Journal of Experimental Botany, Vol. 64, No. 3, pp. 717–730, 2013
doi:10.1093/jxb/ers336 Advance Access publication 16 November, 2012

Journal of Experimental Botany
www.jxb.oxfordjournals.org

REVIEW PAPER

Rubisco activity and regulation as targets for crop improvement

Martin A. J. Parry[1,*], P. John Andralojc[1], Joanna C. Scales[1], Michael E. Salvucci[2], A. Elizabete Carmo-Silva[2], Hernan Alonso[3] and Spencer M. Whitney[3]

[1] Plant Biology and Crop Science, Rothamsted Research, Harpenden, Herts, AL5 2JQ, UK
[2] USDA-ARS, Arid-Land Agricultural Research Center, Maricopa, Arizona, 85138, USA
[3] Plant Science Division, Research School of Biology, Australian National University, Canberra ACT 2600, Australia

* To whom correspondence should be addressed. E-mail: martin.parry@rothamsted.ac.uk

Received 4 September 2012; Revised 22 October 2012; Accepted 24 October 2012

Abstract

Rubisco (ribulose-1,5-bisphosphate (RuBP) carboxylase/oxygenase) enables net carbon fixation through the carboxylation of RuBP. However, some characteristics of Rubisco make it surprisingly inefficient and compromise photosynthetic productivity. For example, Rubisco catalyses a wasteful reaction with oxygen that leads to the release of previously fixed CO_2 and NH_3 and the consumption of energy during photorespiration. Furthermore, Rubisco is slow and large amounts are needed to support adequate photosynthetic rates. Consequently, Rubisco has been studied intensively as a prime target for manipulations to 'supercharge' photosynthesis and improve both productivity and resource use efficiency. The catalytic properties of Rubiscos from diverse sources vary considerably, suggesting that changes in turnover rate, affinity, or specificity for CO_2 can be introduced to improve Rubisco performance in specific crops and environments. While attempts to manipulate plant Rubisco by nuclear transformation have had limited success, modifying its catalysis by targeted changes to its catalytic large subunit via chloroplast transformation have been much more successful. However, this technique is still in need of development for most major food crops including maize, wheat, and rice. Other bioengineering approaches for improving Rubisco performance include improving the activity of its ancillary protein, Rubisco activase, in addition to modulating the synthesis and degradation of Rubisco's inhibitory sugar phosphate ligands. As the rate-limiting step in carbon assimilation, even modest improvements in the overall performance of Rubisco pose a viable pathway for obtaining significant gains in plant yield, particularly under stressful environmental conditions.

Key words: Chloroplast, photosynthesis, Rubisco, Rubisco activase, regulation, specificity, transformation.

Introduction

The primary determinant of crop biomass is the cumulative rate of photosynthesis over the growing season. A comprehensive analysis of free-air CO_2 enrichment experiments unambiguously demonstrated that increasing photosynthesis in C_3 plants increases yield (Ainsworth and Long, 2005). In C_3 photosynthesis, Rubisco (ribulose-1,5-bisphosphate (RuBP) carboxylase/oxygenase) initiates carbon assimilation through the carboxylation of RuBP. Despite the presence of a CO_2-concentrating mechanism in C_4, crassulacean acid metabolism, algal, and cyanobacterial species, the carboxylase activity of Rubisco plays a central role in CO_2 assimilation in all photosynthetic organisms. Even so, some characteristics of Rubisco, including constraints related to its complex reaction mechanism, make it surprisingly inefficient and compromise photosynthetic productivity. For example, Rubisco catalyses a wasteful side reaction with oxygen that leads to

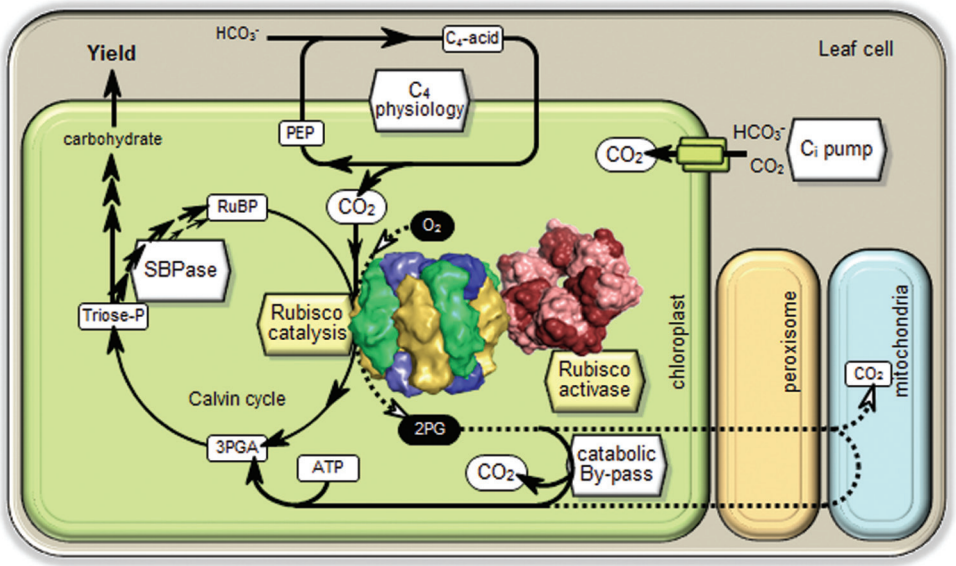

Fig. 1. Biotechnological strategies for improving photosynthetic carbon assimilation in crops. In addition to directly improving Rubisco catalysis, other strategies aim to enhance CO_2 levels around Rubisco to minimize photorespiratory expenses associated with recycling of Rubisco's oxygenase product, 2-phosphoglycolate (2PG, see dashed line). These strategies include: introducing assimilation characteristics from C_4-physiology into C_3 cells (Covshoff and Hibberd, 2012); cyanobacteria inorganic carbon (C_i) pumps into chloroplast membranes (Price *et al.*, 2011); and novel catabolic by-pass pathways (Kebeish *et al.*, 2007). Other strategies include enhancing Calvin cycle RuBP regeneration by increasing sedoheptulose-1,7-bisphosphatase (SBPase) activity (Rosenthal *et al.*, 2011) and increasing the thermotolerance of Rubisco activase to sustain Rubisco activity under moderately elevated temperatures (Kureck *et al.*, 2007; Kumar *et al.*, 2009).

the release of previously fixed CO_2, NH_3, and energy during photorespiration (Fig. 1). Furthermore, Rubisco is slow, and large amounts of the enzyme (accounting for up to 50% of leaf soluble protein, 25% of leaf N) are needed to support adequate photosynthetic rates. In addition, Rubisco requires repeated conformational remodelling to stay active, a process that slows the apparent rate of catalysis.

Rubisco evolved 3 billion years ago in a high-CO_2 environment that contained very little molecular oxygen (Whitney *et al.*, 2011a). As the earth's atmosphere slowly changed, Rubisco gradually evolved to its present-day forms, constrained by a complex reaction mechanism that originated under very different conditions. For over 500 million years, the evolution of Rubisco in land plants to its current L_8S_8 structure, comprising eight 52-kDa large and eight 14–15-kDa small subunits (Andersson and Backlund, 2008), has required multiple and concerted structural changes to both subunits. Given that photosynthetic carbon assimilation is frequently limited by Rubisco catalysis, the acquired evolutionary structural changes to both subunits have had to strike a balance between acquiring those conveying desired catalytic traits without compromising the enzyme's biogenesis or regulation by its ancillary activating protein Rubisco activase (Whitney *et al.*, 2011a). As a result, considerable variation exits in the catalytic properties of Rubisco from diverse vascular plant sources (Fig. 2), based on kinetic measurements from only about 100 species. Even such a small sample has revealed sufficient variation, in principle, to confer superior characteristics to photosynthesis in specific crops and environments (Galmés *et al.*, 2005; Zhu *et al.*, 2010b; Parry *et al.*, 2011). These findings suggest that further variation in Rubisco turnover rate, affinity, or specificity for CO_2 is almost certainly present in the natural environment and awaits discovery by analysing Rubisco structure and function in plants adapted to more extreme growth conditions.

Whilst it may be possible to introduce novel Rubiscos by conventional breeding (e.g. wide crosses), it is most likely that this introduction will require biotechnological approaches. Some attempts to manipulate plant Rubisco by nuclear transformation have had limited success. Modifying Rubisco catalysis by targeted changes to its chloroplast encoded L-subunit gene (*rbcL*) via chloroplast transformation (Fig. 2) has been much more successful – albeit a technique still in need of development for the major food crops (i.e. maize, wheat, and rice). While bioengineering efforts to optimize the catalytic potential of Rubisco itself are pivotal to this approach, so too are several indirect targets for improving Rubisco performance *in planta* (von Caemmerer and Evans, 2011). These include improving the thermal tolerance of Rubisco's ancillary protein, Rubisco activase, and modulating the abundance or effects of naturally occurring sugar phosphate analogues that inhibit Rubisco activity. Ongoing fundamental research in model plant species (*Arabidopsis*, tobacco) and crop species (rice, wheat) are examining these and other strategies for improving photosynthetic carbon assimilation (Fig. 1). The inescapable fact is that the shared goal of almost all of these bioengineering strategies is to enhance CO_2 fixation by Rubisco.

To 'climate proof' crops for the future, innovative biotech approaches are required to go beyond the advances achieved

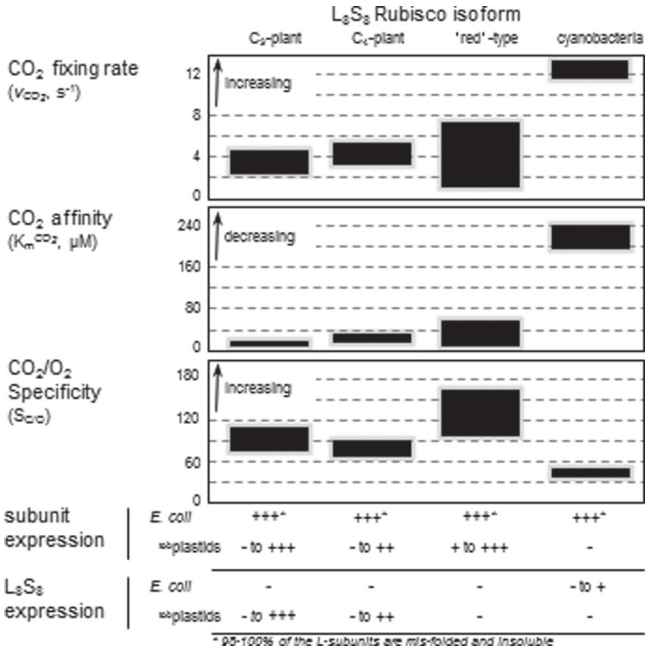

Fig. 2. Variation in L_8S_8 Rubisco catalysis and expression in *Escherichia coli* and in tobacco chloroplasts (^{tob}plastids). During the last 2–3 billion years different L_8S_8 Rubisco isoforms have selected for significant variations in their speed (v_{CO2}), CO_2 affinity (K_m^{CO2}), and specificity for CO_2 over O_2 ($S_{C/O}$) in response to increasing atmospheric O_2, diminishing CO_2, and other environmental and physiological stimuli. Identifying the structure–function detail to account for this variation has been hindered by variations in the extent to which the folding and assembly requirements of recombinant large (L) and small (S) Rubisco subunits can be met by *E. coli* and tobacco chloroplasts. Relative levels of expression are scaled incrementally from nil (–) to maximally expressed (+++). The range of each catalytic parameter for each L_8S_8 isoform summarizes the data from Whitney *et al*. (2011).

either by evolution or by empirical breeding. Rubisco is not optimal for current environmental conditions and agricultural practices, but there is enough variation present in nature to suggest that improvements in the catalytic properties of Rubisco or in its ancillary reactions can be achieved by rational design. Therefore, the goal is to provide a pathway for significantly boosting the CO_2-fixing capability of a crop by introducing tailor-made changes to Rubisco and/or Rubisco activase function that are best suited to the particular crop and its growth environment.

The predicted major benefits include:

- Greater yield under current and future CO_2 levels. Improved Rubisco catalytic efficiencies of 5–25% would increase leaf photosynthetic rates and potentially increase dry matter yield by several-fold when integrated over the growing season in an appropriate crop with adequate nutrient and water supply (Zhu *et al*., 2010b; Parry *et al*., 2011).
- Improved tolerance to higher growing season temperatures and more frequent episodes of extreme heat. Enhancing the thermotolerance of Rubisco activase would increase productivity in current and future climates by reducing inhibition of net CO_2 assimilation by heat stress and allow optimization for a warmer average temperature and/or less precipitation (Ainsworth and Ort 2010; Parry *et al*., 2011).
- Enhanced nitrogen efficiency (more carbon fixed for the same amount of leaf protein; Reynolds *et al*., 2012) and water use efficiency (more CO_2 flux for the same amount of water; Andrews and Whitney, 2003).

Many of the early limitations to Rubisco engineering have been overcome through significant technological advances in our capabilities to modify Rubisco in plant plastids. In this review we examine how these advances have unveiled the evolutionary restrictions that nature, and now bioengineers, face for discovering solutions that improve Rubisco catalysis in crops without hindering its biogenesis. The challenges faced necessitate concerted efforts to fully understand the molecular chaperone requirements of Rubisco biogenesis and improve our comprehension of the natural diversity in sequence–performance relationships among diverse Rubisco isoforms, including how influential the Rubisco small subunit is to catalysis. To meet these challenges requires plastome-transforming capabilities to be developed in key grain crops and the development of higher-throughput technologies involving *in vitro*, *Escherichia coli*, and/or transient plant expression systems. These advances will allow probing of structure–function relationships to identify solutions that benefit Rubisco and Rubisco activase catalysis and their integration into C_3 crops.

Overcoming Rubisco catalysis limitations

Altering leaf Rubisco content

Rubisco represents a major nitrogen investment in crops: it can exceed 25% of leaf nitrogen and comprise as much as 50% of soluble leaf protein (Parry *et al*., 2003). Given that nitrogen is already a major limitation and an expensive nutrient in global agricultural systems, lowering the need for such an abundance of Rubisco is an obvious target for increasing crop nitrogen use efficiency. Modelling studies and data from growth experiments in elevated CO_2 suggest that in some environments there is an overinvestment in Rubisco (Ainsworth and Long, 2005). Evidence from antisense plants with decreased Rubisco content suggests that at low-to-moderate light intensities a reduction in Rubisco in the order of 15–20% would reduce nitrogen demand by as much as 10% without negatively impacting on the capacity for photosynthetic CO_2 fixation (Stitt and Schulze, 1994). This strategy would confer increasing benefit as atmospheric CO_2 concentrations continue to rise.

A different approach may, however, be required in environments characterized by high light intensities, since any reduction in the amount (or activity) of Rubisco under these conditions is likely to decrease photosynthetic rates. A numerical simulation using an evolutionary algorithm that partitioned a fixed amount of protein-associated nitrogen among the enzymes of CO_2 fixation suggested that for optimal photosynthetic rate there was an underinvestment in Rubisco,

sedoheptulose-1,7-bisphosphatase, and fructose-1,6-bisphosphate aldolase and an overinvestment in photorespiratory enzymes (Zhu *et al.*, 2007). In light of that study, rebalancing the investment in leaf proteins and increasing the amount (or activity) of Rubisco would be particularly beneficial, not only in conditions of high irradiance but also of high temperature when intercellular CO_2 concentrations are lower. Any additional investment in Rubisco would need to be offset by decreases in photorespiratory leaf proteins so that there was no overall increase in nitrogen requirement. Clearly, one strategy may not be suitable for all situations. Rather, a modelled optimization of photosynthetic metabolism may be required for specific crops and environments.

Modifying Rubisco performance

Natural variation in L_8S_8 Rubisco catalysis indicates that the enzyme has differentially evolved in response to environmental cues (Tcherkez *et al.*, 2006). It is likely that the complex biogenesis requirements of Rubisco, its high level of expression, and its complex catalytic chemistry have restricted the rate at which beneficial changes have been, and can be, selected (Whitney *et al.*, 2011a). This may explain the apparent slow catalytic adaptation of Rubisco to changing atmospheric CO_2 and O_2 levels. Indeed, many photosynthetic organisms have answered the selection pressure on Rubisco catalysis by preferentially adopting anatomical and biochemical changes that concentrate CO_2 around the enzyme. In C_4 plants, this compromise has led to the selection of coding sequence changes that increase Rubisco CO_2 fixation rates, albeit at the expense of CO_2 affinity (Fig. 2). Using plastome transformation in the tobacco master line ^{cm}trL, the L-subunit Met309Ile substitution has recently been shown to be a trigger for converting Rubisco in *Flaveria* species from C_3- to C_4-like catalysis (Whitney *et al.*, 2011b).

Some Rubisco isoforms in the 'red' L_8S_8 lineage have evolved further and faster than the 'green' L_8S_8 lineage in vascular plants. Compared with C_3 crops, the Rubisco of some red algae has evolved 2-fold improvements in CO_2/O_2 specificity while sustaining adequate rates of carboxylation (v_{CO2}) and CO_2 affinity (Fig. 2). As the folding and assembly requirements of red algae Rubiscos cannot be met by higher plant chloroplasts (Whitney *et al.*, 2001), the challenge is to identify the amino acids responsible for their catalytic properties and transpose these onto C_3 (and C_4) Rubiscos. Unfortunately for Rubisco bioengineers, despite high levels of L- and S-subunit expression, molecular chaperone incompatibilities hinder the production of most L_8S_8 Rubisco isoforms in *E. coli*, restricting use of this system to structure–function studies of certain cyanobacterial forms of Rubisco (Fig. 2). Much like the divergent catalytic properties of cyanobacteria Rubisco relative to other L_8S_8 enzymes, sequence–performance relationships in cyanobacteria Rubisco may also have limited translational relevance to higher plant Rubisco (e.g. Met309Ile mutation, Whitney *et al.*, 2011b).

An expansion in Rubisco engineering capabilities came about with the development of chloroplast-transformation tools to modify the plastome-encoded *rbcL* gene in *Chlamydomonas reinhardtii* and tobacco plastids (Day and Goldschmidt-Clermont, 2011; Maliga and Bock, 2011). Research with *C. reinhardtii* has revealed much about the structure–function of the eukaryotic-type L_8S_8 Rubisco and its regulation by Rubisco activase (Spreitzer *et al.*, 2005). Rubisco engineering in this unicellular green alga is facilitated by the availability of an S-subunit deficient mutant, which enables the study of both L- and S-subunit changes in catalysis (Wostrikoff and Stern, 2009). By exploiting plastome transformation in *C. reinhardtii*, laboratory-directed protein evolution has confirmed that L-subunit amino acid substitutions can improve catalysis (i.e., better v_{CO2}, K_m^{CO2}, and $S_{C/O}$) and these improvements can be transferred to tobacco Rubisco (Zhu *et al.*, 2010a).

Plastome transformation in tobacco has also been used to delete, replace, and modify Rubisco with alternate and modified subunits (Andrews and Whitney, 2003). This pioneering work led to the development of tobacco lines specifically tailored for plastome engineering of Rubisco (Kode *et al.*, 2006; Whitney and Sharwood, 2008). Of these, the high transforming efficiency and non-denaturing PAGE screening simplicity of the ^{cm}trL tobacco master line (Fig. 3A) has emerged as a useful tool for introducing a variety of recombinant Rubiscos (Fig. 3B) and testing the accuracy of leaf photosynthesis assimilation models (see Whitney *et al.*, 1999, 2009, 2011b for examples). Overall, the chloroplast-transformation research in tobacco has shown that changes in L-subunit residues translate into changes in Rubisco catalysis, which directly correlate with plant photosynthesis and growth.

The influence of S-subunits on Rubisco catalysis

Despite the precedence from plastome engineering in tobacco that the L-subunits primarily determine the catalytic prowess of plant L_8S_8 Rubisco (Sharwood *et al.*, 2008; Whitney *et al.*, 2011b), there is strong evidence that Rubisco catalysis can be modulated – possibly stimulated – by the S-subunits. *C. reinhardtii* has been extensively used to show the importance of the S-subunit on catalysis, in particular the important role of the structurally variable βA–βB loop (Spreitzer *et al.*, 2005; Wostrikoff and Stern, 2009; Genkov *et al.*, 2010). Recently, the introduction of a C_4-Rubisco *rbcS* gene from sorghum into the nucleus of rice successfully produced chimeric L_8S_8 Rubisco – comprising both rice and sorghum S-subunits – whose catalysis became more C_4-like (i.e. a higher v_{CO2} and K_m^{CO2}, Ishikawa *et al.*, 2011). In contrast, introducing pea Rubisco S-subunits into *Arabidopsis* impeded Rubisco catalysis (Getzoff *et al.*, 1998). The basis for these conflicting results is unclear.

Recent computational analysis of natural and mutant Rubisco structures also tend to support the assertion that S-subunits can finetune the dynamic structure of the holoenzyme and influence catalysis (van Lun *et al.*, 2011). These properties might explain natural variation in the sequence and expression of different alleles in plant *rbcS* gene families but this has yet to be demonstrated experimentally.

Fully appreciating the extent to which L_8S_8 catalysis can be modulated by S-subunit engineering poses an exciting opportunity because, unlike plastome transformation,

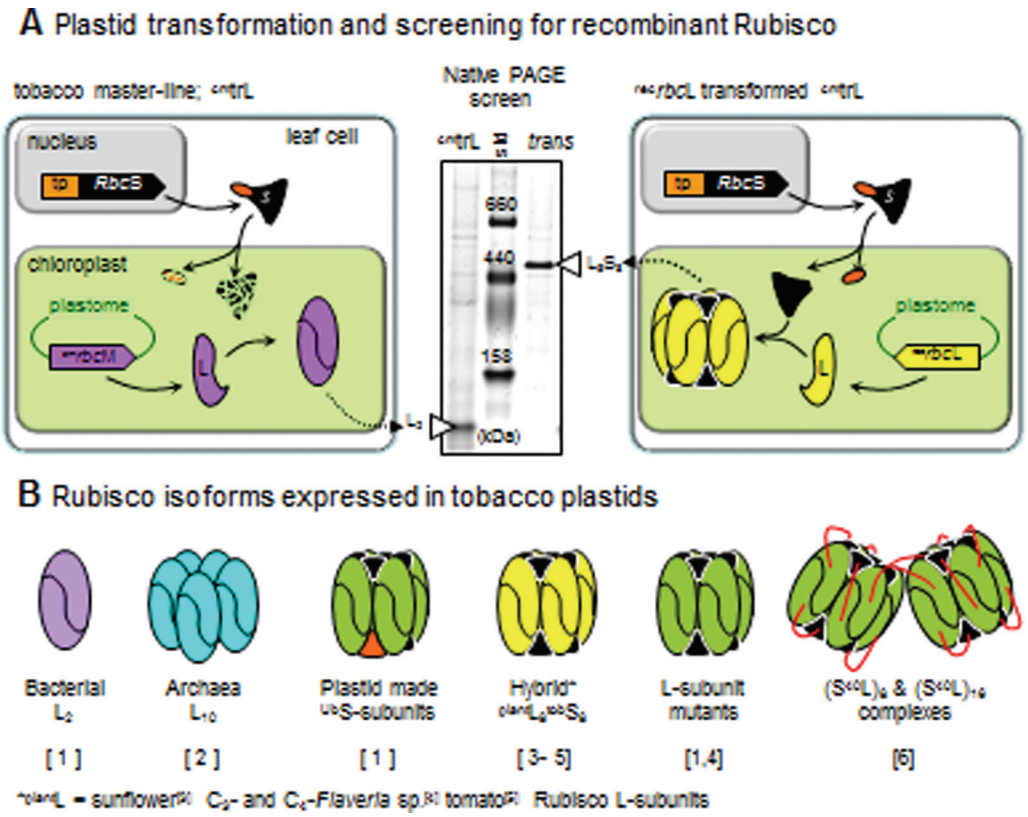

Fig. 3. Plastome engineering of Rubisco in the tobacco master-line ^{cm}trL. (A) Using the homologous recombination route for transforming the chloroplast genome (plastome), the *Rhodospirillum rubrum* L_2 Rubisco encoding ^{cm}*rbcM* in the plastome of the ^{cm}trL tobacco master line (cell on left) can be efficiently replaced with candidate *rbcL* (± *rbcS*) (cell on right). In ^{cm}trL, the cytosolic-made tobacco S-subunits are not needed and therefore degraded (Whitney and Sharwood, 2008). Plastome-transformed (*trans*) ^{cm}trL plants producing alternative larger Rubsico isoforms are easily identified by non-denaturing PAGE of plant soluble protein. (B) Examples of the alternative Rubisco isoforms successfully expressed in tobacco chloroplasts using ^{cm}trL. [1] Whitney and Sharwood (2008); [2] Alonso *et al*. (2009); [3] Sharwood *et al*. (2008); [4] Whitney *et al*. (2011b); [5] Zhang *et al*. (2011); [6] Whitney *et al*. (2009).

nucleus-transforming capabilities are available for most crop species. By contrast, the suitability of testing recombinant *rbcS* genes by plastome transformation is hindered by the inability of S-subunits made in the plastid to effectively compete with the endogenous cytosolic S-subunits for assembly into L_8S_8 holoenzyme (Whitney and Andrews, 2001; Zhang *et al*., 2002; Dhingra *et al*., 2004).

The need to better understand Rubisco biogenesis

The inability of algal and some plant L-subunits to assemble into L_8S_8 complexes in recombinant hosts poses a significant hurdle to the detailed structure–function analysis of these Rubisco isoforms. This was first apparent when chaperone incompatibilities precluded successful replacement of plant Rubisco with a catalytically 'better' enzyme from red algae (Whitney *et al*., 2001). Subsequent transplantation studies have shown that the chaperone requirements of the L-subunits from sunflower, tomato, and various *Flaveria* species Rubiscos can be met by tobacco chloroplasts, albeit to differing extents (Sharwood *et al*., 2008; Whitney *et al*., 2011b; Zhang *et al*., 2011). In contrast, unknown sequence variations within the L-subunit of rice, wheat, and maize Rubiscos preclude their synthesis, folding, and/or assembly with tobacco S-subunits into hybrid L_8S_8 enzymes in tobacco (S. M. Whitney and J. Galmés, unpublished). This is of little surprise when considering that single amino acid changes in *Flaveria* L-subunits significantly influence hybrid L_8S_8 biogenesis in tobacco (Whitney *et al*., 2011b) and that tobacco L-subunit substitutions such as Ser112Phe or Gly322Ser prevent tobacco Rubisco biogenesis (Avni *et al*., 1989; Shikanai *et al*., 1996). Nevertheless, it is encouraging that the inability of some L-subunits to assemble in plastids may be reversed by only one or a few amino acid changes. In support of this is the finding that a number of single and complementary L-subunit mutations can enhance assembly of artificially evolved cyanobacterial Rubisco in *E. coli* by as much as 20-fold (Mueller-Cajar and Whitney, 2008a,b).

Improvements in the assembly of some L_8S_8 Rubiscos in *E. coli* can be achieved by chaperonin overexpression and/or co-expression with the Rubisco-specific molecular chaperone RbcX (Goloubinoff *et al*., 1989; Emlyn-Jones *et al*., 2006; Mueller-Cajar and Whitney, 2008b). In contrast to the BSDII and newly identified RAF1 chaperones, whose functional roles in plastid Rubisco biogenesis remain unqualified (Brutnell *et al*., 1999; Wostrikoff and Stern, 2007; Feiz *et al*., 2012), significant

functional detail has been resolved for RbcX (Saschenbrecker *et al.*, 2007; Bracher *et al.*, 2011). By interacting with post-chaperonin folded L-subunits, RbcX directs their assembly into L_8 cores for holoenzyme assembly with S-subunits (Liu *et al.*, 2011). This level of understanding of the Rubisco chaperone-assembly mechanism and requirements is needed to expand our capabilities to bioengineer Rubisco in plastids, *E. coli*, and cell-free *in vitro* systems to fully explore sequence–performance relationships of different Rubisco isoforms. Progress in our Rubisco engineering capabilities will likely entail exploration and functional characterization of additional molecular chaperones involved in Rubisco biogenesis.

Maintaining Rubisco activity

Amelioration of Rubisco fallover and inhibition

A prominent feature of the evolution of L_8S_8 Rubisco of higher plants has been an increased specificity for RuBP carboxylation relative to oxygenation (the specificity factor; Jordan and Ogren, 1981). Since Rubisco does not bind either gaseous substrate prior to their reaction with the RuBP-derived enediol acceptor (Cleland *et al.*, 1998), discrimination in favour of carboxylation is likely to have been achieved through the selection of an active site configuration with high affinity for a carboxylation transition-state intermediate, in which the incoming CO_2 resembles a carboxylate group (Tcherkez *et al.*, 2006). This strategy has the disadvantages of reducing the rate of catalytic turnover, due to the greater stability of the resulting enzyme–intermediate complex, as well as resulting in the tighter binding of phosphate analogues. These phosphate analogues include the naturally occurring inhibitor 2-carboxy-D-arabinitol 1-phosphate (CA1P; Gutteridge *et al.*, 1986; Berry *et al.*, 1987) and by-products of reaction intermediates, such as xylulose-1,5-bisphosphate (XuBP) formed by misprotonation of the enediol intermediate (Edmondson *et al.*, 1990; Zhu and Jensen, 1991) and D-glycero-2,3-pentodiulose-1,5-bisphosphate (PDBP) formed by the abstraction of H_2O_2 from the peroxyketone intermediate of the oxygenase reaction (Chen and Hartman, 1995; Kim and Portis, 2004). Under some conditions, binding of these analogues and of RuBP itself (Laing and Christeller, 1976) to Rubisco is sufficiently tight to inactivate the enzyme by locking the active site in a closed conformation (Salvucci and Crafts-Brandner, 2004a).

Measurement of RuBP carboxylation *in vitro* reveals a significant decline in activity with time – a phenomenon known as fallover – caused by the progressive inhibition of Rubisco by the accumulation of XuBP and PDBP. This process also occurs *in vivo* during photosynthesis, necessitating the repeated intervention by Rubisco activase to reactivate Rubisco (see below). The autocatalytic inactivation of Rubisco, together with its energy-dependent reactivation, means that the activity of Rubisco can in principle be maintained at a level appropriate to the prevailing capacity to regenerate the co-substrate, RuBP.

A universal catalytic mechanism means that XuBP and PDBP inevitably arise during catalysis by all forms of Rubisco (Kim and Portis, 2004; Pearce, 2006). However, the L_8S_8 Rubisco of vascular plants shows far greater susceptibility to inhibition by these by-products than those from other evolutionary lineages (Pearce, 2006). The absence of fallover in Rubiscos from alternative lineages is accounted for either by increased rates of inhibitor release or by weaker inhibitor binding (Pearce, 2006). The potential for reducing the susceptibility of higher plant Rubisco to fallover has also been demonstrated for tobacco Rubisco incorporating a Leu335Val L-subunit substitution (Pearce and Andrews, 2003). Although posing an impediment to catalysis, the mutation reduced fallover by lowering the enzyme's sensitivity to XuBP inhibition and facilitating the rearrangement of PDBP to its apparently less-inhibitory isomer, carboxytetritol-1,4-bisphosphate. It is also anticipated that targeted improvements to $S_{C/O}$ will reduce fallover by plant Rubisco due to reduced rates of oxygenation and the associated formation of PDBP.

Both PDBP (Kim and Portis, 2004) and XuBP (Schrader *et al.*, 2006) production during catalysis by land plant Rubisco increase at elevated temperatures (35–45 °C). In spite of this, in the presence of excess CO_2 and Mg^{2+}, the relative fall in Rubisco activity was less severe than in parallel experiments conducted at lower temperatures (Kim and Portis, 2006; Schrader *et al.*, 2006), implying that loss of Rubisco activity may not parallel the loss of photosynthetic capacity seen at similarly elevated temperatures. However, at lower, physiological concentrations of CO_2 and Mg^{2+}, the extent of fallover at elevated temperatures was much greater, due in part to increased Rubisco inactivation by decarbamylation (Kim and Portis, 2006), re-emphasizing the relevance of Rubisco activity to photosynthetic performance across a range of temperatures.

The naturally occurring Rubisco inhibitor CA1P binds tightly to the carbamylated form of Rubisco during periods of low light or darkness. CA1P is subsequently released from Rubisco and rendered non-inhibitory by the concerted action of the light-activated enzymes, Rubisco activase (elaborated below) and CA1P phosphatase (CA1Pase; Parry *et al.*, 2008). Details of the biosynthesis of CA1P from CO_2 via the Calvin cycle intermediate fructose-1,6-bisphosphate (FBP) have been elucidated through a combination of *in vitro* radiotracer (Andralojc *et al.*, 1994, 1996; Martindale *et al.*, 1997) and *in vivo* pulse-chase experiments in combination with the demonstration that intermediates of CA1P biosynthesis accumulate in transgenic potato expressing the gene for chloroplast FBP phosphatase in the antisense orientation (Andralojc *et al.*, 2002). The Rubisco-CA1P complex is likely to resemble that between Rubisco and the closely related substrate analogue 2-carboxy-D-arabinitol 1,5-bisphosphate (CABP; Knight *et al.*, 1990), having a closed conformation which is resistant to proteolysis (Mulligan *et al.*, 1988; Khan *et al.*, 1999). It is likely that the tight binding of either PDBP or XuBP to Rubisco also confers resistance to proteolysis.

The sequence and functional characterization of CA1Pase from wheat was recently published (Andralojc *et al.*, 2012), presenting the opportunity not only to manipulate the

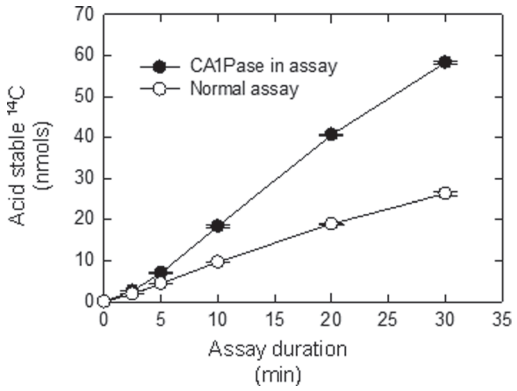

Fig. 4. Activation of CA1P-inhibited Rubisco in the presence and absence of CA1Pase. Activated Rubisco was preincubated for 10 min with a 3-fold excess of CA1P, after which aliquots were taken for measurement of carboxylase activity in the presence and absence of CA1Pase. The total assay volume was 500 µl containing 12.5 µg of Rubisco and 2.5 µg CA1Pase. The reactions were acid-quenched at the indicated times and the remaining ^{14}C (i.e. PGA) determined. Assay buffers and all other details were as described previously (Andralojc *et al.*, 2012). All assays were performed in triplicate.

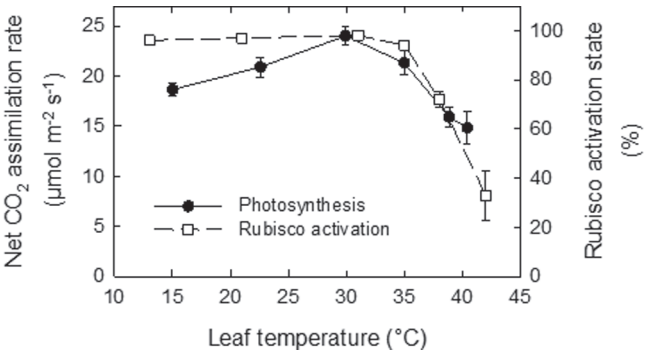

Fig. 5. Temperature response of net CO_2 assimilation rate (filled circles) and Rubisco activation state (open squares) in plants of *Camelina sativa* (L.) Crantz cv. Robinson. Gas exchange was measured in attached leaves at the indicated leaf temperatures using a PPFD of 1800 µmol m^{-2} s^{-1}, a reference CO_2 concentration of 380 µmol mol^{-1}, and 21% (v/v) O_2. Rubisco activation state [(initial activity/total activity) × 100] was determined by measuring Rubisco activities in extracts prepared from leaf discs that were exposed to the same conditions used for the gas-exchange measurements. Data from Carmo-Silva and Salvucci (2012).

abundance of CA1P but also to investigate the interactions between Rubisco, Rubisco activase, and CA1Pase from the same species. Unexpectedly, the cloned CA1Pase was found to diminish PDBP inhibition of Rubisco (fallover), indicating that the phosphatase may play a broader role in Rubisco regulation by removing PDBP generated non-enzymically within the chloroplast (Andralojc *et al*, 2012). CA1Pase has also been found to accelerate the *in vitro* activation of CA1P-inhibited Rubisco (Fig. 4), although the relative contribution of CA1P dephosphorylation and the removal of inhibitory contaminants in the RuBP to this process is unknown. The ability of CA1Pase to remove inhibitors other than CA1P is consistent with the widespread occurrence of putative coding sequences for CA1Pase in plant species, including those that produce little or no detectable levels of CA1P (Andralojc *et al.*, 2012). Therefore, altered expression of CA1Pase may impact on Rubisco activity in the light but, like Rubisco activase, CA1Pase has also been shown to be inactivated at moderately elevated temperatures (Holbrook *et al.*, 1991).

Activation of Rubisco by Rubisco activase

Reactivation of Rubisco active sites that have been locked in closed conformation by the unproductive binding of sugar phosphates requires conformational remodelling by Rubisco activase (Portis, 2003). Rubisco activase is an AAA+ ATPase, which uses the energy from the hydrolysis of ATP to power the remodelling reaction and thereby facilitates the release of the tightly binding sugar phosphate. Because of this dependence on ATP hydrolysis and the inhibition of this activity by ADP (Robinson and Portis, 1989), the activation of Rubisco by Rubisco activase serves an important regulatory function in linking the rate of CO_2 fixation to the rate of electron transport activity via the production of ATP.

Rubisco is typically fully activated in leaves under steady-state conditions and high light, except at high temperatures. As leaf temperatures increase above the optimum for photosynthesis, the activation state of Rubisco decreases in concert with the decrease in CO_2 assimilation rate (Fig. 5). The responses of Rubisco activation state and photosynthesis to supra-optimal temperatures are closely correlated, both qualitatively and quantitatively. Species from warm environments exhibit a higher temperature optimum of activation and CO_2 assimilation than species from colder environments (Feller *et al.*, 1998; Crafts-Brandner and Salvucci, 2000; Haldimann and Feller, 2004; Yamori *et al.*, 2006; Carmo-Silva and Salvucci, 2012; Scafaro *et al.*, 2012).

Inhibition of photosynthesis by heat stress is of practical concern for agriculture since climate change scenarios predict that global temperatures will increase by 2–5 °C by the end of the century (IPCC, 2007), if not sooner (Rowlands *et al.*, 2012). Accompanying this rise, an increased frequency of periods of elevated temperatures is expected. Moreover, these analyses also predict marked changes in water availability in some areas that could exacerbate the general warming trend by reducing the hydration status of the plants and thereby limiting the capacity for leaf cooling via transpiration (Radin *et al.*, 1994; Haldimann *et al.*, 2008; Carmo-Silva *et al.*, 2012). Fig. 6 illustrates this point by showing that Rubisco activation decreases in field-grown cotton cultivars under drought because their lower stomatal conductance reduces evaporative cooling, leading to warmer leaf temperatures. The individual and combined effects of heat and drought on the relationship between the net CO_2 assimilation rate and intercellular CO_2 concentration (C_i) are shown in Fig. 7. Drought-induced stomatal closure lowers

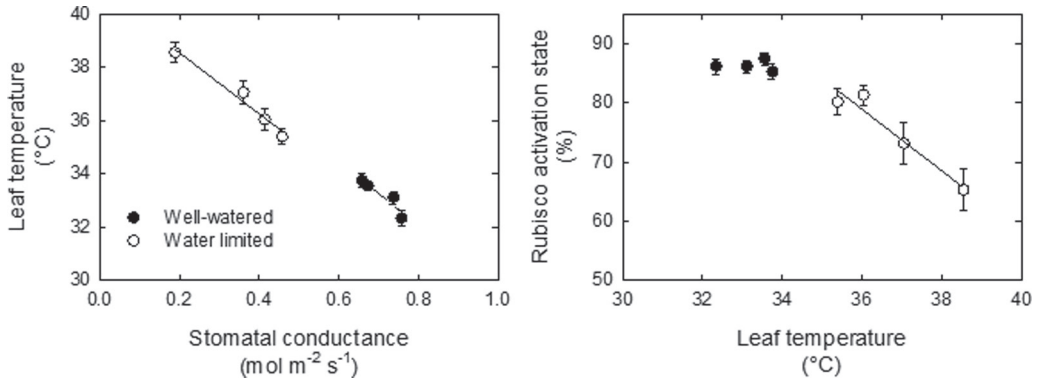

Fig. 6. Relationships between leaf temperature, stomatal conductance and Rubisco activation state in four cotton (*Gossypium barbadense* L.) cultivars grown in a semi-arid environment under well-watered and water-limited conditions. The cultivars measured in the study were: Monseratt Sea Island (MS), Pima 32 (P32), Pima S-6 (S6), and Pima S-7 (S7). Measurements were taken near midday on several occasions throughout the growing season. Conditions for infra-red gas analysis were: reference CO_2 = 380 μmol mol^{-1}, PPFD =1800 μmol m^{-2} s^{-1}, and block temperature = 32 °C. Frozen samples were assayed for Rubisco activity and the activation state was calculated as (initial activity/total activity) × 100. Data from Carmo-Silva *et al.* (2012).

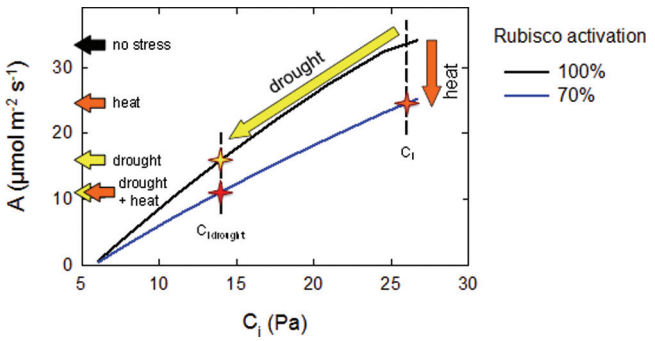

Fig. 7. Individual and combined effects of drought and heat stress on the net CO_2 assimilation rate (A) at a given intercellular CO_2 concentration (C_i). The A–C_i response of cotton Monseratt Sea Island plants under well-watered conditions (data from Carmo-Silva *et al.*, 2012) was modelled. Drought-induced diffusional limitations lower the C_i and heat-induced metabolic limitations caused by Rubisco deactivation decrease the slope of the A–C_i response. In semi-arid environments, drought and heat stress can combine to severely inhibit photosynthesis. Stars denote the values of A and corresponding C_i under each circumstance.

C_i, thereby causing a diffusional limitation to photosynthetic CO_2 assimilation by Rubisco. Heat-induced Rubisco deactivation, on the other hand, causes a metabolic limitation to photosynthesis, reducing the slope of the A–C_i response due to increased restriction to Rubisco activity. When combined, the inhibitory effects of drought and heat stress on photosynthetic performance are additive.

Considerable effort has been made recently on elucidating the biochemical basis for the decrease in Rubisco activation state under heat stress (Salvucci and Crafts-Brandner, 2004a; Sage *et al.*, 2008). Extensive evidence supports the finding that thermal inactivation of Rubisco activity arises primarily from temperature-dependent inactivation of Rubisco activase (Spreitzer and Salvucci, 2002; Portis, 2003). Rubisco activase is exceptionally sensitive to heat inactivation, exhibiting a temperature optimum for activity below 40 °C (Salvucci and Crafts-Brandner, 2004b; Barta *et al.*, 2010; Carmo-Silva and Salvucci, 2011). The optimum temperature varies among plant species in relation to their thermal environment (Fig. 8), within about the same range as photosynthesis (Salvucci and Crafts-Brandner, 2004b). *In planta*, activase stability could be influenced by heat-induced changes either in redox state (Schrader *et al.*, 2007; Zhang *et al.*, 2009; Sharkey and Zhang, 2010) or the concentrations of ions, nucleotides, or other chloroplast constituents (Sage *et al.*, 2008).

The conformational flexibility required to remodel Rubisco at ambient and low temperatures is likely to limit the thermostability of Rubisco activase. It has even been proposed that the thermotolerance of Rubisco activase might even be responsible for restricting the distribution of certain plant species (Sage *et al.*, 2008). The response of Rubisco activase activity to temperature for cotton, a warm-season species, and *Camelina sativa*, a cool-season species, clearly demonstrates this trade-off between thermostability and performance at low temperature (Fig. 8).

Another aspect of photosynthesis affected by the activity of Rubisco activase is the rate of photosynthetic induction during transitions in irradiance level (Woodrow *et al.*, 1996; Mott *et al.*, 1997; Mott and Woodrow, 2000). Compared with electron transport activity, the rate of CO_2 assimilation increases slowly after increasing the irradiance, primarily because of the slow rate of Rubisco activation. Following an abrupt increase in irradiance, slow induction might prevent photoinhibition by allowing stomata time to open. However, a faster response to the transition would increase the amount of CO_2 fixed during sunflecks and in environments where irradiance levels are variable (Roden and Pearcy, 1993; Ögren and Sundin, 1996). Recently, Yamori *et al.* (2012) showed that photosynthetic induction was faster in transgenic rice plants that overexpressed Rubisco activase from

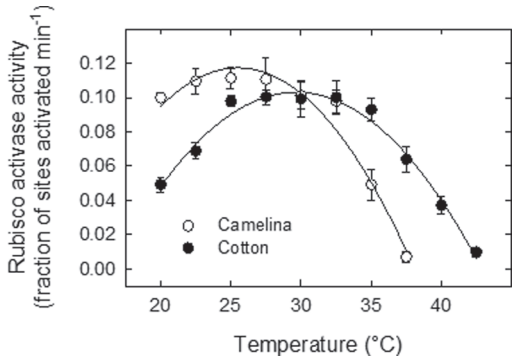

Fig. 8. Temperature response of Rubisco activase activity in leaf extracts of *Camelina sativa* (L.) Crantz cv. Robinson, a cool-season crop species, and *Gossypium hirsutum* L. cv. Coker 100A, a warm-season crop species. Data from Carmo-Silva and Salvucci (2011).

a maize transgene. The performance of these plants under fluctuating irradiance was not analysed in this or a previous study (Fukayama *et al.*, 2012).

Opportunities and limitations for manipulating Rubisco activase

The best evidence for a causal relationship between the thermal instability of Rubisco activase and the inhibition of photosynthesis by heat stress comes from two separate studies with *Arabidopsis*, showing that replacement of the native Rubisco activase with a more thermostable Rubisco activase increased plant tolerance to heat stress (Kurek *et al.*, 2007; Kumar *et al.*, 2009). Moreover, the improvements in photosynthetic performance under heat stress translated into a significant increase in seed yield, showing that assimilate supply from photosynthesis is a primary cause of yield penalty under moderate heat stress. These proof-of-concept experiments with *Arabidopsis* (Kurek *et al.*, 2007; Kumar *et al.*, 2009) demonstrated that improvement of the thermal stability of Rubisco activase by a few degrees would be sufficient in itself to increase photosynthetic performance and yield under moderate heat stress. However, the experiments in these studies were performed with a model plant (*Arabidopsis*) containing no endogenous Rubisco activase (i.e. the null *rca* mutant) that was transformed with constructs that encoded for a more thermostable, but non-regulated, form of Rubisco activase. Thus, it is uncertain if introduction of a more thermostable Rubisco activase will improve the thermotolerance of photosynthesis in a background containing endogenous Rubisco activase. It is also not clear which of the isoforms, regulatory or non-regulatory, would be more effective, particularly under natural conditions of fluctuating irradiance.

Yamori *et al.* (2012) recently showed that the thermal stability of photosynthesis was increased slightly when maize Rubisco activase was overexpressed in the presence of endogenous rice Rubisco activase. Based on the temperature optimum for photosynthesis for these plant species, the introduced Rubisco activase from maize should have a similar temperature optimum to the endogenous Rubisco activase in rice. Thus, as the authors conclude, the improvement in thermal tolerance was probably related to the increased amount of Rubisco activase relative to Rubisco in the transgenic plants (Yamori *et al.*, 2012).

Rubisco activase is a relatively abundant protein representing about 5% of the soluble leaf protein (He *et al.*, 1997), and possibly more (Piques *et al.*, 2009). To lower the investment in nitrogen and thereby avoid reductions in Rubisco amount (Mott and Woodrow, 2000; Fukayama *et al.*, 2012; Yamori *et al.*, 2012), we favour a different engineering strategy involving introduction of a significantly more thermostable Rubisco activase. For example, based on the temperature response shown in Fig. 8, introduction of the Rubisco activase from cotton into a cool-season species like *Camelina* should improve the thermotolerance of photosynthesis by as much as 5 °C (Carmo-Silva and Salvucci, 2012). Expressing cotton Rubisco activase in the presence of endogenous *Camelina* Rubisco activase should broaden the temperature range for activity, avoiding problems with Rubisco activase performance at low temperatures. Improving the thermotolerance of photosynthesis in warm season plants will require engineering new Rubisco activases since these plants already contain the most thermostable Rubisco activase. Recently resolved structural information for Rubisco activase (Blayney *et al.*, 2011; Henderson *et al.*, 2011; Stotz *et al.*, 2011) and its CbbX homologue (that regulates 'red' L_8S_8 Rubisco activity; Mueller-Cajar *et al.*, 2011) provide key advances in understanding the enzyme's structure and interactions with Rubisco – information that is essential for improving Rubisco activase catalysis by rational design.

Conclusions

The central role of Rubisco as the limiting step in CO_2 assimilation under ambient conditions of CO_2 and high light continues to make it the most attractive target for improving photosynthetic activity. Twenty years ago, the thought of modifying Rubisco catalysis in chloroplasts seemed an immense challenge. This changed with the development of plastome-transformation systems that have successfully expanded our Rubisco engineering capabilities. The new challenges include understanding the intricacies of Rubisco biogenesis in plastids to remove the existing constraints on the range of Rubisco isoforms that can be bioengineered *in vitro*, in *E. coli*, and in plastids. Equally important are the needs to measure catalysis from a larger number and greater variety of Rubiscos to identify useful sequence–performance relationships and to fully evaluate the potential of altering catalysis via modification of the S-subunits. These challenges require the development of existing and new higher-throughput recombinant technologies to systematically identify, test, and possibly improve upon Nature's solutions for improving Rubisco catalysis. Unfortunately, efficient and stable plastome transformation is only available for a limited number

of plants (Maliga and Bock, 2011), although recent evidence of horizontal plastome transmission between plant species using grafting approaches may prove beneficial for developing alternative plastome-transforming approaches for crops (Stegemann *et al.*, 2012, Thyssen *et al.*, 2012).

Discovering solutions for enhancing catalysis that can then be tailored into a target Rubisco will therefore likely continue to take advantage of the customized Rubisco-transformation systems available in tobacco (Kode *et al.*, 2006; Whitney and Sharwood, 2008). Being able to take full advantage of such improvements in crops will require co-integration with other biotechnological strategies for improving photosynthetic carbon assimilation (Fig. 1), including the adaptation of Rubisco activase function to meet the temperature changes associated with global climate change. The successful manipulation of the kinetics and regulation of Rubisco play a major role in improving crop photosynthesis and yield and thereby contribute to the challenge of ensuring food security (Parry and Hawkesford, 2010; Parry, 2012; Tiffin, 2012),

Acknowledgements

MAJP and PJA are scientists within 20:20 Wheat which is supported by a BBSRC Institute Strategic Programme. MES and ECS work on Rubisco and Rubisco activase was funded by the Division of Chemical Sciences, Geosciences, and Biosciences, Office of Basic Energy Sciences, of the US Department of Energy (grant no. DE-FG02-10ER20268 to MES). Research by HA and SMW was funded by the Australian Research Council. We thank the Australian Centre for International Agricultural Research (ACIAR) support of the Photosynthesis Workshop in Canberra, 2009, that catalysed this collaboration.

References

Ainsworth EA, Long SP. 2005. What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. *New Phytologist* **165,** 351–371.

Ainsworth EA, Ort DR. 2010. How do we improve crop production in a warming world? *Plant Physiology* **154,** 526–530.

Alonso H, Blayney MJ, Beck JL, Whitney SM. 2009. Substrate-induced assembly of *Methanococcoides burtonii* D-ribulose-1,5-bisphosphate carboxylase/oxygenase dimers into decamers. *Journal of Biological Chemistry* **284,** 33876–33882.

Andersson I, Backlund A. 2008. Structure and function of Rubisco. *Plant Physiology and Biochemistry* **46,** 275–291.

Andralojc PJ, Dawson GW, Parry MAJ, Keys AJ. 1994. Incorporation of carbon from photosynthetic products into 2-carboxyarabinitol-1-phosphate and 2-carboxyarabinitol. *The Biochemical Journal* **304,** 781–786.

Andralojc PJ, Keys AJ, Kossmann J, Parry MAJ. 2002. Elucidating the biosynthesis of 2-carboxyarabinitol 1-phosphate through reduced expression of chloroplastic fructose 1,6-bisphosphate phosphatase and radiotracer studies with $^{14}CO_2$. *Proceedings of the National Academy of Sciences, USA* **99,** 4742–4747.

Andralojc PJ, Keys AJ, Martindale W, Dawson GW, Parry MAJP. 1996. Conversion of D-hamamelose into 2-carboxy-D-arabinitol and 2-carboxy-D-arabinitol 1-phosphate in leaves of *Phaseolus vulgaris* L. *Journal of Biological Chemistry* **271,** 26803–26809.

Andralojc PJ, Madgwick PJ, Tao Y, *et al.* 2012. 2-Carboxy-D-arabinitol 1-phosphate (CA1P) phosphatase: evidence for a wider role in plant Rubisco regulation. *The Biochemical Journal* **442,** 733–742.

Andrews TJ, Whitney SM. 2003. Manipulating ribulose bisphosphate carboxylase/oxygenase in the chloroplasts of higher plants. *Archives of Biochemistry and Biophysics* **414,** 159–169.

Avni A, Edelman M, Rachailovich I, Aviv D, Fluhr R. 1989. A point mutation in the gene for the large subunit of ribulose 1,5-bisphosphate carboxylase/oxygenase affects holoenzyme assembly in *Nicotiana tabacum*. *EMBO Journal* **8,** 1915–1918.

Barta C, Dunkle AM, Wachter RM, Salvucci ME. 2010. Structural changes associated with the acute thermal instability of Rubisco activase. *Archives of Biochemistry and Biophysics* **499,** 17–25.

Berry JA, Lorimer GH, Pierce JP, Seemann JR, Meek J, Freas S. 1987. Isolation, identification, and synthesis of 2-carboxyarabinitol 1-phosphate, a diurnal regulator of ribulose-bisphosphate carboxylase activity. *Proceedings of the National Academy of Sciences, USA* **84,** 734–738.

Blayney M, Whitney SM, Beck J. 2011. NanoESI mass spectrometry of Rubisco and Rubisco activase structures and their interactions with nucleotides and sugar phosphates. *Journal of The American Society for Mass Spectrometry* **22,** 1588–1601.

Bracher A, Starling-Windhof A, Hartl FU, Hayer-Hartl M. 2011. Crystal structure of a chaperone-bound assembly intermediate of form I Rubisco. *Nature Structural and Molecular Biology* **18,** 875–880.

Brutnell TP, Sawers RJH, Mant A, Langdale JA. 1999. BUNDLE SHEATH DEFECTIVE2, a novel protein required for post-translational regulation of the *rbcL* gene of maize. *The Plant Cell* **11,** 849–864.

Carmo-Silva AE, Gore MA, Andrade-Sanchez P, French AN, Hunsaker DJ, Salvucci ME. 2012. Decreased CO_2 availability and inactivation of Rubisco limit photosynthesis in cotton plants under heat and drought stress in the field. *Environmental and Experimental Botany* **83,** 1–11.

Carmo-Silva AE, Salvucci ME. 2011. The activity of Rubisco's molecular chaperone, Rubisco activase, in leaf extracts. *Photosynthesis Research* **108,** 143–155.

Carmo-Silva AE, Salvucci ME. 2012. The temperature response of CO_2 assimilation, photochemical activities and Rubisco activation in *Camelina sativa*, a potential bioenergy crop with limited capacity for acclimation to heat stress. *Planta* **236,** 1433–1445.

Chen Y-R, Hartman FC. 1995. A signature of the oxygenase intermediate in catalysis by ribulose-bisphosphate carboxylase/oxygenase as provided by a site-directed mutant. *Journal of Biological Chemistry* **270,** 11741–11744.

Cleland WW, Andrews TJ, Gutteridge S, Hartman FC, Lorimer GH. 1998. Mechanism of Rubisco: the carbamate as general base. *Chemical Reviews* **98,** 549–561.

Covshoff S, Hibberd JM. 2012. Integrating C_4 photosynthesis into C_3 crops to increase yield potential. *Current Opinion in Biotechnology* **23,** 209–214.

Crafts-Brandner SJ, Salvucci ME. 2000. Rubisco activase constrains the photosynthetic potential of leaves at high temperature and CO_2. *Proceedings of the National Academy of Sciences, USA* **97,** 13430–13435.

Day A, Goldschmidt-Clermont M. 2011. The chloroplast transformation toolbox: selectable markers and marker removal. *Plant Biotechnology Journal* **9,** 540–553.

Dhingra A, Portis AR Jr, Daniell H. 2004. Enhanced translation of a chloroplast-expressed *RbcS* gene restores small subunit levels and photosynthesis in nuclear *RbcS* antisense plants. *Proceedings of the National Academy of Sciences, USA* **101,** 6315–6320.

Edmondson DL, Kane HJ, Andrews TJ. 1990. Substrate isomerisation inhibits ribulosebisphosphate carboxylase-oxygenase during catalysis. *FEBS Letters* **260,** 62–66.

Emlyn-Jones D, Woodger FJ, Price GD, Whitney SM. 2006. RbcX can function as a Rubisco-chaperonin, but is non essential in *Synechococcus* PCC7942. *Plant and Cell Physiology* **47,** 1630–1640.

Feller U, Crafts-Brandner SJ, Salvucci ME. 1998. Moderately high temperatures inhibit ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activase-mediated activation of Rubisco. *Plant Physiology* **116,** 539–546.

Feiz L, Williams-Carrier R, Wostrikoff K, Belcher S, Barkan A, Stern D. 2012. RAF1, a new regulator of Rubisco biogenesis in maize. *The Plant Cell* **24,** 2435–3446.

Fukayama H, Ueguchi C, Nishikawa K, Katoh N, Ishikawa C, Masumoto C, Hatanaka T, Misoo S. 2012. Overexpression of Rubisco activase decreases the photosynthetic CO_2 assimilation rate by reducing Rubisco content in rice leaves. *Plant and Cell Physiology* **53,** 976–986.

Galmés J, Flexas J, Keys AJ, Cifre J, Mitchell RAC, Madgwick PJ, Haslam RP, Medrano H, Parry MAJ. 2005. Rubisco specificity factor tends to be larger in plant species from drier habitats and in species with persistent leaves. *Plant, Cell and Environment* **28,** 571–579.

Genkov T, Meyer M, Griffiths H, Spreitzer RJ. 2010. Functional hybrid Rubisco with plant small subunits and algal large subunits. *Journal of Biological Chemistry* **285,** 19833–19841.

Getzoff TP, Zhu GH, Bohnert HJ, Jensen RG. 1998. Chimeric *Arabidopsis thaliana* Rubisco containing a pea small subunit protein is compromised in carbamylation. *Plant Physiology* **116,** 695–702.

Goloubinoff P, Gatenby AA, Lorimer GH. 1989. GroE heat-shock proteins promote assembly of foreign prokaryotic ribulose bisphosphate carboxylase oligomers in *Escherichia coli*. *Nature* **337,** 44–47.

Gutteridge S, Parry MAJ, Burton S, Keys AJ, Mudd A, Feeney J, Servaites JC, Pierce J. 1986. A nocturnal inhibitor of carboxylation in leaves. *Nature* **324,** 274–276.

Haldimann P, Feller U. 2004. Inhibition of photosynthesis by high temperature in oak (*Quercus pubescens*) leaves grown under natural conditions closely correlates with a reversible heat-dependent reduction of the activation state of ribulose-1,5-bisphosphate carboxylase/oxygenase. *Plant, Cell and Environment* **27,** 1169–1183.

Haldimann P, Gallé A, Feller U. 2008. Impact of an exceptionally hot dry summer on photosynthetic traits in oak (*Quercus pubescens*) leaves. *Tree Physiology* **28,** 785–795.

He ZL, von Caemmerer S, Hudson GS, Price GD, Badger MR, Andrews TJ. 1997. Ribulose-1,5-bisphosphate carboxylase/oxygenase activase deficiency delays senescence of ribulose-1,5-bisphosphate carboxylase/oxygenase but progressively impairs its catalysis during tobacco leaf development. *Plant Physiology* **115,** 1569–1580.

Henderson JN, Kuriata AM, Fromme R, Salvucci ME, Wachter RM. 2011. Atomic resolution x-ray structure of the substrate recognition domain of higher plant ribulose-bisphosphate carboxylase/oxygenase (Rubisco) activase. *Journal of Biological Chemistry* **286,** 35683–35688.

Holbrook GP, Galasinski SC, Salvucci ME. 1991. Regulation of 2-carboxyarabinitol 1-phosphatase. *Plant Physiology* **97,** 894–899.

IPCC. 2007. *Climate change 2007: synthesis report. Contribution of working groups I, II and III to the fourth assessment report of the Intergovernmental Panel on Climate Change*. Geneva: IPCC, Core Writing Team, Pachauri RK, Reisinger A, eds.

Ishikawa C, Hatanaka T, Misoo S, Miyake C, Fukayama H. 2011. Functional incorporation of sorghum small subunit increases the catalytic turnover rate of Rubisco in transgenic rice. *Plant Physiology* **156,** 1603–1611.

Jordan DB, Ogren WL. 1981. Species variation in the specificity of ribulose bisphosphate carboxylase/oxygenase. *Nature* **291,** 513–515.

Kebeish R, Niessen M, Thiruveedhi K, Bari R, Hirsch H-J, Rosenkranz R, Staebler N, Schoenfeld B, Kreuzaler F, Peterhaensel C. 2007. Chloroplastic photorespiratory bypass increases photosynthesis and biomass production in *Arabidopsis thaliana*. *Nature Biotechnology* **25,** 593–599.

Khan S, Andralojc PJ, Lea PJ, Parry MAJ. 1999. 2'-Carboxy-D-arabinitol 1-phosphate (CA1P) protects ribulose-1,5-bisphosphate carboxylase/oxygenase against proteolytic breakdown. *European Journal of Biochemistry* **266,** 840–847.

Kim K, **Portis AR Jr**. 2004. Oxygen-dependent H_2O_2 production by Rubisco. *FEBS Letters* **571,** 124–128.

Kim K, **Portis AR Jr**. 2006. Kinetic analysis of the slow inactivation of Rubisco during catalysis: effects of temperature, O_2 and Mg^{++}. *Photosynthesis Research* **87,** 195–204.

Knight S, Andersson I, Brändén C-I. 1990. Crystallographic analysis of ribulose 1,5-bisphosphate carboxylase from spinach at 2.4 Å resolution. *Journal of Molecular Biology* **215,** 113–160.

Kode V, Mudd EA, Iamtham S, Day A. 2006. Isolation of precise plastid deletion mutants by homology-based excision: a resource for site-directed mutagenesis, multi-gene changes and high-throughput plastid transformation. *The Plant Journal* **46,** 901–909.

Kumar A, **Li C**, **Portis AR Jr**. 2009. *Arabidopsis thaliana* expressing a thermostable chimeric Rubisco activase exhibits enhanced growth and higher rates of photosynthesis at moderately high temperatures. *Photosynthesis Research* **100,** 143–153.

Kurek I, Thom KC, Bertain SM, Madrigal A, Liu L, Lassner MW, Zhu G. 2007. Enhanced thermostability of *Arabidopsis* Rubisco activase improves photosynthesis and growth rates under moderate heat stress. *The Plant Cell* **19,** 3230–3241.

Laing WA, Christeller JT. 1976. A model for the kinetics of activation and catalysis of ribulose 1,5-bisphosphate carboxylase. *The Biochemical Journal* **159,** 563–570.

Liu C, Young AL, Starling-Windhof A, *et al.* 2011. Coupled chaperone action in folding and assembly of hexadecameric Rubisco. *Nature* **463,** 197–202.

Maliga P, Bock R. 2011. Plastid biotechnology: food, fuel, and medicine for the 21st Century. *Plant Physiology* **155,** 1501–1510.

Martindale W, Parry MAJ, Andralojc PJ, Keys AJ. 1997. Synthesis of 2^1-carboxy-D-arabinitol-1-phosphate in French bean (*Phaseolus vulgaris* L.): a search for precursors. *Journal of Experimental Botany* **48,** 9–14.

Mott KA, Snyder GW, Woodrow IE. 1997. Kinetics of Rubisco activation as determined from gas-exchange measurements in antisense plants of *Arabidopsis thaliana* containing reduced levels of Rubisco activase. *Australian Journal of Plant Physiology* **24,** 811–818.

Mott KA, Woodrow IE. 2000. Modelling the role of Rubisco activase in limiting non-steady-state photosynthesis. *Journal of Experimental Botany* **51,** 399–406.

Mueller-Cajar O, Stotz M, Wendler P, Hartl FU, Bracher A, Hayer-Hartl M. 2011. Structure and function of the AAA+ protein CbbX, a red-type Rubisco activase. *Nature* **479,** 194–199.

Mueller-Cajar O, Whitney SM. 2008a. Directing the evolution of Rubisco and Rubisco activase: first impressions of a new tool for photosynthesis research. *Photosynthesis Research* **98,** 667–675.

Mueller-Cajar O, Whitney SM. 2008b. Evolving improved *Synechococcus* Rubisco functional expression in *Escherichia coli*. *The Biochemical Journal* **414,** 205–214.

Mulligan RM, Houtz RL, Tolbert NE. 1988. Reaction-intermediate analogue binding by ribulose bisphosphate carboxylase/oxygenase causes specific changes in proteolytic sensitivity: the amino-terminal residue of the large subunit is acetylated proline. *Proceedings of the National Academy of Sciences, USA* **85,** 1513–1517.

Ögren E, Sundin U. 1996. Photosynthetic responses to variable light: a comparison of species from contrasting habitats. *Oecologia* **106,** 18–27.

Parry MAJ. 2012. Food and energy security: exploring the challenges of attaining secure and sustainable supplies of food and energy. *Food and Energy Security* **1,** 1–2.

Parry MAJ, Andralojc PJ, Mitchell RAC, Madgwick PJ, Keys AJ. 2003. Manipulation of Rubisco: the amount, activity, function and regulation. *Journal of Experimental Botany* **54,** 1321–1333.

Parry MAJ, Hawkesford MJ. 2010. Food security; increasing yield and improving resource use efficiency. *Proceedings of the Nutrition Society* **69,** 592–600.

Parry MAJ, Keys AJ, Madgwick PJ, Carmo-Silva AE, Andralojc PJ. 2008. Rubisco regulation: a role for inhibitors. *Journal of Experimental Botany* **59,** 1569–1580.

Parry MAJ, Reynolds M, Salvucci ME, Raines C, Andralojc PJ, Zhu X-G, Price GD, Condon AG, Furbank RT. 2011. Raising yield potential of wheat. II. Increasing photosynthetic capacity and efficiency. *Journal of Experimental Botany* **62,** 453–467.

Pearce FG. 2006. Catalytic by-product formation and ligand binding by ribulose bisphosphate carboxylases from different phylogenies. *The Biochemical Journal* **399,** 525–534.

Pearce FG, Andrews TJ. 2003. The relationship between side reactions and slow inhibition of ribulose-bisphosphate carboxylase revealed by a loop 6 mutant of the tobacco enzyme. *Journal of Biological Chemistry* **278,** 32526–32536.

Piques M, Schulze WX, Höhne M, Usadel B, Gibon Y, Rohwer J, Stitt, M. 2009. Ribosome and transcript copy numbers, polysome occupancy and enzyme dynamics in *Arabidopsis*. *Molecular Systems Biology* **5**, 314.

Portis AR Jr. 2003. Rubisco activase – Rubisco's catalytic chaperone. *Photosynthesis Research* **75,** 11–27.

Price GD, Badger MR, von Caemmerer S. 2011. The prospect of using cyanobacterial bicarbonate transporters to improve leaf photosynthesis in C_3 crop plants. *Plant Physiology* **155,** 20–26.

Radin JW, Lu Z, Percy RG, Zeiger E. 1994. Genetic variability for stomatal conductance in Pima cotton and its relation to improvements of heat adaptation. *Proceedings of the National Academy of Sciences, USA* **91,** 7117–7221.

Reynolds M, Foulkes J, Furbank R, Griffiths S, King J., Murchie E, Parry MAJ, Slafer G. 2012. Achieving yield gains in wheat. *Plant, Cell and Environment* **35,** 1799–01823.

Robinson SP, **Portis AR Jr**. 1989. Adenosine triphosphate hydrolysis by purified Rubisco activase. *Archives of Biochemistry and Biophysics* **268,** 93–99.

Roden JS, Pearcy RW. 1993. Photosynthetic gas exchange responses of poplars to steady-state and dynamic light environments. *Oecologia* **93,** 208–214.

Rosenthal D, Locke A, Khozaei M, Raines C, Long S, Ort D. 2011. Over-expressing the C_3 photosynthesis cycle enzyme sedoheptulose-1–7 bisphosphatase improves photosynthetic carbon gain and yield under fully open air CO_2 fumigation (FACE). *BMC Plant Biology* **11,** 123.

Rowlands DJ, Frame DJ, Ackerley D, *et al.* 2012. Broad range of 2050 warming from an observationally constrained large climate model ensemble. *Nature Geoscience* **5,** 256–260.

Sage RF, Way DA, Kubien DS. 2008. Rubisco, Rubisco activase, and global climate change. *Journal of Experimental Botany* **59,** 1581–1595.

Salvucci ME, Crafts-Brandner SJ. 2004a. Inhibition of photosynthesis by heat stress: the activation state of Rubisco as a limiting factor in photosynthesis. *Physiologia Plantarum* **120,** 179–186.

Salvucci ME, Crafts-Brandner SJ. 2004b. Relationship between the heat tolerance of photosynthesis and the thermal stability of Rubisco activase in plants from contrasting thermal environments. *Plant Physiology* **134,** 1460–1470.

Saschenbrecker S, Bracher A, Rao KV, Rao BV, Hartl FU, Hayer-Hartl M. 2007. Structure and function of RbcX, an assembly chaperone for hexadecameric Rubisco. *Cell* **129,** 1189–1200.

Scafaro AP, Yamori W, Carmo-Silva AE, Salvucci ME, von Caemmerer S, Atwell BJ. 2012. Rubisco activity is associated with

photosynthetic thermotolerance in a wild rice (*Oryza meridionalis*). *Physiologia Plantarum* **146,** 99–109.

Schrader SM, Kane HJ, Sharkey TD, von Caemmerer S. 2006. High temperature enhances inhibitor production but reduces fallover in tobacco Rubisco. *Functional Plant Biology* **33,** 921–929.

Schrader SM, Kleinbeck KR, Sharkey TD. 2007. Rapid heating of intact leaves reveals initial effects of stromal oxidation on photosynthesis. *Plant, Cell and Environment* **30,** 671–678.

Sharkey TD, Zhang R. 2010. High temperature effects on electron and proton circuits of photosynthesis. *Journal of Integrative Plant Biology* **52,** 712–722.

Sharwood RE, von Caemmerer S, Maliga P, Whitney SM. 2008. The catalytic properties of hybrid Rubisco comprising tobacco small and sunflower large subunits mirror the kinetically equivalent source Rubiscos and can support tobacco growth. *Plant Physiology* **146,** 83–96.

Shikanai T, Foyer CH, Dulieu H, Parry MAJ, Yokota A. 1996. A point mutation in the gene encoding the Rubisco large subunit interferes with holoenzyme assembly. *Plant Molecular Biology* **31,** 399–403.

Spreitzer RJ, Peddi SR, Satagopan S. 2005. Phylogenetic engineering at an interface between large and small subunits imparts land-plant kinetic properties to algal Rubisco. *Proceedings of the National Academy of Sciences, USA* **102,** 17225–17230.

Spreitzer RJ, Salvucci ME. 2002. Rubisco: structure, regulatory interactions, and possibilities for a better enzyme. *Annual Reviews of Plant Biology* **53,** 449–475.

Stegemann S, Keuthe M, Greiner S, Bock R. 2012. Horizontal transfer of chloroplast genomes between plant species. *Proceedings of the National Academy of Sciences, USA* **109,** 2434–2438.

Stitt M, Schulze E-D. 1994. Does Rubisco control the rate of photosynthesis and plant growth? An exercise in molecular ecophysiology. *Plant, Cell and Environment* **17,** 465–487.

Stotz M, Mueller-Cajar O, Ciniawsky S, Wendler P, Hartl FU, Bracher A, Hayer-Hartl M. 2011. Structure of green-type Rubisco activase from tobacco. *Nature Structural and Molecular Biology* **18,** 1366–1370.

Tcherkez G, Farquhar GD, Andrews TJ. 2006. Despite slow catalysis and confused substrate specificity, all ribulose bisphosphate carboxylases may be nearly perfectly optimized. *Proceedings of the National Academy of Sciences, USA* **103,** 7246–7251.

Thyssen G, Svab Z, Maliga P. 2012. Cell-to-cell movement of plastids in plants. *Proceedings of the National Academy of Sciences, USA* **109,** 2439–2443.

Tiffin R. 2012. Economists are not dismal, the world is not a Petri dish and other reasons for optimism. *Food and Energy Security* **1,** 3–8.

van Lun M, van der Spoel D, Andersson I. 2011. Subunit interface dynamics in hexadecameric Rubisco. *Journal of Molecular Biology* **411,** 1083–1098.

von Caemmerer S, Evans JR. 2011. Enhancing C_3 photosynthesis. *Plant Physiology* **154,** 589–592.

Whitney SM, Andrews TJ. 2001. The gene for the ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) small subunit relocated to the plastid genome of tobacco directs the synthesis of small subunits that assemble into Rubisco. *The Plant Cell* **13,** 193–205.

Whitney SM, Baldet P, Hudson GS, Andrews TJ. 2001. Form I Rubiscos from non-green algae are expressed abundantly but not assembled in tobacco chloroplasts. *The Plant Journal* **26,** 535–547.

Whitney SM, Houtz RL, Alonso H. 2011a. Advancing our understanding and capacity to engineer nature's CO_2-sequestering enzyme, Rubisco. *Plant Physiology* **155,** 27–35.

Whitney SM, Kane HJ, Houtz RL, Sharwood RE. 2009. Rubisco oligomers composed of linked small and large subunits assemble in tobacco plastids and have higher affinities for CO_2 and O_2. *Plant Physiology* **149,** 1887–1895.

Whitney SM, Sharwood RE. 2008. Construction of a tobacco master line to improve Rubisco engineering in chloroplasts. *Journal of Experimental Botany* **59,** 1909–1921.

Whitney SM, Sharwood RE, Orr D, White SJ, Alonso H, Galmés J. 2011b. Isoleucine 309 acts as a C_4 catalytic switch that increases ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) carboxylation rate in *Flaveria*. *Proceedings of the National Academy of Sciences, USA* **108,** 14688–14693.

Whitney SM, von Caemmerer S, Hudson GS, Andrews TJ. 1999. Directed mutation of the Rubisco large subunit of tobacco influences photorespiration and growth. *Plant Physiology* **121,** 579–588.

Woodrow IE, Kelly ME, Mott KA. 1996. Limitation of the rate of ribulosebisphosphate carboxylase activation by carbamylation and ribulosebisphosphate carboxylase activase activity: development and test of a mechanistic model. *Australian Journal of Plant Physiology* **23,** 141–149.

Wostrikoff K, Stern DB. 2007. Rubisco large-subunit translation is autoregulated in response to its assembly state in tobacco chloroplasts. *Proceedings of the National Academy of Sciences, USA* **104,** 6466–6471.

Wostrikoff K, Stern DB. 2009. Rubisco. In: EH Harris, DB Stern, GB Witman, eds, *The Chlamydomonas sourcebook*, 2nd edn. London: Academic Press, 303–332.

Yamori W, Masumoto C, Fukayama H, Makino A. 2012. Rubisco activase is a key regulator of non-steady-state photosynthesis at any leaf temperature and, to a lesser extent, of steady-state photosynthesis at high temperature. *The Plant Journal* **71,** 870–880.

Yamori W, Suzuki K, Noguchi K, Nakai M, Terashima I. 2006. Effects of Rubisco kinetics and Rubisco activation state on the temperature dependence of the photosynthetic rate in spinach leaves from contrasting growth temperatures. *Plant, Cell and Environment* **29,** 1659–1670.

Zhang R, Cruz JA, Kramer DM, Magallanes-Lundback ME, Dellapenna D, Sharkey TD. 2009. Moderate heat stress reduces the pH component of the transthylakoid proton motive force in light-adapted, intact tobacco leaves. *Plant, Cell and Environment* **32,** 1538–1547.

Zhang XH, **Ewy RG**, **Widholm JM**, **Portis AR Jr**. 2002. Complementation of the nuclear antisense rbcS-induced photosynthesis deficiency by introducing an rbcS gene into the tobacco plastid genome. *Plant and Cell Physiology* **43,** 1302–1313.

Zhang XH, Webb J, Huang Y-H, Lin L, Tang R-S, Liu A. 2011. Hybrid Rubisco of tomato large subunits and tobacco small subunits is functional in tobacco plants. *Plant Science* **180,** 480–488.

Zhu G, Jensen RG. 1991. Xylulose 1,5-bisphosphate synthesized by ribulose 1,5-bisphosphate carboxylase/oxygenase during catalysis binds to decarbamylated enzyme. *Plant Physiology* **97,** 1348–1353.

Zhu G, Kurek I, Liu L. 2010a. Engineering photosynthetic enzymes involved in CO_2-assimilation by gene shuffling. In: CA Rebeiz, C Benning, HJ Bohnert, H Daniell, JK Hoober, HK Lichtenthaler, AR Portis Jr, BC Tripathy, eds, *The chloroplast: basics and application (Advances in photosynthesis and respiration.)* Dordrecht: Springer, 307–322.

Zhu X-G, de Sturler S, Long SP 2007. Optimizing the distribution of resources between enzymes of carbon metabolism can dramatically increase photosynthetic rate: a numerical simulation using an evolutionary algorithm. *Plant Physiology* **145,** 513–526.

Zhu X-G, Long SP, Ort DR. 2010b. Improving photosynthetic efficiency for greater yield. *Annual Review of Plant Biology* **61,** 235–261.