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Rubisco regulation: a role for inhibitors

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Abstract

In photosynthesis Rubisco catalyses the assimilation of CO_2 by the carboxylation of ribulose-1,5-bisphosphate. However, the catalytic properties of Rubisco are not optimal for current or projected environments and limit the efficiency of photosynthesis. Rubisco activity is highly regulated in response to short-term fluctuations in the environment, although such regulation may not be optimally poised for crop productivity. The regulation of Rubisco activity in higher plants is reviewed here, including the role of Rubisco activase, tight binding inhibitors, and the impact of abiotic stress upon them.

Key words: Abiotic stress, CA1P, inhibitors, Rubisco, Rubisco activase.

Introduction

The most abundant protein, Rubisco [ribulose-1,5-bisphosphate (RuBP) carboxylase/oxygenase; EC 4.1.1.39] catalyses the assimilation of CO_2, by the carboxylation of ribulose-1,5-bisphosphate (RuBP) in photosynthetic carbon assimilation (Ellis, 1979). However, the catalytic limitations of Rubisco compromise the efficiency of photosynthesis (Parry *et al*., 2007). Compared to other enzymes of the Calvin cycle, Rubisco has a low turnover number, meaning that relatively large amounts must be present to sustain sufficient rates of photosynthesis. Furthermore, Rubisco also catalyses a competing and wasteful reaction with oxygen, initiating the process of photorespiration, which leads to a loss of fixed carbon and consumes energy. Although Rubisco and the photorespiratory enzymes are a major N store, and can account for more than 25% of leaf nitrogen, Rubisco activity can still be limiting. Furthermore, growth studies with transgenic plants with decreased amounts of Rubisco have confirmed that, under field conditions with intense or variable irradiance, photosynthetic rate is highly correlated with the amount of Rubisco (Hudson *et al*., 1992). This relationship cannot be ignored in attempts to improve resource use, particularly of nitrogen and water (Lea and Azevedo, 2006, 2007; Parry *et al*., 2005, 2007; Parry and Reynolds, 2007; Tambussi *et al*., 2007; Tuberosa *et al*., 2007).

In higher plants, Rubisco has a hexadecameric structure, being composed of eight large, chloroplast-encoded subunits arranged as four dimers and eight small, nuclear-encoded subunits. This is also known as Form I Rubisco. Each large subunit has two major structural domains, an N-terminal domain and a larger C-terminal domain which is an alpha/beta barrel. Most of the active site residues (that interact with substrate and/or substrate analogues) are contributed by loops at the mouth of the alpha/beta barrel with the remaining residues being supplied by two loop regions in the N-terminal domain of the second large subunit within a dimer. The availability of high resolution 3-D structures has provided detailed insight into the catalytic mechanisms of the enzyme and enabled properties to be related to sequences (Parry *et al*., 1987; see comprehensive reviews by Andersson, 1996, 2008; Cleland *et al*., 1998; Spreitzer and Salvucci, 2002; Portis and Parry, 2007).

Rubisco is highly regulated to control flux through the photosynthetic carbon reduction cycle in response to short-term fluctuations in the environment (Geiger and Servaites, 1994). The potential Rubisco activity is determined by the amount of Rubisco protein which, in turn, is determined by the relative rate of biosynthesis and degradation. These processes are regulated by gene expression (Sheen, 1990; Krapp *et al*., 1993), mRNA stability, polypeptide synthesis, post-translational modification, assembly of subunits into

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an active holoenzyme, and various factors which impact upon protein degradation (Mehta *et al*., 1992; Eckardt and Pell, 1995; Desimone *et al*., 1996). In the short term, regulating Rubisco activity is essential to match the capacity for RuBP regeneration with the prevailing rate of RuBP utilization. This is not achieved solely by the availability of substrate (Badger *et al*., 1984; Mott *et al*., 1984) since Rubisco in excess of that needed to sustain photosynthesis in the prevailing environment is deactivated (Sage *et al*., 1990). From an agronomic standpoint, Rubisco regulation may not be optimallly poised for crop productivity (Parry *et al*., 2007). Whilst Rubisco regulation has been previously reviewed (Portis, 1992, 2003; Parry *et al*., 1999*b*; Spreitzer and Salvucci, 2002) this review focuses on the regulation of Rubisco activity in higher plants including the role of Rubisco activase, tight binding inhibitors, and the corresponding impact of abiotic stress upon them.

The mechanisms involved in Rubisco regulation are summarized in Fig. 1. Rubisco (E) activity *in vivo* is modulated either by the carbamylation of an essential lysine residue at the catalytic site and subsequent stabilization of the resulting carbamate by a Mg^{2+} ion, forming a catalytically active ternary complex ($E.CO_2.Mg^{2+}$); or through the tight binding of low molecular weight inhibitors (I). Note that the CO_2 involved in active site carbamylation is distinct from CO_2 reacting with the acceptor molecule, RuBP, during catalysis. Inhibitors bind either before (E.I) or after carbamylation ($E.CO_2.Mg^{2+}$.I)

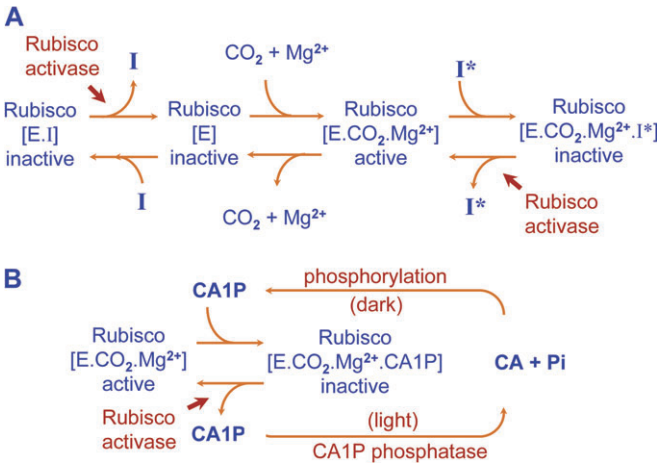

Fig. 1. (A) Principles of regulation of Rubisco catalytic activity. For full explanation, see text. [E], unmodified enzyme ('decarbamylated' Rubisco); [E.I], decarbamylated enzyme with substrate (RuBP) or misfire product (XuBP) bound at active sites—in this context both compounds are inhibitors (I); [$E.CO_2.Mg^{2+}$], ternary complex with catalytically competent active site geometry; [$E.CO_2.Mg^{2+}$ I], carbamylated enzyme with catalytic site occupied by tight binding inhibitor (CA1P, PDBP and possibly KABP). (B) Reversible inhibition of carbamylated Rubisco by CA1P, showing the light-dependent removal and dephosphorylation of CA1P, mediated by Rubisco activase and CA1P phosphatase, respectively. CA can be rephosphorylated to CA1P in a subsequent period of darkness.

and block the active site of the enzyme, preventing carbamylation and/or substrate binding. The removal of tightly bound inhibitors from the catalytic site of the carbamylated and decarbamylated forms of Rubisco requires Rubisco activase and the hydrolysis of ATP. In this way Rubisco activase ensures that the Rubisco active site is not blocked by inhibitors and so free either to become carbamylated or to participate directly in catalysis.

Rubisco inhibitors

The analysis of various 3-D structures has revealed that, after substrate binding, major structural changes occur in loop 6, at the mouth of a large subunit alpha/beta barrel, in which this loop folds or slides over the active site and loses its mobility. This closed conformation is maintained by residues of the C-terminal tail and of loops in the N-terminal domain of the partnering large subunit. In the reaction between bound (enediol) RuBP and either CO_2 or O_2, cleavage of the C2–C3 bond triggers site opening. By analogy to the high resolution structures for the Rubisco–carboxyarabinitol-1,5-bisphosphate ($E.CO_2.Mg^{2+}$.CABP) complex, a number of naturally occurring sugar phosphates that are tight binding inhibitors cause the active site of carbamylated or decarbamylated Rubisco to adopt a closed conformation and impact on Rubisco regulation (Fig. 2). Tight binding inhibitors resemble transition state intermediates of catalysis and prolonged dialysis or gel filtration are insufficient to release them from the catalytic site of purified Rubisco. Other sugar phosphates and some inorganic anions also interact with the catalytic site *in vitro* and affect carbamylation. These have been regarded as effectors and are usually competitive inhibitors with respect to RuBP at higher concentrations, but either increase (positive effectors, e.g. 6-phosphogluconate, NADPH and inorganic orthophosphate), or decrease (negative effectors, e.g. ribose-5-phosphate and fructose 6-phosphate), the extent of carbamylation at sub-saturating concentrations of CO_2 and Mg^{2+} (Hatch and Jensen, 1980; Badger and Lorimer, 1981; Jordan *et al*.,1983). Parry *et al*. (1985) showed that inorganic orthophosphate (Pi) increased the activity of wheat Rubisco without increasing the extent of carbamylation and thus revitalized the idea that allosteric effects might be involved in the response of Rubisco activity to effectors. Allosteric effects have been reported with Rubiscos from spinach (Yokota *et al*., 1992) and from cyanobacteria (Marcus and Gurevitz, 2000). A phosphate binding site on cyanobacterial Rubisco has been found that is distinct from sites binding the phosphate groups of substrate RuBP and is involved in regulation of activity (Marcus *et al*., 2005). The effectors, 6-phosphogluconate, NADPH, and Pi are of particular interest for the regulation of Rubisco *in vivo* as they are

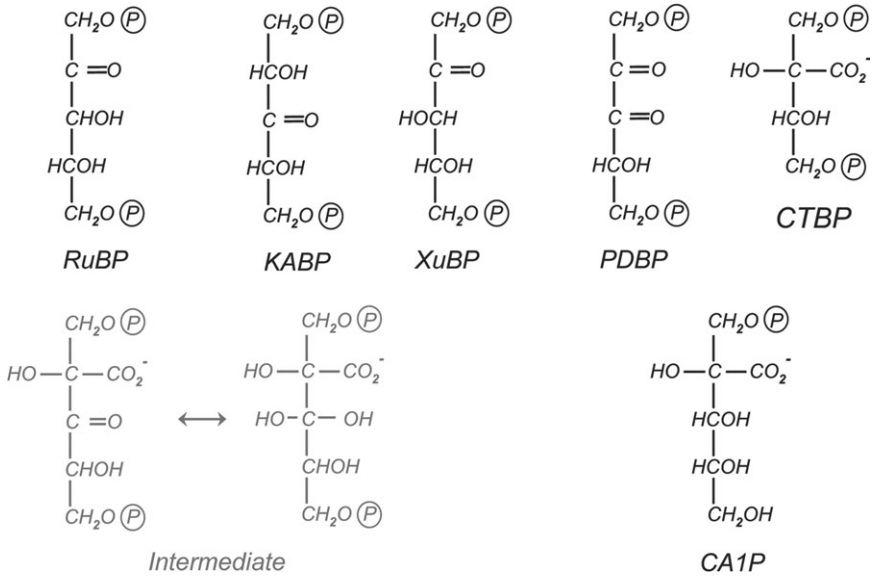

Fig. 2. Structures of naturally occurring inhibitors of Rubisco. For full explanation, see text. RuBP, ribulose-1,5-bisphosphate; KABP, 3-ketoarabinitol-1,5-bisphosphate; XuBP, D-xylulose-1,5-bisphosphate; PDBP, D-glycero-2,3-pentodiulose-1,5-bisphosphate; CTBP, 2-carboxytetritol-1,4-bisphosphate; CA1P, 2-carboxy-D-arabinitol 1-phosphate. Also shown are alternative structures of a transient intermediate of the carboxylase reaction.

present in the chloroplasts. The extent of their involvement in Rubisco regulation still needs to be fully determined. *In vitro*, sulphate ions remove such effectors, as well as tight binding inhibitors, from their respective sites of interaction (Parry *et al*., 1997) but the inclusion of modest concentrations (25–50 mM) of sulphate ions to Rubisco extraction buffers has been shown to prevent the binding of the naturally occurring inhibitor, 2-carboxy-D-arabinitol 1-phosphate (CA1P) by Rubisco, but not to cause the release of CA1P already bound to Rubisco (Moore and Seemann, 1994).

Identifying the role of tight binding inhibitors in Rubisco regulation followed the observation of a diurnal variation in the total extractable Rubisco activity (McDermitt *et al*., 1983; Vu *et al*., 1983) and of the inhibition of carbamylation by the prior (tight) binding of RuBP (Jordan and Chollet, 1983). Servaites (1985) and Seemann *et al*. (1985) demonstrated that the diurnal variation in Rubisco activity was caused by the production of a phosphorylated inhibitor in the dark that was subsequently identified as CA1P (Gutteridge *et al*., 1986; Berry *et al*., 1987).

CA1P is the best characterized of the Rubisco inhibitors. CA1P is only found in the chloroplast (Moore *et al*., 1995; Parry *et al.,* 1999a) and is formed by phosphorylation of 2-carboxy-D-arabinitol (CA) during periods of low irradiance or darkness (Moore and Seemann, 1992). Once formed CA1P binds tightly to the active site of carbamylated Rubisco. The route for the *de novo* biosynthesis of CA1P was unambiguously established by pulse-chase experiments that sequentially followed the flow of ^{14}C from newly assimilated $^{14}CO_2$ into fructose 1,6-bisphosphate followed by hamamelose bisphosphate, hamamelose monophosphate, hamamelose, and CA (Andralojc *et al*., 1994, 1996, 2002) (Fig. 3). At low irradiances or in darkness, CA is converted into CA1P (Moore and Seemann, 1992; Andralojc *et al*., 1996; Martindale *et al*., 1997). Measurements using a variety of species show that the abundance of leaf CA is greater (and in some cases by at least an order of magnitude) than the corresponding CA1P concentration (Moore *et al*., 1991, 1992; Andralojc *et al*., 1994, 2002). Whilst this may simply reflect the distribution of CA across several intracellular compartments, compared to the exclusive occurrence of CA1P in the chloroplast (Moore *et al*., 1992, 1995; Parry *et al*., 1999*a*) it is possible that these intermediates serve other purposes. (Andralojc *et al*., 2002).

The extent of carbamylation of Rubisco *in vivo* may be determined by comparing the initial activity (determined immediately following extraction) with the total activity (determined after incubation with saturating concentrations of CO_2 and Mg^{2+} to carbamylate vacant catalytic sites fully). Diurnal variation in total Rubisco activity can occur as a result of RuBP binding to decarbamylated Rubisco forming E.RuBP (Jordan and Chollet, 1983; Brooks and Portis, 1988). Diurnal variation can also occur as a result of CA1P binding to carbamylated Rubisco forming $E.CO_2.Mg^{2+}$.CA1P (Servaites *et al*., 1986).

In the light, CA1P is removed from the active site of Rubisco by Rubisco activase and then dephosphorylated by a specific CA1P phosphatase. The CA thus liberated is

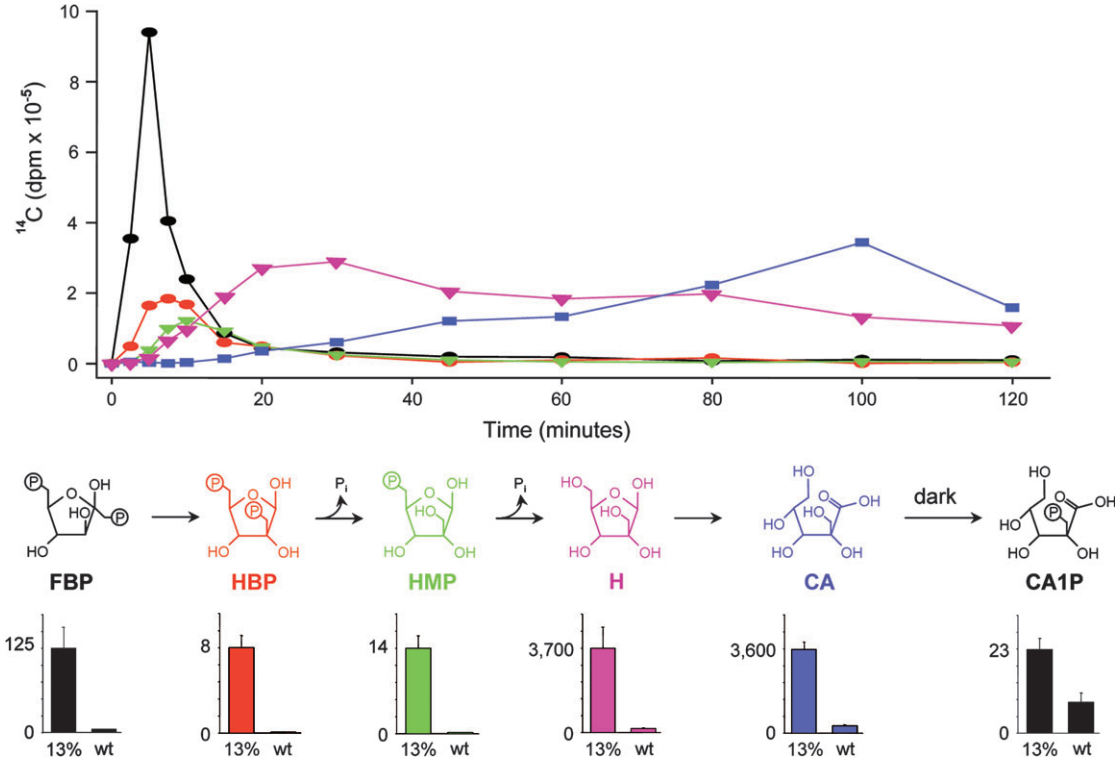

Fig. 3. Biosynthesis of CA1P. The pathway intermediates have been coloured (centre) to highlight the passage of radiolabelled carbon in a pulse-chase experiment under conditions of steady-state photosynthesis (upper) and the greater abundance of these intermediates (lower) in plants with only 13% of the wt chloroplastic fructose-1,6-bisphosphatase (FBPase) activity. Amounts (μmol m^{-2}) indicated on the ordinate axis apply to the leaves with less FBPase (Andralojc *et al*., 2002).

then available for re-phosphorylation, when the light intensity falls (Fig. 1b). The overall process of CA1P removal is light-dependent and is inhibited by DCMU or methyl viologen, which block photosynthetic electron transport (Seemann *et al*., 1985; Salvucci and Anderson, 1987). CA1P phosphatase is redox regulated, activated by NADPH (Holbrook *et al*., 1989; Kingston-Smith *et al*., 1992), DTT or glutathione (Holbrook *et al*., 1991) and reduced thioredoxin (Heo and Holbrook, 1999). The redox response is mediated by redox sensitive thiol/disuphide residues (Heo and Holbrook, 1999).

For reasons which are unclear, the amount of CA1P that accumulates during the night varies from species to species. Whilst many species (e.g. rice, soya, potato, tobacco, French bean, and tomato) contain sufficient CA1P to have a significant effect on Rubisco activity others (e.g. maize and wheat) do not (Servaites *et al*., 1986). Curiously, members of the latter group may still contain the CA1P-specific phophatase (Charlet *et al*., 1997) whilst a wide ranging survey suggests that all vascular plants contain CA (Moore *et al*., 1992, 1993). Such observations imply functions unrelated to the regulation of Rubisco or the metabolism of CA1P.

As well as the nocturnal decline, a depression in the total Rubisco activity (Keys *et al*., 1995; Hrstka *et al*., 2007) has been reported during the day and also in response to stress (Parry *et al*., 1993, 1997, 2002; Medrano *et al*., 1997) (Fig. 4). In wheat and French bean this depression in total activity was caused by a phosphorylated inhibitor, but not by CA1P or xylulose-1,5- bisphosphate (XuBP) (Keys *et al*., 1995). This phosphorylated daytime inhibitor was subsequently identified as pentadiulose-1,5-bisphosphate (PDBP) (Kane *et al*., 1998) whose formation from RuBP had previously been reported in Form II Rubisco from an autotrophic bacterium (Chen and Hartman, 1995). Products of catalytic 'misfire' arise at various points in the catalytic pathway of both carboxylation and oxygenation and are themselves potent and tight binding inhibitors. XuBP is formed by misprotonation of the initial enediol intermediate (Edmondson *et al*., 1990). Although XuBP is released from the catalytic site (Zhu *et al*., 1998) and has been shown to act as a (poor) substrate, being slowly carboxylated to form 3-phosphoglycerate by carbamylated Rubisco (Yokota, 1991) it can (like RuBP) also bind tightly to uncarbamylated Rubisco (Edmondson *et al*., 1990; Zhu and Jensen, 1991; Pearce and Andrews, 2003). 3-Ketoarabinitol-1,5-bisphosphate (KABP) has been proposed to be derived from the breakdown of an unstable peroxy intermediate of the oxygenase reaction (Zhu and Jensen, 1991; Zhu *et al*., 1998). However, it has been reported that identification of KABP as an inhibitor may be incorrect, on account of the

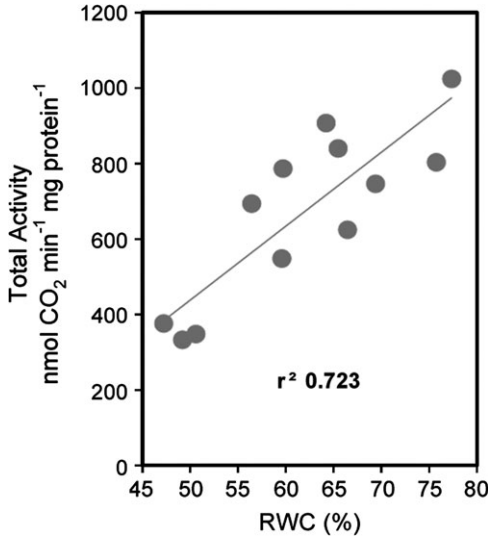

Fig. 4. The relationship between total Rubisco activity and relative water content (RWC) in field-grown tobacco plants subjected to drought stress.

stereoselective reduction of the true inhibitor in the presence of borate ions at low ionic strength. Similar analyses at high ionic strength are regarded to be unbiased and show a distribution of reduction products consistent with PDBP as the inhibitor, not KABP (Chen and Hartman, 1995, and reiterated by Pearce and Andrews, 2003; Kim and Portis, 2004). For this reason, other identifications of KABP as a physiologically relevant inhibitor, based on the products of borohydride reduction (Zhu and Jensen, 1991; Zhu *et al*., 1998) are open to question.

The 'daytime' inhibitor, pentadiulose 1,5-bisphosphate (PDBP) is formed by the elimination of H_2O_2 from a peroxyketone intermediate of the oxygenation reaction and, on average, occurs once in every 260 turnovers (Kane *et al*., 1998; Pearce and Andrews, 2003; Kim and Portis, 2004). PDBP is thought to accumulate progressively at the catalytic site causing progressive inhibition, known as 'fallover' (Robinson and Portis, 1989*a*; Edmondson *et al*., 1990; Zhu *et al*., 1998; Kim and Portis, 2004). Therefore, any conditions that favour the oxygenase reaction and photorespiration, such as stomatal closure (increasing O_2/CO_2) and high temperature (because of the increased relative solubility of O_2 compared to CO_2 and the decreased specificity of Rubisco) will lead to increased PDBP production and will promote fallover. Likewise, Rubiscos with lower specificity factors have been shown to generate more PDBP (Kim and Portis, 2004). These properties are consistent with a depression in the total Rubisco activity reported for drought-stressed wheat and tobacco (Parry *et al*., 1993, 2002). Although the production of all misfire products (including PDBP) increases with temperature, the corresponding fallover has been shown to become less severe, because at the higher temperatures the structure of Rubisco becomes more mobile so that the same inhibitors do not bind so tightly (Schrader *et al*., 2006). Whilst the enzymes from all species produce misfire products under RuBP-saturated conditions these products do not cause fallover in Form I Rubisco from cyanobacteria and red algae, or the Form II Rubisco (composed of a single large subunit dimer) from autotrophic bacteria (Pearce and Andrews, 2003; Pearce, 2006).

While the presence of PDBP is undoubtedly associated with Rubisco inactivation, 2-carboxytetritol-1,4-bisphosphate (CTBP) derived from a benzilic acid-type rearrangement (Harpel *et al*., 1995) of enzyme bound PDBP (Pearce and Andrews, 2003) is likely to be a tighter binding inhibitor than PDBP itself (Pearce and Andrews, 2003). From Fig. 2 it can be seen that CTBP closely resembles both the transition state intermediate of the carboxylase reaction and CABP, rationalizing its high affinity for Rubisco and its potency as an inhibitor.

The structural changes that occur during catalytic turnover make the protein more compact and protect Rubisco from proteolytic breakdown *in vitro* (Houtz and Mulligan, 1991; Khan *et al*., 1999). Since tight binding inhibitors cause similar structural changes involving immobilization of hitherto exposed structural elements, it is not surprising that they also protect Rubisco from degradation by exogenous and endogenous proteases. Judging by the effect of CA1P (Khan *et al*., 1999) other tight binding inhibitors may also confer protection against inactivation by active oxygen species.

Rubisco activase

The importance of Rubisco activase for complete activation of Rubisco *in vivo*, was first recognized during the analysis of an *Arabidopsis* (*rca*) mutant that was unable to survive under ambient CO_2 (Somerville *et al*., 1982). Salvucci *et al*. (1985) showed this to be due to the absence of a novel enzyme, Rubisco activase. It has subsequently been shown that Rubisco activase is essential for the activation and maintenance of Rubisco catalytic activity by promoting the removal of any tightly bound, inhibitory, sugar phosphates from the catalytic site of both the carbamylated and decarbamylated forms of Rubisco (Robinson and Portis, 1988*a*, 1989*a*; Edmondson *et al*., 1990; Wang and Portis, 1992; Mate *et al*., 1993). Rubisco activase has been detected in all plant species examined thus far and is a member of the AAA^+ super family whose members perform chaperone like functions (Spreitzer and Salvucci, 2002).

Although no stable complexes between Rubisco and Rubisco activase have yet been isolated, Rubisco activase must interact directly with Rubisco to alter the enzyme

structure, making the active site accessible for carbamylation or catalysis (Portis *et al*., 2008). Whilst early electron micrographs (Büchen-Osmond *et al*, 1992) suggested a complex between Rubisco and Rubisco activase, there is little additional direct evidence for an interaction between them (Parry *et al*., 1999*b*). However, the subunits of Rubisco activase have been cross-linked to the large subunit of Rubisco using disuccinimidyl suberate (Yokota and Tsujimoto, 1992) and the Rubisco large and small subunits were subsequently co-precipitated using Rubisco activase antisera (Zhang and Komatsu, 2000) providing evidence for direct interaction (Spreitzer and Salvucci, 2002). Interactions between Rubisco and Rubisco activase have been revealed through studies involving genetic recombination which exploited differences in compatibility between Rubisco and Rubisco activase from different species (Li *et al*., 2005). Studies have shown that the C-terminus of Rubisco activase is important in Rubisco recognition (Esau *et al*., 1998) and that, although the N-terminus is not needed for ATP hydrolysis, it is necessary for Rubisco activation. A highly conserved Rubisco activase residue Trp 16 (tobacco) or Trp 12 (spinach) is important in Rubisco recognition (van de Loo *et al*., 1996; Esau *et al*., 1996). Elucidating the mechanism by which Rubisco activase triggers inhibitor release from Rubisco remains a major challenge. However, experience with Rubisco suggests that even high resolution 3-D structures for Rubisco activase may not provide all the answers for this dynamic interaction.

In many species, including *Arabidopsis*, spinach, and rice (Wernke *et al*., 1988, 1989; Werneke *et al*., 1989; To *et al*., 1999) Rubsico activase occurs in two isoforms which result from the alternative splicing of a single gene transcript. In *Arabidopsis* the isoforms are of MW 43 kDa and 46 kDa. The additional amino acids of the larger isoform occur at the C-terminus. Activase activity is enhanced by illumination (Lan *et al*., 1992) and by electron transport through PSI which provides the basis for the light regulation of Rubisco (Campbell and Ogren, 1990). Furthermore, the ATP/ADP ratio influences the activity of Rubisco activase (Robinson and Portis, 1989*b*) and this effect is mediated by the C-terminal extension of the larger activase isoform (Shen *et al*., 1991) due to an interaction with the nucleotide-binding pocket (Wang and Portis, 2006). The report that reactivation of Rubisco in lysed spinach chloroplasts was dependent on the presence of ADP and Pi, together with illumination (Parry *et al*., 1988) and of the involvement of stromal ATP in intact chloroplasts (Robinson and Portis, 1988*b*) are both consistent with the currently held model for activase function.

In vitro, the additional residues at the C-terminus of the 46 kDa isoform of Rubisco activase render both ATPase and Rubisco activation activities responsive to a combination of the ATP/ADP ratio, and the ambient redox status. The redox response is mediated by thioredoxin-f, which, combined with dithiothreitol (DTT), make the ATP/ADP ratio much less inhibitory, causing an increase in both activities of this isoform. Conversely, a combination of thioredoxin-f and oxidized glutathione makes the ambient ATP/ADP ratio more inhibitory, suppressing activase activities (Zhang and Portis, 1999). This response invloves a redox-sensitive disulphide bond formed between two cysteine residues in the C-terminus unique to the larger isoform (Zhang and Portis, 1999). Substitution of either one of these C-terminal cysteine residues for alanine diminished the ATP/ADP sensitivity of activase (Zhang and Portis, 1999) and the light responsiveness of Rubisco activity *in vivo* (Zhang *et al*., 2002). Other manipulations using *Arabidopsis rca* plants have been described, with the introduction of either the 43 kDa or the 46 kDa isoform of Rubisco activase. Rubisco activity in plants expressing only the shorter isoform was not down-regulated following a light–dark transition, while the activity in plants expressing the larger isoform was strongly down-regulated (Zhang *et al*., 2002). *In vitro*, a ratio of Rubisco activase isoforms of 1:1 displayed between 4-fold and 6-fold changes in activity in response to redox status (Zhang and Portis, 1999; Zhang *et al*., 2002). However, assayed alone, the 43 kDa isoform was not redox sensitive. The large change in activity of a 1:1 mixture of the two isoforms in response to redox status indicates that the 46 kDa isoform allosterically regulates the activity of the 43 kDa isoform. *In vivo*, the expression of both isoforms in *rca* plants conferred a light-dependent activation of Rubisco very similar to that observed in the wild type. Wild-type *Arabidopsis* (wt) contains roughly equal amounts of both isoforms (Eckardt *et al*., 1997) and, since functional activase is believed to be multimeric, it has been proposed that the 46 kDa isoform allosterically modulates the activity of neighbouring 43 kDa isoforms in activase complexes containing both isoforms.

Manipulation of Rubisco activase expression has also provided the opportunity to examine the release of CA1P from Rubisco during light-induction of photosynthesis, since this process is slowed sufficiently in antisense Rubisco activase tobacco to be resolved from accompanying changes in the rate of CO_2 assimilation attributable both to the (rapid) onset of RuBP supply and to the (slow) activation of non-carbamylated Rubisco (Hammond *et al*., 1998). Since the dephosphorylation of CA1P subsequent to its release from Rubisco was slower than Rubisco activation, it was concluded that Rubisco activase drives the activation of CA1P-inhibited Rubisco, rather than the dephosphorylation of CA1P by the CA1P-specific phosphatase. This suggests that attempts to manipulate Rubisco activity by altering the expression of CA1P-phosphatase alone are unlikely to be successful. By contrast, a promising role for altered Rubisco activase expression in enhancing crop performance is apparent

from the correlation between greatly improved grain yield in maize grown in the field—obtained after 20 agronomic selection cycles for improvement of grain yield—and parallel increases in abundance of Rubisco activase and activity of Rubisco, both of which were increased more than 2-fold over the 40 d period following anthesis (i.e. during grain filling) relative to the initial cultivar (Martinez-Barajas *et al*., 1997).

Abiotic stress

The abundance and ease with which Rubisco can be purified, quantified, and assayed, make it an ideal model protein in studies of the effects of stress on photosynthetic metabolism. The extent to which the continual process of Rubisco protein turnover (a function of synthesis, maintenance, and degradation) represents a drain on cellular resources is uncertain, but on account of its abundance could be considerable, particularly in the presence of a relatively oxidising environment induced by stress (Feller *et al*., 2008).

In the light, under conditions which might promote heat and cold stress, an increase in active oxygen species in the chloroplast is likely to cause increased oxidative damage to thylakoid-bound and stromal proteins. Stress-induced oxidative modification of specific residues on Rubisco mark the enzyme for degradation (Mehta *et al*., 1992; Desimone *et al*., 1996; Ishida *et al*., 1999; Moreno and Spreitzer, 1999; Marin-Navarro and Moreno, 2003; Moreno *et al*., 2008). For example, the importance of cysteine 172 of the large subunit was demonstrated by creating a C172S substitution in *Chlamydomonas reinhardtii* (Moreno and Spreitzer, 1999). While growth rates under normal conditions were unchanged, the mutated Rubisco was more resistant to proteinase K at low redox potential *in vitro* and also showed delayed stress-induced degradation (by hydrogen peroxide or mannitol) *in vivo* (Moreno and Spreitzer, 1999). The additional observation that the modified Rubisco inactivation was faster than the control at elevated temperatures (40 °C and 50 °C) may have resulted either from inherent differences in the large subunit or altered interactions with Rubisco activase. Site-directed mutagenesis was also used to investigate the role of large subunit residues Cys 449 and Cys 459 in *Chlamydomonas*. When both these residues were substituted by serine, Rubisco degradation and the polymerization of the enzyme during salt stress were both increased (Marin-Navarro and Moreno, 2006).

Apart from the oxidative modifications which initiate these processes, the mechanisms involved in such accelerated Rubisco degradation remain obscure, although the process may involve association of oxidation-damaged Rubisco with chloroplast membranes (Mehta *et al*., 1992). Oxidative stress also impacts on Rubisco abundance through reduced transcript levels for the small subunit, possibly as the result of increased ethylene levels (Glick *et al*., 1995). It is becoming clear, however, that Rubisco expression is regulated by elements which also influence the regulation of other chloroplast and cytosolic enzymes. For example, in *Chlamydomonas*, translation of Rubisco large subunit transcript has been shown to be transiently arrested by active oxygen species and/or a reduction in stromal signals, resulting from high-light stress (Irihimovitch and Shapira, 2000). In addition, the drought-induced decrease of rbcS transcript is accompanied by increased expression of cytosolic glutamine synthetase (Bauer *et al*., 1997). This highlights the co-ordinated changes in expression of enzymes of primary metabolism initiated by oxidative stress.

A plausible model for Rubisco turnover in cereal leaves by Irving and Robinson (2006) was shown to describe previously published experimental data accurately (Friedrich and Huffaker, 1980; Mae *et al*., 1983, 1989; Suzuki *et al*., 2001). According to this model, control of Rubisco concentration is exerted by altering biosynthesis, with degradation being regarded as a simple first-order decay process, where the amount of Rubisco at any time (t) would be given by $[\text{Rubisco}]_t=[\text{Rubisco}]_0e^{-kt}$, where k is the first-order rate constant for degradation and $[\text{Rubisco}]_0$ is the initial concentration of Rubisco. The protection of Rubisco against proteolysis afforded by CA1P (Khan *et al*., 1999) and the variation in protease sensitivity accompanying changes in redox status (Moreno and Spreitzer, 1999; Marin-Navarro and Moreno, 2003, 2006), both imply that the rate constant for degradation cannot be fixed but must assume different values according to catalytic site occupancy or redox status. Protection of Rubisco from degradation has only been demonstrated using CA1P, but it is likely that the fallover inhibitors would have a similar effect. This may explain why transformed tobacco with reduced expression of Rubisco activase contained more Rubisco with lower catalytic activity, throughout leaf development (He *et al*., 1997), the accumulation of inhibitors at the catalytic site would confer resistance against the normal process of degradation.

Rubisco is exceptionally heat stable, since the purified, carbamylated spinach enzyme suffers no loss in activity after incubation for 1 h at 57 °C (Eckardt and Portis, 1997). In contrast, for over a decade it has been known that Rubisco activase is exceptionally heat labile: both activities of the purified enzyme (Rubisco activation and ATP hydrolysis) falling by 80% after only 5 min at 40 °C (Robinson and Portis, 1989*b*). Assayed separately, the smaller activase isoform has been shown to be considerably more heat-labile than the larger isoform, while the temperature sensitivity of a mixture containing equal amounts of each isoform resembled that of the larger isoform (Crafts-Brandner *et al*., 1997). In other words, the larger isoform confers increased thermal stability to the

smaller isoform of activase. The demonstration of increased amounts of the larger isoform during heat shock in young maize leaves suggests that it may have a protective effect *in vivo* (Jimenéz *et al*., 1995) preventing large decreases in activase activity during heat stress. However, the evidence supporting an additional hypothesis that Rubisco activase is a molecular chaperone, maintaining Rubisco activity during and after heat shock (Jimenéz *et al*., 1995) has been questioned by Eckardt and Portis (1997) who showed that Rubisco activase is likely to be totally inactivated long before the irreversible loss of Rubisco activity and that Rubisco activase did not restore the activity of heat-denatured Rubisco. The report that elevated temperatures cause the functional association between Rubisco activase and thylakoid-bound polysomes (Rokka *et al*., 2001) and that the bound activase assumed the role of a molecular chaperone, omitted to demonstrate this specific function by activase. The demonstration that such an association was more rapid for the smaller than for the larger isoform of activase (Rokka *et al*., 2001) is reminiscent of the heat-denaturation characteristics of the two activase isoforms (Crafts-Brandner *et al*., 1997), suggesting that this association may be an intermediate state of activase heat inactivation.

The relationship between the effects of high temperature on Rubisco activase and Rubisco activity *in vivo* has been investigated using leaves of cotton and wheat (Feller *et al*., 1998). The very close correlation between CO_2 assimilation and Rubisco activity over a range of temperatures from 28 °C to 45 °C (Law and Crafts-Brandner, 1999) provides further evidence that elevated temperatures inhibit Rubisco (and photosynthesis) primarliy as a result of the temperature sensitivity of Rubisco activase. Furthermore, in agreement with the correlation between Rubisco activase content and grain yield in maize (Martinez-Barajas *et al*., 1997), higher Rubisco activities, *in vitro*, were observed between 25 °C and 42 °C in the presence of higher concentrations of activase (Crafts-Brandner and Salvucci, 2000). In addition, post-transcriptional mechanisms are involved in modulating Rubisco activase gene expression in cotton and maize which may contribute to acclimation of photosynthesis to heat stress (Vargas-Suarez *et al*., 2004; DeRidder *et al*., 2006). Such studies provide compelling evidence that the activity of Rubisco is limiting to net photosynthesis at elevated temperatures and that this is determined by the activity of Rubisco activase. Indeed, when allowance was made for the decline in Rubisco activity at elevated temperatures, the kinetic properties of Rubisco accurately predicted the experimentally determined rate of photosynthesis over the same temperature range (Crafts-Brandner and Salvucci, 2000). The potential for improving Rubisco activity, and therefore photosynthesis, at elevated temperatures by engineering an increase in the abundance of activase is clear. Indeed, recent reports have confirmed that photosynthesis at elevated temperatures is increased by the introduction of *rca* variants [generated by gene shuffling (Kurek *et al*., 2007) or chimeric constructs (Portis *et al*., 2007)] with improved thermostability even at moderate temperatures. However, others (e.g. Cen and Sage, 2005; Sage and Kubien, 2007) have reported that electron transport, RuBP pool size, and the RuBP-to-PGA ratio declined with increasing temperature consistent with the hypothesis that the reduction in the activation state of Rubisco at high temperature is caused by limitation in RuBP regeneration. The reasons for the conflicting reports remain unclear, but may relate species differences and preconditioning.

Conclusion

Rubisco activity is not always poised for optimal crop productivity. The effect of naturally occurring inhibitors on catalytic activity is dependent on the prevailing activity of Rubisco activase. The production of tight binding inhibitors and the activity of activase are intricately choreographed in response to environmental changes. Manipulating Rubisco activase and the abundance of inhibitors, by targeting their synthesis or degradation, offers opportunities to modulate Rubisco activity and also to control the stability of Rubisco under stress.

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References

Andersson I. 1996. Large structures at high resolution: the 1.6 angstrom crystal structure of spinach ribulose-1,5-bisphosphate carboxylase/oxygenase complexed with 2-carboxyarabinitol bisphosphate. *Journal of Molecular Biology* **259,** 160–174.

Andersson I. 2008. Catalysis and regulation in Rubisco. *Journal of Experimental Botany* **59,** (in press).

Andralojc PJ, Dawson GW, Parry MAJ, Keys AJ. 1994. Incorporation of carbon from photosynthetic products into 2-carboxyarabinitol-1-phosphate and 2-carboxyarabinitol. *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 MAJ. 1996. Conversion of D-hamamelose into 2-carboxy-D-arabinitol and 2-carboxyarabinitol 1-phosphate in leaves of *Phaseolus vulgaris* L. *Journal of Biological Chemistry* **271,** 26803–26810.

Badger M, Lorimer GL. 1981. Interaction of sugar phosphates with the catalytic site of ribulose-1,5-bisphosphate carboxylase. *Biochemistry* **20,** 2219–2225.

Badger M, Sharkey T, von Caemmerer S. 1984. The relationship between steady-state gas exchange of bean leaves and the level of carbon reduction cycle intermediates. *Planta* **160,** 305–313.

Bauer D, Biehler K, Fock H, Carrayol E, Hirel B, Migge A, Becker TW. 1997. A role for cytosolic glutamine synthetase in the remobilization of leaf nitrogen during water stress in tomato. *Physiologia Plantarum* **99,** 241–248.

Berry JA, Lorimer GH, Pierce J, Seemann J, 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.

Brooks A, Portis AR. 1988. Protein-bound ribulose bisphosphate correlates with deactivation of ribulose bisphosphate carboxylase in leaves. *Plant Physiology* **87,** 244–249.

Büchen-Osmond C, Portis AR, Andrews TJ. 1992. Rubisco activase modifies the appearance of Rubisco in the electron microscope. *Proceedings of the International Congress on Photosynthesis*. Nagoya Dordrecht: Kluwer, 653–656.

Campbell WJ, Ogren WL. 1990. Electron transport through photosystem I stimulates light activation of ribulose bisphosphate carboxylase/oxygenase (Rubisco) by Rubisco activase. *Plant Physiology* **94,** 479–484.

Charlet T, Moore BD, Seemann JR. 1997. Carboxyarabinitol 1-phosphate phosphatase from leaves of *Phaseolus vulgaris* and other species. *Plant and Cell Physiology* **38,** 511–517.

Cen YP, Sage RF. 2005. The regulation of Rubisco activity in response to variation in temperature and atmospheric CO_2 partial pressure in sweet potato. *Plant Physiology* **139,** 979–990.

Chen YR, 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.

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.

Crafts-Brandner SJ, Van De Loo FJ, Salvucci ME. 1997. The two forms of ribulose-1,5-bisphosphate carboxylase/oxygenase activase differ in sensitivity to elevated temperature. *Plant Physiology* **114,** 439–444.

DeRidder BP, Salvucci ME. 2007. Modulation of Rubisco activase gene expression during heat stress in cotton (*Gossypium hirsutum* L.) involves post-transcriptional mechanisms. *Plant Science* **172,** 246–254.

Desimone M, Henke A, Wagner E. 1996. Oxidative stress induces partial degradation of the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase in isolated chloroplasts of barley. *Plant Physiology* **111,** 789–796.

Eckardt N, Pell E. 1995. Oxidative modification of Rubisco from potato foliage in response to ozone. *Plant Physiology and Biochemistry* **33,** 273–282.

Eckardt NA, Portis AR. 1997. Heat denaturation profiles of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and Rubisco activase and the inability of Rubisco activase to restore activity of heat-denatured Rubisco. *Plant Physiology* **113,** 243–248.

Eckardt NA, Snyder GW, Portis AR, Ogren WL. 1997. Growth and photosynthesis under high and low irradiance of *Arabidopsis thaliana* antisense mutants with reduced ribulose-1,5-bisphosphate carboxylase/oxygenase activase content. *Plant Physiology* **113,** 575–586.

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

Ellis RJ. 1979. The most abundant protein in the world. *Trends in Biochemical Science* **4,** 241–244.

Esau B, Snyder GW, Portis AR. 1996. Differential effects of N- and C-terminal deletions on the two activities of Rubisco activase. *Archives of Biochemistry and Biophysics* **326,** 100–105.

Esau B, Snyder GW, Portis AR. 1998. Activation of ribulose bisphosphate carboxylase/oxygenase (Rubisco) with chimeric activase proteins. *Photosynthesis Research* **58,** 175–181.

Feller U, Anders I, Mae T. 2008. Rubiscolytics: fate of Rubisco after its enzymatic function in a cell is terminated. *Journal of Experimental Botany* **59,** (in press).

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.

Friedrich JW, Huffaker RC. 1980. Photosynthesis, leaf resistances, and ribulose-1,5-bisphosphate carboxylase degradation in senescing barley leaves. *Plant Physiology* **65,** 1103–1107.

Geiger D, Servaites JC. 1994. Dynamics of self-regulation of photosynthetic carbon metabolism. *Plant Physiology and Biochemistry* **32,** 173–183.

Glick RE, Schlagnhaufer CD, Arteca RN, Pell EJ. 1995. Ozone-induced ethylene emission accelerates the loss of ribulose-1,5-bisphosphate carboxylase/oxygenase and nuclear-encoded mRNAs in senescing potato leaves. *Plant Physiology* **109,** 891–898.

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.

Hammond E, Andrews T, Mott K, Woodrow I. 1998*a*. Regulation of Rubisco activation in antisense plants of tobacco containing reduced levels of Rubisco activase. *The Plant Journal* **14,** 101–110.

Hammond ET, Andrews TJ, Woodrow IE. 1998*b*. Regulation of ribulose-1,5-bisphosphate carboxylase/oxygenase by carbamylation and 2-carboxyarabinitol 1-phosphate in tobacco: insights from studies of antisense plants containing reduced amounts of Rubisco activase. *Plant Physiology* **118,** 1463–1471.

Harpel MR, Chen YR, Hartman FC. 1985*b*. Stabilization and partitioning of the ribulose 1,5-bisphosphate (RuBP) carboxylase oxygenase (Rubisco) oxygenation intermediate and related side products. *FASEB Journal* **9,** a1483–a1483.

Harpel MR, Serpersu EH, Lamerdin JA, Huang ZH, Gage DA, Hartman FC. 1995*a*. Oxygenation mechanism of ribulose-bisphosphate carboxylase oxygenase: structure and origin of 2-carboxytetritol 1,4-bisphosphate, a novel O_2–dependent side product generated by a site-directed mutant. *Biochemistry* **34,** 11296–11306.

Hatch AL, Jensen RG. 1980. Regulation of ribulose-1,5-bisphosphate carboxylase from tobacco: changes in pH response and affinity for CO_2 and Mg^{2+} induced by chloroplast intermediates. *Archives of Biochemistry and Biophysics* **205,** 587–594.

He Z, von Caemmerer S, Hudson G, Price G, Badger M, Andrews TJ. 1997. Ribulose bisphosphate carboxylase/oxygenase activase deficiency delays senesence of ribulose bisphosphate carboxylase/oxygenase but progressively impairs its catalysis during tobacco leaf development. *Plant Physiology* **115,** 1569–1580.

Heo J, Holbrook GP. 1999. Regulation of 2-carboxy-D-arabinitol 1-phosphate phosphatase and interaction with thiol reagents. *Biochemical Journal* **338,** 409–416.

Holbrook GP, Bowes G, Salvucci ME. 1989. Degradation of 2-carboxyarabinitol 1-phosphate by a specific chloroplast phosphatase. *Plant Physiology* **90,** 673–678.

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

Houtz RL, Mulligan RM. 1991. Protection of tryptic-sensitive sites in the large subunit of ribulose bisphosphate carboxylase/oxygenase by catalysis. *Plant Physiology* **96,** 335–339.

Hrstka M, Urban O, Petriu E, Babak B. 2007. Diurnal regulation of ribulose-1,5-bisphosphate carboxylase/oxygenase activity and its content in Norway spruce needles. *Photosynthetica* **45,** 334–339.

Hudson GS, Evans JR, von Caemmerer S, Arvidsson YBC, Andrews TJ. 1992. Reduction of ribulose-1,5-bisphosphate carboxylase oxygenase content by antisense RNA reduces photosynthesis in transgenic tobacco plants. *Plant Physiology* **98,** 294–302.

Irihimovitch V, Shapira M. 2000. Glutathione redox potential modulated by reactive oxygen species regulates translation of Rubisco large subunit in the chloroplast. *Journal of Biological Chemistry* **275,** 16289–16295.

Irving LJ, Robinson D. 2006. A dynamic model of Rubisco turnover in cereal leaves. *New Phytologist* **169,** 493–504.

Ishida H, Makino A, Mae T. 1999. Fragmentation of the large subunit of ribulose-1,5-bisphosphate carboxylase by reactive oxygen species occurs near gly-329. *Journal of Biological Chemistry* **274,** 5222–5226.

Jimenéz ES, Medrano L, Martínez-Barajas E. 1995. Rubisco activase, a possible new member of the molecular chaperone family. *Biochemistry* **34,** 2826–2831.

Jordan D, Chollet R. 1983. Inhibition of ribulose bisphosphate carboxylase by substrate ribulose bisphosphate. *Journal of Biological Chemistry* **258,** 13752–13758.

Jordan DB, Chollet R, Ogren WL. 1983. Binding of phosphorylated effectors by active and inactive forms of ribulose-1,5-bisphosphate carboxylase. *Biochemistry* **22,** 3410–3418.

Kane H, Wilkin J, Portis AR, Andrews TJ. 1998. Potent inhibition of ribulose bisphosphate carboxylase by an oxidized impurity in ribulose 1,5-bisphosphate. *Plant Physiology* **117,** 1059–1069.

Keys AJ, Major I, Parry MAJ. 1995. Is there another player in the game of rubisco regulation. *Journal of Experimental Botany* **46,** 1245–1251.

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

Kingston-Smith AH, Major I, Parry MAJ, Keys AJ. 1992. Purification and properties of a phosphatase in French bean (*Phaseolus vulgaris* L.) leaves that hydrolyses 2′-carboxy-D-arabinitol 1-phosphate. *Biochemical Journal* **287,** 821–825.

Krapp A, Hoffman B, Schafer C, Stitt M. 1993. Regulation of the expression of *rbc*S and other photosynthetic genes by carbohydrates: a mechanism for sink regulation of photosynthesis. *The Plant Journal* **3,** 817–828.

Kurek I, Chang TK, 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.

Lan Y, Woodrow IE, Mott KA. 1992. Light-dependent changes in ribulose bisphosphate carboxylase activase activity in leaves. *Plant Physiology* **99,** 304–309.

Law RD, Crafts-Brandner SJ. 1999. Inhibition and acclimation of photosynthesis to heat stress is closely correlated with activation of ribulose-1,5-bisphosphate carboxylase/oxygenase. *Plant Physiology* **120,** 173–181.

Lea PJ, Azevedo RA. 2006. Nitrogen use efficiency. 1. Uptake of nitrogen from the soil. *Annals of Applied Biology* **149,** 243–247.

Lea PJ, Azevedo RA. 2007. Nitrogen use efficiency. 2. Amino acid metabolism. *Annals of Applied Biology* **151,** 269–275.

Li C, Salvucci ME, Portis AR. 2005. Two residues of Rubisco activase involved in recognition of the Rubisco substrate. *Journal of Biological Chemistry* **280,** 24864–24869.

Mae T, Kamei C, Funaki K, Miyadai K, Makino A, Ohira K, Ojima K. 1989. Degradation of ribulose-1,5-bisphosphate carboxylase/oxygenase in the lysates of the chloroplasts isolated mechanically from wheat (*Triticum aestivum* L.) leaves. *Plant and Cell Physiology* **30,** 193–200.

Mae T, Makino A, Ohira K. 1983. Changes in the amounts of ribulose bisphosphate carboxylase synthesized and degraded during the life-span of rice leaf (*Oryza sativa* L.). *Plant and Cell Physiology* **24,** 1079–1086.

Marcus Y, Altman-Gueta H, Finkler A, Gurevitz M. 2005. Mutagenesis at two distinct phosphate-binding sites unravels their differenctial roles in regulation of Rubisco activation and catalysis. *Journal of Bacteriology* **187,** 4222–4228.

Marcus Y, Gurevitz M. 2000. Activation of cyanobacterial RuBP-carboxylase/oxygenase is facilitated by inorganic phosphate via two independent mechanisms. *European Journal of Biochemistry* **267,** 5995–6003.

Marin-Navarro J, Moreno J. 2003. Modification of the proteolytic fragmentation pattern upon oxidation of cysteines from ribulose 1,5-bisphosphate carboxylase/oxygenase and the translocation of the enzyme to membranes during stress. *Plant. Cell and Environment* **29,** 898–908.

Marin-Navarro J, Moreno J. 2006*a*. Cysteines 449 and 459 modulate the reduction–oxidation conformational changes of ribulose 1.5-bisphosphate carboxylase/oxygenase and the translocation of the enzyme to membranes during stress. *Plant. Cell and Environment* **29,** 898–908.

Marin-Navarro J, Moreno J. 2006*b*. Cysteines 449 and 459 modulate the reduction–oxidation conformational changes of ribulose-bisphosphate carboxylase/oxygenase. *Biochemistry* **42,** 14930–14938.

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

Martínez-Barajas E, Molina-Galán J, Sánvchez De Jiménez E. 1997. Regulation of Rubisco activity during grain-fill in maize: possible role of Rubisco activase. *Journal of Agricultural Science* **128,** 155–161.

Mate CJ, Hudson GS, Von Caemmerer S, Evans JR, Andrews TJ. 1993. Reduction of ribulose bisphosphate carboxylase activase levels in tobacco (*Nicotiana tabacum*) by antisense RNA reduces ribulose bisphosphate carboxylase carbamylation and impairs photosynthesis. *Plant Physiology* **102,** 1119–1128.

McDermitt DK, Zeiher CA, Porter CA. 1983. Physiological activity of RuBP carboxylase in soybeans. In: **Randall DD, Blevins DG, Larson R, eds.** *Current topics in plant biochemistry and physiology*, Vol. 1. Columbia: University of Missouri Press, 230.

Medrano H, Parry MAJ, Socias X, Lawlor DW. 1997. Long-term water stress inactivates Rubisco in subterranean clover. *Annals of Applied Biology* **131,** 491–501.

Mehta RA, Fawcett TW, Porath D, Mattoo AK. 1992. Oxidative stress causes rapid membrane translocation and *in vivo* degradation of ribulose-1,5-bisphosphate carboxylase/oxygenase. *Journal of Biological Chemistry* **267,** 2810–2816.

Moore BD, Isidoro E, Seemann J. 1993. Distribution of 2-carboxyarabinitol among plants. *Phytochemistry* **34,** 703–707.

Moore BD, Kobza J, Seemann J. 1991. Measurement of 2-carboxyarabinitol 1-phosphate in plant leaves by isotope dilution. *Plant Physiology* **96,** 208–213.

Moore BD, Seemann J. 1992. Metabolism of 2′-carboxyarabinitol in leaves. *Plant Physiology* **99,** 1551–1555.

Moore BD, Seemann J. 1994. Evidence that 2-carboxyarabinitol 1-phosphate binds to ribulose-1,5-bisphosphate carboxylase *in vivo*. *Plant Physiology* **105,** 731–737.

Moore BD, Sharkey TD, Kobza J, Seemann J. 1992. Identification and levels of 2′-carboxyarabinitol in leaves. *Plant Physiology* **96,** 1546–1550.

Moore BD, Sharkey TD, Seemann J. 1995. Intracellular localization of CA1P and CA1P phosphatase activity in leaves of *Phaseolus vulgaris* L. *Phytochemistry* **34,** 703–707.

Moreno J, García-Murria MJ, Marín-Navarro J. 2008. Redox modulation of Rubisco conformation and activity through its cysteine residues. *Journal of Experimental Botany* **59,** (in press).

Moreno J, Spreitzer RJ. 1999. C172S substitution in the chloroplast-encoded large subunit affects stability and stress-induced turnover of ribulose-1,5-bisphosphate carboxylase/oxygenase. *Journal of Biological Chemistry* **274,** 26789–26793.

Mott J, Ogren W, O'Leary J, Berry J. 1984. Photosynthesis and ribulose 1,5-bisphosphate concentrations in intact leaves of *Xanthium strumarium* L. *Plant Physiology* **76,** 968–971.

Parry MAJ, Andralojc PJ, Khan S, Lea PJ, Keys AJ. 2002. Rubisco activity: effects of drought stress. *Annals of Botany* **89,** 833–839.

Parry MAJ, Andralojc PJ, Lowe HM, Keys AJ. 1999*a*. The localisation of 2-carboxy-D-arabinitol 1-phosphate and inhibition of Rubisco in leaves of *Phaseolus vulgaris* L. *FEBS Letters* **444,** 106–110.

Parry MAJ, Andralojc PJ, Parmar S, Keys AJ, Habash D, Paul MJ, Alred R, Quick WP, Servaites JC. 1997. Regulation of Rubisco by inhibitors in the light. *Plant, Cell and Environment* **20,** 528–534.

Parry MAJ, Delgado E, Vadell J, Keys AJ, Lawlor DW, Medrano H. 1993. Water-stress and the diurnal activity of ribulose-1,5-bisphosphate carboxylase in field-grown *Nicotiana tabacum* genotypes selected for survival at low CO_2 concentrations. *Plant Physiology and Biochemistry* **31,** 113–120.

Parry MAJ, Loveland JE, Andralojc PJ. 1999*b*. Regulation of Rubisco. In: **Bryant JA, Burrell MM, Kruger NJ, eds.** *Plant carbohydrate biochemistry*. Oxford: BIOS Scientific Publishers Ltd, 127–145.

Parry MAJ, Flexas J, Medrano H. 2005. Prospects for crop production under drought. *Annals of Applied Biology* **147,** 211–278.

Parry MAJ, Keys AJ, Foyer CH, Furbank RT, Walker DA. 1988. Regulation of ribulose-1,5-bisphosphate carboxylase activity by the activase system in lysed spinach chloroplasts. *Plant Physiology* **87,** 558–561.

Parry MAJ, Madgwick PJ, Carvahlo JFC, Andralojc PJ. 2007. Prospects for increasing photosynthesis by overcoming the limitations of Rubisco. *Journal of Agricultural Science* **145,** 31–43.

Parry MAJ, Reynolds MP. 2007. Improving resource use efficiency. *Annals of Applied Biology* **151,** 133–135.

Parry MAJ, Schmidt CNG, Cornelius MJ, Keys AJ, Millard BN, Burton S, Gutteridge S, Dyer TA, Keys AJ. 1987. Variations in properties of ribulose-1,5 bisphosphate carboxylase from species related to differences in amino acid sequences. *Journal of Experimental Botany* **38,** 1260–1271.

Parry MAJ, Schmidt CNG, Cornelius MJ, Keys AJ, Millard BN, Gutteridge S. 1985. Stimulation of ribulose bisphosphate carboxylase by inorganic orthophosphate without increase in bound-activating CO_2: co-operativity between subunits of the enzyme. *Journal of Experimental Botany* **36,** 1396–1404.

Pearce FG. 2006. Catalytic by-product formation and ligand binding by ribulose bisphosphate carboxylases from different phylogenies. *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.

Portis AR. 1992. Regulation of ribulose 1,5-bisphosphate carboxylase/oxygenase activity. *Annual Review of Plant Physiology and Plant Molecular Biology* **43,** 415–437.

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

Portis AR, Kumar A, Li C. 2007. The rate of photosynthesis remains relatively high at moderately high temperatures in *Arabidopsis thaliana rca* mutant expressing thermostable chimeric Rubisco activase. *Photosynthesis Research* **91,** 317–317.

Portis AR, Parry MAJ. 2007. Discoveries in Rubisco (ribulose 1,5-bisphosphate carboxylase/oxygenase): a historical perspective. *Photosynthesis Research* **94,** 121–143.

Portis AR, Li C, Wang D, Salvucci ME. 2008. Regulation of Rubisco activase and its interaction with Rubisco. *Journal of Experimental Botany* **59,** (in press).

Robinson SP, Portis AR. 1988*a*. Release of the nocturnal inhibitor, carboxyarabinitol-1-phosphate, from ribulose bisphosphate carboxylase/oxygenase by Rubisco activase. *FEBS Letters* **233,** 413–416.

Robinson SP, Portis AR. 1988*b*. Involvement of stromal ATP in the light activation of ribulose-1,5-bisphosphate carboxylase/oxygenase in intact isolated chloroplasts. *Plant Physiology* **86,** 293–298.

Robinson SP, Portis AR. 1989*a*. Ribulose-1,5-bisphosphate carboxylase/oxygenase activase protein prevents the *in vitro* decline in activity of ribulose-1,5-bisphosphate carboxylase/oxygenase. *Plant Physiology* **90,** 968–971.

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

Rokka A, Zhang L, Aro EM. 2001. Rubisco activase: an enzyme with a temperature-dependent dual function? *The Plant Journal* **25,** 463–471.

Sage RF, Kubien DS. 2007. The temperature response of C_3 and C_4 photosynthesis. *Plant, Cell and Environment* **30,** 1086–1106.

Sage RF, Sharkey TD, Seemann JR. 1990. Regulation of ribulose-1,5-bisphosphate carboxylase activity in response to light intensity and CO_2 in the C_3 annuals *Chenopodium album* L. and *Phaseolus vulgaris* L. *Plant Physiology* **94,** 1735–1742.

Salvucci ME, Anderson JC. 1987. Factors affecting the activation state and the level of total activity of ribulose bisphosphate carboxylase in tobacco protoplasts. *Plant Physiology* **85,** 66–71.

Salvucci ME, Portis AR, Ogren WL. 1985. A soluble chloroplast protein catalyzes ribulosebisphosphate carboxylase/oxygenase activation *in vivo*. *Photosynthesis Research* **7,** 193–201.

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.

Seemann JR, Berry JA, Freas SM, Krump MA. 1985. Regulation of ribulose bisphosphate carboxylase activity *in vivo* by a light-modulated inhibitor of catalysis. *Proceedings of the National Academy of Sciences USA* **82,** 8024–8028.

Servaites JC. 1985. Binding of a phosphorylated inhibitor to ribulose bisphosphate carboxylase/oxygenase at night. *Plant Physiology* **78,** 839–843.

Servaites JC, Parry MAJ, Gutteridge S, Keys AJ. 1986. Species variation in the predawn inhibition of ribulose bisphosphate carboxylase. *Plant Physiology* **82,** 1161–1163.

Sheen J. 1990. Metabolic repression to transcription in higher plants. *The Plant Cell* **2,** 1027–1038.

Shen JB, Orozco EM, Ogren WL. 1991. Expression of the two isoforms of spinach ribulose 1,5-bisphosphate carboxylase activase and essentiality of the conserved lysine in the consensus nucleotide-binding domain. *Journal of Biological Chemistry* **266,** 8963–8968.

Somerville CR, Portis AR, Ogren WL. 1982. A mutant of *Arabidopsis thaliana* which lacks activation of RuBP carboxylase *in vivo*. *Plant Physiology* **70,** 381–387.

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

Suzuki Y, Makino A, Mae T. 2001. Changes in the turnover of Rubisco and levels of mRNAs of *rbc*L and *rbc*S in rice leaves from emergence to senescence. *Plant. Cell and Environment* **24,** 1353–1360.

Tambussi A, Bort J, Araus JL. 2007. Water use efficiency in C_3 cereals under Mediterranean conditions: a review of physiological aspects. *Annals of Applied Biology* **150,** 307–321.

To KY, Suen DF, Chen SCG. 1999. Molecular characterisation of ribulose-1,5-bisphosphate carboxylase/oxygenase activase in rice leaves. *Planta* **209,** 66–76.

Tuberosa R, Giuliani S, Parry MAJ, Araus JL. 2007. Improving water use efficiency in Mediterranean agriculture: what limits the adoption of new technologies? *Annals of Applied Biology* **150,** 157–162.

van de Loo FJ, Salvucci ME. 1996. Activation of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) involves Rubisco activase Trp16. *Biochemistry* **35,** 8143–8148.

Vargas-Suarez M, Ayala-Ochoa A, Lozano-Franco J, Garcia-Torres I, Diaz-Quinonez A, Ortiz-Navarrete VF, Sanchez-de-Jimenez E. 2004. Rubisco activase chaperone activity is regulated by a post-translational mechanism in maize leaves. *Journal of Experimental Botany* **55,** 2533–2539.

Vu CV, Allen Jr LH, Bowes G. 1983. Effects of light and elevated atmospheric CO_2 on the ribulose bisphosphate carboxylase activity and ribulose bisphosphate level of soybean leaves. *Plant Physiology* **73,** 729–734.

Wang D, Portis AR. 2006. Increased sensitivity of oxidized large isoform of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activase to ADP inhibition is due to an interaction between its carboxyl extension and nucleotide-binding pocket. *Journal of Biological Chemistry* **281,** 25241–25249.

Wang ZY, Portis AR. 1992. Dissociation of ribulose-1,5-bisphosphate bound to ribulose-1,5-bisphosphate carboxylase/oxygenase and its enhancement by ribulose-1,5-bisphosphate carboxylase/oxygenase activase-mediated hydrolysis of ATP. *Plant Physiology* **99,** 1348–1353.

Werneke JM, Chatfield JM, Ogren WL. 1989. Alternative mRNA splicing generates the two ribulose bisphosphate carboxylase/oxygenase activase polypeptides in spinach and *Arabidopsis*. *The Plant Cell* **1,** 815–825.

Werneke JM, Zielinski R, Ogren WL. 1988. Structure and expression of spinach leaf cDNA encoding ribulose bisphosphate caboxylase/oxygenase activase. *Proceedings of the National Academy of Sciences, USA* **85,** 787–791.

Yokota A. 1991. Carboxylation and detoxification of xylulose bisphosphate by spinach ribulose bisphosphate carboxylase/oxygenase. *Plant and Cell Physiology* **32,** 755–762.

Yokota A, Higashioka M, Wadano A. 1992. Regulation of the activity of ribulose-1,5-bisphosphate carboxylase/oxygenase through cooperative binding of 6-phosphogluconate to its regulatory sites. *European Journal of Biochemistry* **208,** 547–557.

Yokota A, Tsujimoto N. 1992. Characterization of ribulose-1,5-bisphosphate carboxylase/oxygenase carrying ribulose 1,5-bisphosphate at its regulatory sites and the mechanism of interaction of this form of the enzyme with ribulose-1,5-bisphosphate carboxylase/oxygenase activase. *European Journal of Biochemistry* **204,** 901–909.

Zhang N, Kallis RP, Ewy RG, Portis AR. 2002. Light modulation of Rubisco in *Arabidopsis* requires a capacity for redox regulation of the larger Rubisco activase isoform. *Proceedings of the National Academy of Sciences, USA* **99,** 3330–3334.

Zhang N, Portis AR. 1999. Mechanism of light regulation of Rubisco: a specific role for the larger Rubisco activase isoform involving reductive activation by thioredoxin-f. *Proceedings of the National Academy of Sciences, USA* **96,** 9438–9443.

Zhang Z, Komatsu S. 2000. Molecular cloning and characterization of cDNAs encoding two isoforms of ribulose-1,5-bisphosphate carboxylase/oxygenase activase in rice (*Oryza sativa* L.). *Journal of Biochemistry* **128,** 383–389.

Zhu GH, Bohnert HJ, Jensen RG, Wildner GF. 1998. Formation of the tight-binding inhibitor, 3-ketoarabinitol-1,5-bisphosphate carboxylase/oxygenase is O_2-dependent. *Photosynthesis Research* **55,** 67–74.

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

Zhu GH, Jensen RG, Bohnert HJ, Wildner GF, Schlitter J. 1998. Dependence of catalysis and CO_2/O_2 specificity of Rubisco on the carboxy-terminus of the large subunit different temperatures. *Photosynthesis Research* **57,** 71–79.