. Arntzen at the University of Illinois at Urbana-Champaign. That work was published by Bennett, Katherine E. Steinback and Arntzen in 1980. Control of both protein phosphorylation and excitation energy distribution by the redox state of the plastoquinone pool was first established in 1980 during the author's visit to Arntzen's laboratory. The experiments were prompted by the realization that coupling between redox state of an inter-photosystem electron carrier and excitation energy distribution provides a concrete mechanism for adaptations known as state transitions. This work was published by Allen, Bennett, Steinback, and Arntzen in 1981. This discovery and its background are discussed, together with some implications for photosynthesis and for research generally. This minireview is a personal account of the Urbana-Warwick and related collaborations in 1979–83: it includes impressions, conjectures, and acknowledgements for which the author is solely responsible.

Discovery: University of Illinois, Urbana, Illinois

At about 3 o'clock on a bright afternoon early in February 1980, I sat in the Biology library of the University of Illinois at Urbana-Champaign and had an idea. I had gone to the library intending to learn how, in practical terms, to measure changes in Emerson enhancement, and was reading a review article by Jack Myers called 'Enhancement studies in photosynthesis' (Myers 1971). I think I found the paper simply by looking through the contents pages of *Annual Reviews of Plant Physiology*. It was a fine review, written with great clarity and a few gems of dry, throwaway wit. I felt slightly guilty that it was a good read but that I still did not know which experiment to do next, or how to do it. It was rather as if I had taken the afternoon off without intending to do so. Myers explained distribution of absorbed excitation energy between Photosystem I and Photosystem II. He also reviewed evidence that this distribution could change under photosystem-selective illumination (Cecilia Bonaventura and Myers 1969; Norio Murata 1969a). Myers (1971) said nothing about chloroplast protein phosphorylation, for the good reason that there was then nothing to say. The paper also said nothing about redox control. Therefore, I was not doing my job. It did not feel good. I had not been looking to broaden my education that day.

To add to other problems, I was preoccupied with the annoying fact that my experiments over the previous weeks had repeatedly failed to confirm the

Figure 1. Contemporary snapshots. (A) The author on December 22, 1979, in Mendocino, California, en route from Warwick to Urbana. (B) John Bennett in 1977. (C) John Mullet with Charles J. Arntzen. (D) Jan Watson. (E) Kit Steinback with friend. (F) Kit Steinback with the author, March 1980. (G) Charles J. Arntzen (then at Michigan State University), Alison Telfer (Imperial College London), Richard Williams (Warwick University), at a meeting in Leiden, The Netherlands, April 1982. Photographs provided by the author (A, G), John Bennett (B), Charles J. Artntzen (C–F).

obvious and reasonable guess that chloroplast protein phosphorylation (Bennett 1977) was switched on by reduction of ferredoxin or thioredoxin. The more I added 2,6-dichlorophenol indophenol (DCPIP) and ascorbate as an electron donor to Photosystem I, the more the light-induced kinase activity refused to come back after inhibition by 3-(3,4-dichlorophenyl)-1,1,-dimethylurea (DCMU; Diuron). At face value, this repeatable result was inconsistent with the assumption that the site of activation lay on the acceptor side of Photosystem I. I had done a number of controls: the experiment kept giving the wrong answer. At least the recently established correlation between activation of protein phosphorylation and changes in low-temperature fluorescence emission (Bennett et al. 1980) continued to hold. So I decided that I could perhaps rescue something from my time as a United States Department of Agriculture (USDA) visiting researcher, and a postdoc on loan from Warwick University in England (Figure 1A), by independently confirming the changes in excitation energy distribution previously seen as differences between fluorescence spectra. I knew that these changes should also cause changes in Emerson enhancement, but I also knew that I did not entirely understand how to predict what the enhancement changes would be. I had prepared the ground and was satisfied that I could measure enhancement. It had been fun repeating classic experiments. My Urbana host laboratory was handsomely equipped. By following absorbance at 340 nm using an Aminco-Chance DW2 difference absorption spectrophotometer with side illumination and interference filters, I had confirmed that the red-drop and enhancement of nicotinamide adenine dinucleotide phosphate $(NADP)^+$ reduction is present when water is the electron donor (Govindjee et al. 1964), but absent when $DCPIPH_2$ is the electron donor. I had never used fiber optics before. Luxury – and the right answer. And on the great Robert Emerson's home ground, too (see Emerson et al. 1957; Emerson and Rabinowitch 1960; Govindjee and Rabinowitch 1960). However, there was not much of a market in 1980 for more evidence for the Z-scheme, which was rock-solid, and did not need further support, least of all mine. I wanted to know how enhancement could change. Hence the trip to the library.

I continued to worry whether redox-activation of chloroplast protein phosphorylation could come from somewhere between the photosystems, rather than from the acceptor side of Photosystem I. I have to say I did not like that conclusion. I had been properly educated in the BobWhatley-and-Dan Arnon tradition that ferredoxin, the Photosystem I electron acceptor, is the key to everything interesting in photosynthesis (see Whatley 1995). In addition, there had been recent demonstrations from Bob Buchanan and coworkers (see Buchanan 1991; also see Buchanan et al. this issue) that ferredoxin controls many reactions via thioredoxin. It was obvious: ferredoxin had to be controlling protein phosphorylation, too. Whoever heard of anything being regulated by the redox state of a quinone? Another reason to doubt the obvious inference of my own protein phosphorylation measurements was the evidence, from John Bennett (Figure 1B), that ferredoxin plus NADPH would activate the thylakoid protein kinase in darkness (Bennett 1979). Furthermore, I had already shown, back in Warwick, that protein phosphorylation in intact chloroplasts was stimulated when CO_2 fixation was blocked, as it should be if reduced ferredoxin is the activating factor (Allen and Bennett 1981). It should have been easy to see control at the acceptor side of Photosystem I. Perhaps I was doing something wrong.

With these things on my mind, I learned from Myers's review, for the first time, what state transitions were all about. I had never understood them before, though could recite what other people said were key words: these included 'stacking,' 'spillover,' and 'cations.' 'Enhancement studies in photosynthesis' (Myers 1971) took quite a different approach, and was a revelation. According to Myers (1971), state transitions were simply about α, the proportion of total absorbed light energy that reaches the reaction center of Photosystem II. Thus Photosystem I gets the remainder, $(1\text{-}\alpha)$ of all the absorbed energy that reaches the two reaction centers. As a necessary condition for maximal efficiency (and excluding cyclic electron flow for the moment, just for the sake of argument) it follows from the Z-scheme that $\alpha = 0.5$. But α must have different values for different wavelengths of light – this follows from the two distinct pigment systems. A second, short-wave beam of light enhances quantum yield from a first beam, of wavelength beyond the red drop, because α for the first beam is less than 0.5. State transitions are what happens when you change wavelengths so that you 'fool' the system and make α either less than (light 1), or greater than (light 2), 0.5. The system refuses to be 'fooled,' however, and adjusts, restoring α to 0.5, or as near as it can get it, over a time-course of a few minutes. How does it do that?

I think everyone knows the feeling. It might be when you realize finally where you actually are on a map that you were not even sure was the right map. However, this is nothing as compared to finding a solution and simultaneously, and for the first time, understanding the problem it solves. And nobody set you up for it: the problem is about the world, which is described, but not made, by people. And nobody told you either the problem or the solution. You thought of both yourself. Had I been in a bath and not in a library, I could quite easily have got out and forgotten to dress. I did not say 'Eureka!,' making do, quite unselfconsciously, with 'Bloody hell. . . .' In a state of agitation I mentally connected a number of things that had never previously been fitted together – not by me, nor anyone, as far as I could tell. Then there were predictions about other things nobody had tried, but which were completely obvious, and not difficult to do, once you had the key. The key was this.

Assume that plastoquinone becomes reduced when α is greater than 0.5; that is, when Photosystem II gives electrons to plastoquinone faster than Photosystem I takes them away. This is a reasonable assumption about the effect of light 2 since you get net reduction – of anything – when electron input exceeds output. Then accept the inference from my preliminary experiments, and assume that reduced plastoquinone, not ferredoxin, activates the chloroplast thylakoid protein kinase. Following reduction of plastoquinone under light 2, phosphorylation of light harvesting complex (LHC) II will then redirect excitation energy from Photosystem II to Photosystem I (the redirection itself was a given – Bennett et al. 1980). Thus the value of α decreases, tending to return back to 0.5. The state in which α has thus been decreased to 0.5 is state 2. Therefore, light 2 induces state 2. Conversely, if the pendulum swings too far, or if you add light 1, then α becomes less than 0.5. Then plastoquinone becomes oxidized, any phospho-LHC II becomes dephosphorylated and excitation energy is redirected to Photosystem II. That is just another way of saying that α increases back up towards 0.5. The state in which the value of α has been increased to 0.5 is state 1. Therefore, light 1 induces state 1. *Quod erat demonstrandum.*

So these clever and purposeful-looking light states were just chemistry: phosphate transfer to an amino-acid side chain (giving state 2) and phosphate ester hydrolysis (giving state 1). All one had to assume was that the phosphate transfer itself is promoted when something between the photosystems becomes reduced. It was so neat, it just had to be that way. If there was really no prior evidence for quinone-level redox control, then tough luck, and all the better – the idea just had to be true. Plastoquinone redox control instantly stopped being annoying and inexplicable. It was a mechanism, a prediction, and unprecedented. And quite beautiful. At least to me.

There was one person whom I knew might understand, listen, and tell me if I was crazy or wrong. I quite literally ran back to the lab on the second floor of Morrill Hall. I think I remember the route was through an elevated, third-floor hallway that spanned a pathway separating Microbiology and Botany (now Plant Biology). This would have been quite fitting, I now realize. You could imagine throwing in chemistry, biochemistry, biophysics, and plant physiology and it would have made an even better bridge.

Charlie Arntzen's response was something like 'Hmmm. . . interesting. . .,' which was downbeat and chastening. Perhaps I had been looking slightly unhinged, and Charlie (Figure 1C) was dapper and liked things to be under control. Perhaps he was thinking about something else. At least he could not say immediately why my idea was wrong. That was something. Charlie had even said, a few weeks before, when I outlined the results that seemed to rule out ferredoxin, 'Gee, it would be interesting if it was plastoquinone. . . .' I did not then see why, but did now. I asked him why he had made the remark, and he did not seem to remember. Anyway, Charlie came back with several suggestions over the next few days. One was to try duroquinol, an analogue of plastoquinol, as donor in place of reduced dichlorophenolindophenol ($DCPIPH_2$). It worked: it activated phosphorylation in the dark and restored phosphorylation after inhibition by DCMU in the light. Another great idea of Charlie's was to correlate the ^{32}P-labelling with the area above the room temperature fluorescence induction curve. I would never have thought of that, nor known how to do it. So Charlie made me an assistant for a few days to his entertaining, attractive, and supercompetent technician, Jan Watson (Figure 1D), who knew exactly what to do. That worked too. The area above the curve is a measure of the proportion of the plastoquinone pool that is in its oxidized state. The measured area correlated inversely with the quantity of ^{32}P remaining with the thylakoid membranes after washing and centrifugation. Nobody worried about making the fluorometer cuvette radioactive – this was a lab of positive thinking, where you did not look for

reasons not to do an experiment. It is surprising how rare that is.

Charlie's enthusiasm grew. Each successive experiment seemed to suggest itself. It was a good time. Charlie's postdoc in his collaboration with John Bennett was Kit Steinback (Figures 1E, F). I think Kit was in mild despair at having to keep me in order, but she was helpful with techniques and lab protocols. Besides ordering (γ-^{32}P)ATP and being encouraging about the enhancement results, Kit seemed impressed at my total recall of the reagents and treatments of each of my dozen or so samples. Since 'dark' meant precisely that, I could not read the labels on the tubes for part of the time, and committing them to memory was not difficult given the logical construction of each experiment with its in-built controls. Kit also showed me where things were and how to run the formidable SLM (Spencer-Laker-Mitchel) fluorescence spectrometer. This was an early version of the instrument, and looked as if it had been built in someone's garage. It had a light-path through boiling liquid nitrogen that introduced me to the advantages of smoothing spectra by computer. I never liked the idea, thinking real data have warts and all – just not that many. Maarib Bazzaz from Govindjee's lab was kind, and patiently explained principles of fluorescence spectroscopy, and which emission band came from which photosystem.

One Saturday in March, Colin Wraight kindly helped me to use his redox train to do the experiment that I thought would confirm the site of redox control once and for all – a redox titration. Unfortunately, we found no labeling under any redox conditions. I think there was a problem with moving the sample across the room to Eppendorf tubes with trichloroacetic acid (TCA) waiting to stop the reaction. It was an experiment that should have worked. It did, later, but elsewhere; so Colin got nothing out of it, unfortunately, while I learned a lot.

Urbana was a great place to be at that time. I do not think I was as grateful a guest as I should have been. Let me say 'thank you' now, to those who remember. From Charlie's lab, Ken Leto and his wife Robin were unflaggingly generous with their time, met me when I arrived, and took us foreigners to Chicago and St. Louis. Phil Howarth arrived as an Arntzen postdoc a few weeks after me, and, with other 'aliens,' we shared incredulous stories about America and the Mid-West. John Mullet (Figure 1C) was busy isolating Photosystem I at night, but we had cordial conversations on the change of shift. Sylvia Darr was Charlie's new graduate student. From Urbana, I also remember people passing through, some to give seminars, including Gernot Renger and Achim Trebst. Trebst was a sage who knew my old mentor Bob Whatley from the great days of the Dan Arnon lab in Berkeley (Whatley 1995). Trebst asked 'How many proteins are there in a chloroplast thylakoid membrane?' The question seemed to me an odd one and unimportant at the time, but not now. Also Klaus Pfister, who, in a brief visit, showed that D1 protein (psbA) was the herbicide-binding protein, by cross-linking D1 with azido-atrazine (Pfister et al. 1981). On leaving Urbana, I handed over my share of a rented apartment to Bill Rutherford, then working in Tony Crofts's lab. The apartment was shared with a Glaswegian, David Patterson, and a microbiologist, Tony Pope. The latter, by bizarre coincidence, remembered, from the University of Cardiff Microbiology Department, a student called Carol Smith, who had arrived at Warwick at the same time as I. Carol tells me that John Bennett had given her my Urbana address and said he thought I might be homesick and like to hear from her. How did he know that?

Toward the end of my stay in Urbana, Kit (Figure 1F) whisked me off one afternoon to Agronomy, to meet an enthusiastic Canadian working with Bill Ogren on a model system to screen for mutants with restricted photorespiration. The postdoc was Chris Somerville, and his model was *Arabidopsis thaliana* (Somerville and Ogren 1982). I also remember giving an Urbana photosynthesis seminar on my old PhD and first postdoc work on superoxide and oxygen reduction, and receiving a complimentary response from Govindjee. Govindjee had Tom Wydrzynski in his lab. The lab itself was right next door to Charlie's, and full of awesome Cary spectrophotometers and various home-made fluorometers. I guessed, incorrectly, that the lab had been the setting for the showdown-'collaboration' between Robert Emerson and Otto Warburg in the late 1940s, where it was proved for all time (but only by Warburg) that the quantum requirement of photosynthesis was four, and not eight or more, as in the real world. The true location of that decisive experiment was, however, in another building, the Natural History Building (see Govindjee 1999). On my last day, Govindjee wrote, with a flourish, on a piece of official American Photobiology Society notepaper (he was then President of that Society), 'To John: I enjoyed your stay in Urbana. Govindjee.' I still have that piece of paper. Thanks, Govindjee. I enjoyed it, too.

I had a return journey to England arranged, via Berkeley, California, and left Urbana at the end of March, leaving Jan Watson to run a gel with my samples. We needed to confirm by autoradiography that my crude membrane labeling was phospho-LHC II.

Background: Warwick University, Department of Biological Sciences

Warwick University, too, was a great place to be. I arrived back with data, tales to tell, and some thioredoxin that Bob Buchanan had given me in Berkeley, saying 'Just add some and see what happens.' He had also told me to ask the Pan Am flight attendant to put the stuff in the cabin ice-box. To my amazement, she did. I added some thioredoxin to samples in my next experiment. It made no difference.

The collaboration in which I participated was between Charles J. Arntzen (University of Illinois, Urbana, Figures 1C,G) and John Bennett (Warwick University, Figure 1B). I was one of John's two postdocs. The other was Andrew C. Cuming, who worked on LHC II synthesis (Cuming and Bennett 1981). John Bennett was an Australian biochemist who, a few years earlier, had graduated from postdoc to lecturer in Warwick. After the first paper describing chloroplast phosphoproteins (Bennett 1977), he had done some excellent biochemical characterizations (Bennett 1979a, b, 1980). The original discovery was a by-product of a project on chloroplast RNA synthesis, the latter topic fitting nicely within the work of the Warwick 'chloroplast lab.' However, the ^{32}P-nucleoside triphosphate had labeled the membrane fraction better than the RNA fraction, and John had had the sense, and the encouragement from the group leader, John Ellis, to follow the part that worked and look at autoradiographs of protein gels. Around 20 years later an excellent visiting postdoc, Thomas Pfannschmidt, found plastoquinone redox control of chloroplast transcription in my Lund laboratory (Pfannschmidt et al. 1999). Photosystem I and II reaction center gene transcriptions are complementary, just like excitation energy distribution, so you would not expect an effect on total RNA.

One collaboration that John Bennett set up was with Patrick Williams at London University's Chelsea College, as it was then. With Patrick I started my regular role as a sort of ^{32}P-runner, taking label, gloves, Benchcote, yellow warning tape and Gilson pipettes to UK labs equipped to do spectroscopy and photosynthesis measurements, but not set up to run gels. I returned to Warwick with labeled TCA-precipitated samples in a steel box in dry ice in the back of my Citröen 2CV. I designed and did the experiments with my hosts, drove home, ran the gels, made autoradiographs, and cut out and counted the bands. The state 2 transition correlated nicely with LHC II phosphorylation in *Chlorella*, too (Saito et al. 1983). The phenomenon just had to be something quite general, and thus important. Though the redox titration failed in Chelsea, too.

In early June 1980, John Bennett's parting shot before going on sabbatical to University of California at Los Angeles (UCLA), to Philip Thornber's lab, was 'Get in touch with Peter Horton and see if there's anything you can do.' Peter had been studying a mysterious ATP-induced but uncoupler-insensitive chlorophyll fluorescence quenching (Horton and Black 1981), and it made complete sense to see if this had anything to do with John Bennett's LHC II phosphorylation. I wrote to this unknown (to me) new lecturer in Biochemistry in Sheffield University, outlining the Urbana experiments, the relevance of plastoquinone redox control of LHC II phosphorylation, and how this explained state transitions. By return I received an enthusiastic response and we spoke on the telephone. We agreed the redox titration was essential, and we should be able to titrate both ^{32}P-labeling and ATP-induced fluorescence quenching to see if either or both matched the mid-point of plastoquinone. When I got back from Sheffield, I had the labeled samples and Peter had the raw fluorescence data. I remember reading out the values for my bands from the Warwick scintillation counter over the telephone. The match with the normalized fluorescence values was perfect (Horton et al. 1981).

Not long after that first trip to Sheffield, Horton and Mike Black published an experiment with just a redox titration of ATP-induced fluorescence quenching (Horton and Black 1980), and with the essence of my Urbana idea in the title. To Peter's credit, their paper acknowledged John Bennett for discussion, and me for communicating results that gave rise to the same conclusion. Peter and I later checked out more systematically Charlie's suggestion to try duroquinol. I am pleased with that oxygen electrode trace (Allen and Horton 1981). It is really my one bit of true photosynthesis research wholly done in Warwick.

In the fall of 1980, Charlie Arntzen visited Warwick and he, John Bennett, and I then agreed on a

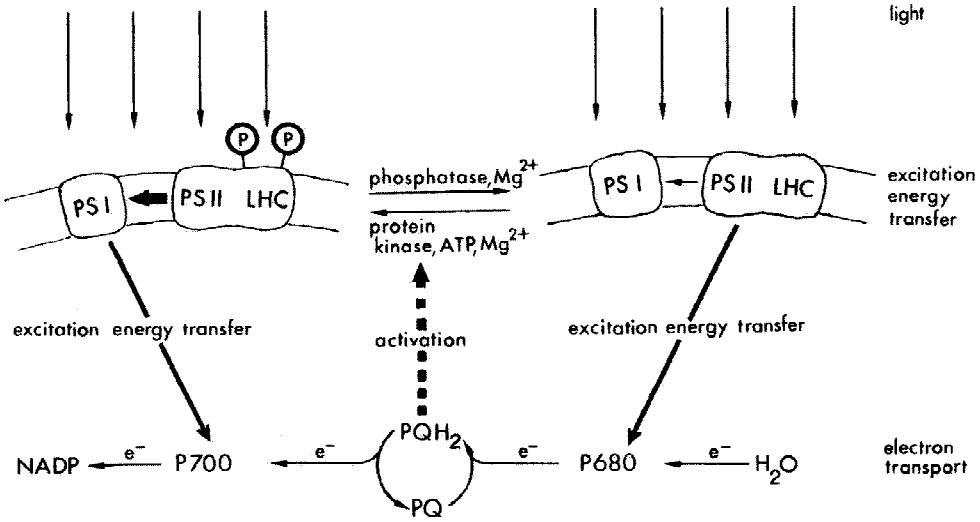

Figure 2. The scheme for regulation of excitation energy distribution between Photosystem I and Photosystem II, as published in 1981 by Allen et al. Today most authors would separate phosphorylated LHC from PS II, and envisage lateral movement of LHC (LHC II) between the two photosystems. This is not a trivial detail, since the diagram shown above suggests a mechanism – 'Spillover' – that is almost certainly incorrect (see, for example, Allen and Forberg 2001). Simply transposing 'LHC' and 'PS II' would improve the picture, but would also make it difficult to depict energy transfer from Photosystem II to Photosystem I, the essence of 'spillover.' The failure to separate 'LHC' from an LHC-PS II 'supercomplex' and the gratuitous addition of Mg^{2+} as a cofactor for both kinase and phosphatase (correct but irrelevant to the hypothesis) may have allowed inclusion of what the conventions of 1981 held to be essential key words. In contrast, no attempt is made to implicate membrane stacking. The general picture is still in agreement with available evidence. Control of distribution of absorbed light energy by means of plastoquinone-redox control of LHC phosphorylation has been an influential hypothesis that, in general terms, is now based upon results obtained in many different laboratories. A pdf file of a complete publisher's reprint of the paper from which the figure is taken (Allen et al. 1981) can be downloaded, for individual use, from http://plantcell.lu.se/john/pdf/13.pdf. Original figure legend (Allen et al. 1981): *A model for control of distribution of excitation energy in photosynthesis, in which reversible phosphorylation of LHC couples the redox state of plastoquinone (PQ) to the distribution of excitation energy between the photosystems. The mechanism we propose ensures maximal quantum efficiency of non-cyclic electron transport from water to NADP, mediated by P680 and P700, the reaction centres of Photosystem II (PS II) and Photosystem I (PS I), respectively. Reduction of plastoquinone by Photosystem II leads to kinase activation and LHC phosphorylation, and hence to increased excitation of Photosystem I. Conversely, oxidation of plastohydroquinone (PQH_2) by Photosystem I inactivates the kinase; the phosphatase then dephosphorylates LHC and hence excitation of Photosystem II is increased relative to that of Photosystem I. Since the redox state of plastoquinone will be determined in part by the distribution of excitation energy between the two photosystems, reversible LHC phosphorylation completes a feedback loop by means of which any imbalance in this distribution will tend to be self-correcting.*

manuscript, including all the essential details of my Urbana results. I suggested *Nature*. Charlie and John had a long discussion about the model. John wanted to have phospho-LHC II leaving Photosystem II and joining Photosystem I in state 2 – a change in absorption cross-section. Charlie held out for phospho-LHC II and Photosystem II together passing excitation energy to Photosystem I, reminiscent of 'spillover,' and more in line with the influential ideas of Warren Butler (Butler 1976). Charlie won (Figure 2). I did not much mind, thinking plastoquinone redox control was the big issue, and that the distinction they were arguing over was arcane. I was quite wrong, and would now side with John (Allen and Forsberg 2001).

Nature records that our manuscript was received on 22 December 1980. Three sets of reviewers' comments came in the New Year. Two were positive. The third was emphatically hostile. The third referee's argument was that the work was derived, without acknowledgement, entirely from that of others, notably Peter Horton, and that it was, besides, wrong, since it was inconsistent with key features of state transitions. I phoned Peter to ask if he would be willing to state that the work was not derived from his, and he said he would. I then drafted a long, scholarly reply. Among detailed points, I outlined an element of inconsistency in something being laundered from the established, reputable work of others, while at the same time being a piece of junk. John Bennett applied Australian plain speaking and cut most of my long-winded draft. I had written 'The allegation... [of stealing the idea] ... is wholly without foundation'. I remember John crossing out 'wholly without foundation' and substituting 'complete rubbish.' Gloves off. I thank him. It worked.

The paper was accepted largely intact, and as a full Article, on 18 February 1981, almost exactly one year after the afternoon in the Urbana library. The paper appeared in May (Allen et al. 1981), and even got coverage in *The Times* (1981). The page numbers in the journal are 25–29, but two other citations are common and correspond to the pagination of the reprints (1–5) and the publishers own, incorrect index (21–25) that appeared on the reprints themselves (see legend to Figure 2).

Implications: pieces of the puzzle

History, as Andy Cuming would say, is written by the victors. So if the rest is history, it is still being written. In any case, I try to think of science as exploration and progress, not war. I made more car journeys to do experiments. First with Alison Telfer (Figure 1G), who was then Jim Barber's postdoc at Imperial College, London. This collaboration began after a seminar I gave at King's College London in early 1981. I said you should be able to see state transitions induced by lights 1 and 2 in isolated thylakoids provided you gave them a suitable electron acceptor and ATP – and that the state 1 transition should then be inhibited by fluoride, an inhibitor of the phosphoprotein phosphatase. Correct on both counts. In addition, the kinetics of LHC II phosphorylation and dephosphorylation matched those of the state 2 and state 1 transitions, respectively (Telfer et al. 1983). Then, in late summer 1982 and early 1983, Richard Cogdell was a generous host in Glasgow, where we found phosphoproteins in purple bacterial membranes labeled with [γ-^{32}P]ATP and cells labeled with [^{32}P]Pi. Almost everything in chromatophores seemed to be under redox control, including the mobility of the Coomassie-stained bands. Redox reagents had never influenced the electrophoretic mobility of chloroplast thylakoid polypeptides. I regret not publishing the work with Richard Cogdell, despite its loose ends. Applying the chloroplast model to photosynthetic prokaryotes later became a central objective of my independent research.

To return to state transitions in oxygenic photosynthesis, I found in preparing the *Nature* manuscript (Allen et al. 1981) that control of excitation energy distribution by redox state of an inter-photosystem electron carrier had been suggested as a theoretical possibility by L.N.M. Duysens (1972) and by Ried and Reinhardt (1980). In retrospect, John Bennett's chloroplast protein phosphorylation was a final piece of jigsaw that completed, at one level, a picture of what was going on. Norio Murata had made a clever comparison between state transitions in the red alga *Porphyridium cruentum* (Murata 1969a) and alterations in chlorophyll fluorescence of chloroplasts induced by changes in free cation concentration (Murata 1969b). However, if cation efflux from thylakoids is part of the mechanism, then the transition to only one of the two light-states should have a definite quantum requirement, since the other, reverse transition should then be a passive return to equilibrium. This is not the case: both the state 1 and state 2 transitions have a definite quantum requirement (Ried and Reinhardt 1980). So one obstacle in solving the problem of state transitions as posed by Myers, Murata, Duysens, and others was an important-looking piece of jigsaw, namely cation effects, that the literature said was relevant but which turned out to be from a different puzzle, or no a puzzle at all. Beware of being told you have to make things fit.

Acknowledgments

I should like to end this sketch of a decisive few years with some explicit, personal acknowledgements. To Charlie Arntzen I owe a lot. Apart from the work and the insight on thylakoid structure and function (e.g. Staehelin and Arntzen 1979), Charlie totally disarmed an 'English chauvinist' (his phrase) with his infectious optimism and pure, American 'can-do' philosophy – quite a paradox, I learned later, for a Minnesotan, loyal to his Norwegian roots. I am also grateful to John Bennett, a very bright, ambitious, and motivated man, intense and difficult to get on with. John hired me as a postdoc, which changed everything. Though I thought I had found a friend when I first met him, I suspect we were, in some aspects of personality, too alike for comradeship, while in others, too different. R. John Ellis, the head of the Warwick chloroplast group, supported people, and had plenty of ideas of his own (e.g. Barraclough and Ellis 1980). 'RJE' seemed big enough not to feel threatened by good work he did not control, and took vicarious pleasure in the achievements of others. This trait is rarer than one would think. Equally, photosynthesis never became a priority in Warwick, although postdoc Richard Williams (Figure 1G) continued with LHC II. In 1983 John Bennett left for Brookhaven National Laboratory, and I left for a lectureship in Leeds.

One gratuitous 'without whom' out of many: Bob Whatley, student of Robin Hill, codiscoverer of photophosphorylation, and a pervasive, earlier influence on points of science and style. After Warwick, my participation in this field was initially aided by my excellent Leeds PhD students Conrad Mullineaux, Michael Harrison, and Nikos Tsinoremas, and postdoc Nigel Holmes. John Bennett reviewed the field itself and subsequent developments in 1991 (Bennett 1991), and I did so in 1992 (Allen 1992). While I believe that the discoveries related here steered aspects of regulation of photosynthesis onto the right track, it is clear that many fundamental problems remain. It would be inappropriate to list these here. Some current issues and new opportunities are discussed elsewhere (e.g. Allen and Forsberg 2001).

When Carol Smith and I married in June 1982, most of the Warwick chloroplast lab came to the reception, including John Bennett, and Philip Thornber with Elaine Tobin, who were visiting John on a return sabbatical. For me, photosynthesis continues to be a brilliant field to work in, and a unique and privileged viewpoint on life and its evolution. Carol and I have moved more than we would wish, and traveled less, but we have many good friends in photosynthesis research. Scientific careers today make absurd demands on families. The deeper the commitment to each of these spheres, the greater its potential cost to the other – and our commitments are deep. I believe this dilemma, recent but now widespread, is the true glass ceiling, wastefully setting careers against human values and aspirations. Carol's extraordinary personal dedication and generosity have complemented and supported my science over two decades. This is my final acknowledgement, and greatest debt.

This personal minireview was written at the invitation of Govindjee, who was also its editor.

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