Plant, Cell & Environment

Plant, Cell and Environment (2015) **38**, 1817–1832 doi: 10.1111/pce.12425

Review

Optimizing Rubisco and its regulation for greater resource use efficiency

Elizabete Carmo-Silva, Joanna C. Scales, Pippa J. Madgwick & Martin A. J. Parry

Plant Biology and Crop Science, Rothamsted Research, Harpenden, Herts AL5 2JQ, UK

ABSTRACT

Rubisco catalyses the carboxylation of ribulose-1,5-bisphosphate (RuBP), enabling net CO_2 assimilation in photosynthesis. The properties and regulation of Rubisco are not optimal for biomass production in current and projected future environments. Rubisco is relatively inefficient, and large amounts of the enzyme are needed to support photosynthesis, requiring large investments in nitrogen. The competing oxygenation of RuBP by Rubisco decreases photosynthetic efficiency. Additionally, Rubisco is inhibited by some sugar phosphates and depends upon interaction with Rubisco activase (Rca) to be reactivated. Rca activity is modulated by the chloroplast redox status and ADP/ATP ratios, thereby mediating Rubisco activation and photosynthetic induction in response to irradiance. The extreme thermal sensitivity of Rca compromises net CO_2 assimilation at moderately high temperatures. Given its central role in carbon assimilation, the improvement of Rubisco function and regulation is tightly linked with irradiance, nitrogen and water use efficiencies. Although past attempts have had limited success, novel technologies and an expanding knowledge base make the challenge of improving Rubisco activity in crops an achievable goal. Strategies to optimize Rubisco and its regulation are addressed in relation to their potential to improve crop resource use efficiency and climate resilience of photosynthesis.

Key-words: carbon; crop; enzyme; metabolism; productivity; Rubisco activase.

INTRODUCTION

Ribulose-1,5-bisphosphate (RuBP) carboxylase/oxygenase (Rubisco, EC 4.1.1.39) catalyses the photosynthetic assimilation of CO_2 into organic compounds. This is often the rate-limiting step in photosynthesis at the top of the canopy in field-grown crops (Spreitzer & Salvucci 2002). Although Rubisco is the most abundant protein on Earth (Ellis 1979; Raven 2013), it is characterized by a number of severe limitations. Natural and synthetic alternative carbon fixation pathways that overcome some of these limitations have been postulated (e.g. Bar-Even *et al*. 2010), yet Rubisco is still the only enzyme capable of supporting the net assimilation of carbon that leads to biomass gain. Optimizing the functionality of Rubisco has large implications regarding the improvement of plant productivity and resource use efficiency (Parry *et al*. 2007; Whitney *et al*. 2011a).

Rising global temperatures, changes in water availability and more variable weather events will adversely impact plant productivity and carbon assimilation. Environmental changes, in addition to continued population growth (e.g. http://www.census.gov/popclock/), mean that more effort is required to optimize sustainable biomass production and deliver climate-smart agriculture and forestry. It is timely for *Rubiscologists* to make full use of the enormous potential of the most advanced technologies to drive Rubisco research and deliver improved plant productivity.

Rubisco has been the subject of a considerable number of reviews over the past decades (Supporting Information Table S1). Here, we review the limitations of Rubisco, the knowledge gained through genetic engineering and through characterization of its catalytic diversity in nature, and the possibilities for optimizing photosynthesis and crop productivity by maximizing Rubisco functionality. We address the regulation of Rubisco activity as a promising strategy for maintaining CO_2 assimilation in response to variable environments. Optimal photosynthesis requires a fine balance between the activity of Rubisco and that of the Calvin cycle (Salvucci 1989; Raines 2003), and maximal plant biomass production further depends upon adequate sink capacity to use increased photoassimilates efficiently. Thus, while we concentrate on Rubisco function and regulation, we recognize that this is only one aspect of the combined efforts to improve photosynthesis and maximize plant productivity and resource use efficiency in crop and forest production systems.

RUBISCO FUNCTIONALITY AND CATALYTIC INEFFICIENCIES

Rubisco is characterized by a relatively slow catalytic turnover rate, k_{cat} (e.g. McNevin *et al*. 2006), and as a consequence, large amounts of the enzyme are required to sustain adequate photosynthetic rates. This constitutes a large investment in nitrogen and impacts upon the environmental and production costs of agriculture. The catalytic cycles initiated by Rubisco [the carboxylation and oxygenation of ribulose-1,5-bisphosphate (RuBP)] are complex and involve a number of steps and transition states (reviewed in Andersson 2008; Tcherkez 2013). Rubisco's reaction with CO_2 produces two

Correspondence: E. Carmo-Silva. E-mail: elizabete.carmo-silva @rothamsted.ac.uk

Table 1. Rubisco inefficiencies and corresponding research targets for improving its functionality and optimizing photosynthesis and plant growth

Rubisco inefficiency	Research target
Slow turnover rate (k_{cat})	Rubisco with faster k_{cat}
Oxygenase reaction	Rubisco with higher carboxylation to oxygenation ratio
Low affinity for CO_2	Rubisco with affinity comparable to that of other carboxylases, e.g. phospho*enol*pyruvate carboxylase
Inhibition by tight binding of sugar phosphates	Optimize Rubisco regulation

molecules of 3-phosphoglycerate, whereas the competing reaction with O_2 results in the formation of one molecule of 3-phosphoglycerate and one molecule of 2-phosphoglycolate. The latter enters the photorespiratory carbon oxidation cycle that leads to a net loss of assimilated CO_2, release of NH_3 and considerable consumption of energy (Keys 1986; Wingler *et al*. 2000). The CO_2-concentrating mechanisms present in cyanobacteria, algae, C_4 and CAM plants efficiently decrease the oxygenation of RuBP and therefore the proportion of photorespiration in relation to net photosynthesis (Edwards *et al*. 1985; Nobel 1991; Carmo-Silva *et al*. 2008; Hagemann *et al*. 2013; Moroney *et al*. 2013). The ratio of carboxylation to oxygenation in the presence of the two gaseous substrates, CO_2 and O_2, is described by the specificity factor (Laing *et al*. 1974): $S_{C/O} = V_cK_o/V_oK_c$, where V_c and V_o represent the maximum velocities of the carboxylase and oxygenase reactions, respectively, and K_c and K_o are the Michaelis–Menten constants for CO_2 [$K_{m(CO_2)}$ or K_c] and O_2 [$K_{m(O_2)}$ or K_o].

The inefficiencies of Rubisco and corresponding research targets for improving its functionality are summarized in Table 1. Attempts to address these targets by engineering a more efficient Rubisco have resulted in limited success (Andrews & Whitney 2003; Parry *et al*. 2007; Mueller-Cajar & Whitney 2008; Whitney *et al*. 2011a; Mueller-Cajar *et al*. 2014). An account of genetic modifications to improve Rubisco catalysis is provided in Supporting Information Table S2. Much of the engineering work has focused upon Rubisco synthesis and assembly (e.g. Kanevski & Maliga 1994; Whitney & Sharwood 2008; Feiz *et al*. 2012). The valuable information gained from these studies, combined with increasingly comprehensive genome and plastome sequence information [e.g. IWGSC (The International Wheat Genome Sequencing Consortium) 2014; Middleton *et al*. 2014] and the development of new technologies such as chloroplast transformation for crop species (Hanson *et al*. 2013), will play a crucial role in advancing engineering of improved Rubiscos.

The complete catalytic properties of Rubisco are only available for surprisingly few plant species, and mostly at a single temperature, thus the overall extent of natural diversity remains poorly understood. While much of the observed difference in catalysis of well-characterized Rubiscos is comparatively small, even this could be exploited to improve crops (e.g. Parry *et al*. 2007). Importantly, these limited studies also suggest that further useful variation is likely to occur in nature (Parry *et al*. 2007, 2013). For example, some C_3 plants that are native to arid environments have evolved Rubiscos that discriminate more strongly against O_2 (i.e. higher $S_{C/O}$; Galmés *et al*. 2005). Rubiscos from C_4 plants are generally characterized by faster rates of carboxylation but higher sensitivity to O_2 (lower $S_{C/O}$) than Rubiscos from C_3 plants (Jordan & Ogren 1983; Seemann *et al*. 1984; von Caemmerer 2000; Sage 2002; Ghannoum *et al*. 2005; Kubien *et al*. 2008; Carmo-Silva *et al*. 2010). Correlation analysis between the catalytic properties of Rubiscos from diverse origins suggests that there is a trade-off, whereby increased carboxylation turnover rate is associated with lower affinity for the CO_2 substrate (e.g. Savir *et al*. 2010). Hence, the challenge is to identify forms of Rubisco characterized by catalytic properties that maximize carboxylation rates in the chloroplast of the target crop and allow the plant to photosynthesize optimally within its environment (Galmés *et al*. 2014; Sharwood & Whitney 2014).

Ongoing research aims to characterize the natural diversity in Rubisco catalytic properties across diverse plant lineages and link this variation with specific amino acid residue changes in the large (e.g. Whitney *et al*. 2011b) and/or small subunits (e.g. Ishikawa *et al*. 2011). Recent evidence suggests that positive selection of amino acid changes occurs in adaptation to the cellular environment, which, in turn, varies in response to external environmental conditions (Galmés *et al*. 2005, 2014; Tcherkez *et al*. 2006; Kapralov & Filatov 2007; Kapralov *et al*. 2012). Structural studies have shown that the active sites of Rubisco are on the large subunits (Andersson 2008); however, the small subunits also affect catalysis (Spreitzer 2003). The catalytic properties are inherent to the amino acid sequence of each enzyme and are independent of plant age and growth conditions. However, the nuclear genome contains multiple copies of the Rubisco small subunit gene (*rbc*S) and their individual expression does change in response to development and environment (Yoon *et al*. 2001; Sawchuk *et al*. 2008). Hence, it is plausible that where these encode different *rbc*S isoforms, the catalytic properties of Rubisco may be altered.

RUBISCO REGULATORY PROPERTIES AND INTERACTIONS

Many cellular components interact with Rubisco and its *in vivo* activity is modulated by carbamylation and/or by tight binding of inhibitors. Carbamylation involves the binding of an activator CO_2 molecule to a lysine residue in the catalytic site of the enzyme (E), which is stabilized by the subsequent binding of a Mg^{2+} ion, forming a catalytically competent ternary complex (Enzyme–CO_2–Mg^{2+}; ECM). Sugar phosphates may bind to the non-carbamylated (E.X) or carbamylated (ECM.X) enzyme, blocking the active site and preventing carbamylation or catalysis. Rubisco activase (Rca) restores catalytic competence to Rubisco *in vivo* by removing the tightly bound inhibitors from the catalytic sites in an ATP-dependent manner.

A number of natural sugar phosphates have been described that bind Rubisco active sites tightly (Table 2). The

Table 2. Sugar phosphates that bind the non-carbamylated (E) or carbamylated (ECM) forms of Rubisco, inhibiting its activity

Inhibitor	Abbreviation	Rubisco form	Additional information	Reference
Ribulose-1,5-bisphosphate	RuBP	E	ECM-R, substrate; E-R, inhibitor	Jordan & Chollet (1983)
2-Carboxy-D-arabinitol 1-phosphate	CA1P	ECM	Nocturnal/Low light	Gutteridge *et al*. (1986); Berry *et al*. (1987)
D-xylulose-1,5-bisphosphate	XuBP	E, ECM	Misprotonation of enediol (ECM-R); poor substrate	Edmondson *et al*. (1990b); Zhu & Jensen (1991); Pearce & Andrews (2003)
D-glycero-2,3-pentodiulose-1,5-bisphosphate	PDBP	ECM	Oxygenation by-product	Paech *et al*. (1978); Kane *et al*. (1998)
2-Carboxytetritol-1,4-bisphosphate	CTBP	ECM	Re-arrangement of PDBP; tighter binding	Harpel *et al*. (1995); Pearce & Andrews (2003)
3-Ketoarabinitol-1,5-bisphosphate	KABP	ECM	Conflicting information. True inhibitor?	Zhu & Jensen (1991); Zhu *et al*. (1998); Pearce & Andrews (2003); Kim & Portis (2004)

substrate, RuBP, has a high affinity for the inactive, non-carbamylated form of Rubisco and acts as an efficient inhibitor of catalytic activity when Rubisco carbamylation is low (Jordan & Chollet 1983; Brooks & Portis 1988; Portis *et al*. 1995). The inhibitor 2-carboxyarabinitol-1-phosphate (CA1P; Gutteridge *et al*. 1986; Berry *et al*. 1987; Moore *et al*. 1992) is not ubiquitous throughout the plant kingdom, but inhibits Rubisco in certain species when exposed to low light or darkness (Vu *et al*. 1984; Seemann *et al*. 1985; Servaites *et al*. 1986; Holbrook *et al*. 1992; Sage & Seemann 1993). In species where CA1P is abundant, it might function to inhibit Rubisco activity in the lower canopy layers, which are exposed to limiting light levels.

During the daytime, misfire products of Rubisco catalysis also lock carbamylated active sites in unproductive forms (Keys *et al*. 1995; Parry *et al*. 1997; Pearce & Andrews 2003; Kim & Portis 2004). The most important of the inhibitors arising from catalytic misfire is likely to be D-glycero-2,3-diulose-1,5-bisphosphate (PDBP; Kane *et al*. 1998; Andralojc *et al*. 2012), which can be converted into 2-carboxytetritol-1,4-bisphosphate (Harpel *et al*. 1995; Pearce & Andrews 2003). A number of other chloroplast metabolites and some inorganic ions have also been shown to interact positively or negatively with Rubisco and regulate its activity (Hatch & Jensen 1980; Badger & Lorimer 1981; Jordan *et al*. 1983; Servaites & Geiger 1995; Parry *et al*. 2008).

Tight-binding inhibitors have been shown to protect Rubisco from proteolytic breakdown (Khan *et al*. 1999) and subsequently suggested to play an important role in preventing degradation of the enzyme under stress conditions (e.g. Parry *et al*. 2008). An oxidized chloroplast environment, as is frequently observed under stress conditions, would promote protein degradation (Moreno *et al*. 2008). If the binding of inhibitors to catalytic sites makes Rubisco less prone to proteolysis, it would help to maintain stable amounts of the protein during stress events. This is particularly relevant under transient stress, as Rubisco can be re-activated when optimal conditions are re-established, without the expense of *de novo* synthesis.

As a consequence of misfire product formation, in the presence of saturating concentrations of RuBP and CO_2, the *in vitro* activity of Rubiscos from flowering plants decreases progressively with time until a steady-state rate is reached (this progressive inhibition of Rubisco *in vitro* has been termed 'fallover'; Robinson & Portis 1989; Edmondson *et al*. 1990a). In line with its function *in vivo*, Rubisco activase counteracts fallover, both preventing and reversing the *in vitro* decline in Rubisco catalytic activity (Robinson & Portis 1989). In a similar way, CA1P phosphatase (CA1Pase) was recently shown to maintain the activity of Rubisco *in vitro* by dephosphorylating inhibitory sugar phosphates and preventing these from binding to Rubisco active sites (Andralojc *et al*. 2012).

Elevated temperatures favour oxygenation over carboxylation of RuBP (due to increased solubility of O_2 relative to CO_2 in the chloroplast stroma, and decreased specificity of Rubisco towards CO_2; Keys 1999) and promote faster overall rates of catalysis, resulting in faster formation of misfire products (Kim & Portis 2004; Salvucci & Crafts-Brandner 2004a; Schrader *et al*. 2006). However, because Rubisco also becomes more flexible at higher temperatures, sugar phosphates bind less tightly to the active sites and their spontaneous dissociation is faster (Jordan & Chollet 1983; Schrader *et al*. 2006). Overall, this means that the decline in Rubisco activity *in vitro* does not increase with temperature. Other factors, such as decreased availability of Mg^{2+} at high temperatures (Kim & Portis 2006), have also been suggested as causal factors for the inactivation of Rubisco under these conditions *in vivo*. It is possible that a negative interaction with heat-inhibited Rca (Salvucci *et al*. 2001) could contribute or even explain the negative impact of elevated temperatures on Rubisco activation state *in planta*.

Pearce (2006) compared different types of Rubisco (from algae and cyanobacteria) and argued that proteins with increased flexibility produce by-products in larger quantity, but are less prone to inhibition, due to the facilitated release of sugar phosphates from the catalytic site. Accordingly, a tobacco Rubisco enzyme in which Leu-335 has been substituted by Val

(Whitney *et al*. 1999) had a more flexible catalytic site and bound inhibitors less tightly (Pearce & Andrews 2003). This type of residue substitution may be useful for manipulating the regulation of Rubisco activity by altering the tight binding of inhibitors, provided there is no impact on the enzyme's catalytic properties. Altering the amount and properties of Rca and CA1Pase provide alternative options for modulating the capacity to regulate Rubisco catalysis.

RUBISCO'S CATALYTIC CHAPERONE

Rca belongs to the AAA+ protein superfamily (ATPases associated with a variety of cellular activities; Neuwald *et al*. 1999). It is a catalytic chaperone that uses the energy from ATP hydrolysis to remodel the conformation of Rubisco and promote the release of inhibitory sugar phosphates from active sites. The Rca holoenzyme is typically composed of a shorter redox-insensitive β-isoform and a longer α-isoform that contains a redox-sensitive C-terminal extension with two cysteine residues (Zhang & Portis 1999). Changes in the redox status and ADP/ATP ratio of the chloroplast modulate the activity of Rca, thereby mediating the regulation of Rubisco activation and net CO_2 assimilation in response to the prevailing irradiance (Salvucci *et al*. 1985; Robinson & Portis 1988; Woodrow *et al*. 1996; Mott & Woodrow 2000; Zhang *et al*. 2002; Carmo-Silva & Salvucci 2013; Scales *et al*. 2014). The activity of Rca is extremely thermally sensitive and the enzyme becomes inactive and limits net CO_2 assimilation at moderately high temperatures. Hence, Rca has become a target for optimizing irradiance use efficiency and for probing Rubisco activity to adapt to rising temperatures.

The number of Rca isoforms is not ubiquitous across the plant kingdom. Immunoblotting analyses first showed the presence of the longer α-isoform and shorter β-isoform in a number of plant species, including *Arabidopsis*, spinach, soybean, kidney bean, pea, celery, oat and barley (Salvucci *et al*. 1987). The same authors mentioned pigweed, purslane, dandelion, sorghum and crabgrass as containing both long and short isoforms. Their immunoblotting analyses also indicated that the ratio between the abundance of these isoforms was species-specific, and that in tobacco and maize, only the short Rca isoform was present. Subsequent studies have extended the list of plant species known to contain two isoforms of different lengths to include apple (Watillon *et al*. 1993), cotton (Feller *et al*. 1998; Salvucci *et al*. 2003; DeRidder & Salvucci 2007), rice (To *et al*. 1999), wheat (Law & Crafts-Brandner 2001), creosote bush and Antarctic grass (Salvucci & Crafts-Brandner 2004b), red maple (Weston *et al*. 2007), black cottonwood (Hozain *et al*. 2010), sweet potato (Xu *et al*. 2010; Jiang *et al*. 2013) and grass-leaved arrowhead (Wang *et al*. 2014).

The number and structure of *Rca* genes present in diverse plant species and the respective isoforms they encode are also variable. In many species, including *Arabidopsis* and spinach, alternative splicing of a single pre-mRNA was shown to produce either the long or the short Rca isoforms (Werneke *et al*. 1989). In barley, in addition to an *Rca* gene that is alternatively spliced and produces both isoforms, an additional gene is present that produces only the short isoform (Rundle & Zielinski 1991). In cotton and soybean, there is no evidence for alternative splicing, instead two *Rca* genes encode the long and short isoforms separately (Salvucci *et al*. 2003; Yin *et al*. 2010). Tobacco contains at least three genes, all of which encode short isoforms only (Wang *et al*. 1992; Qian & Rodermel 1993). In maize, despite the initial observations that only a short isoform gene would be present (Ayala-Ochoa *et al*. 2004), analysis of the species genome revealed the presence of a second gene encoding a long isoform (Yin *et al*. 2014). Recent evidence suggested that alternative splicing of the *Rca* gene can also produce short β-isoforms that vary in C-terminus sequence and length (DeRidder *et al*. 2012). In most cases, these differences are likely to be too small to be resolvable by electrophoresis. Thus, thorough dissection of genome and transcriptome information may reveal the presence of a more diverse range of *Rca* isoforms.

The availability of wheat genome sequence data [IWGSC (The International Wheat Genome Sequencing Consortium) 2014] enabled us to characterize the *Rca* gene structure in this important crop (Fig. 1). Two isoforms of Rca, a 46 kDa α-isoform and a 42 kDa β-isoform, have been reported to be present in non-stressed leaves of *Triticum aestivum* L. (wheat), with Rca-α representing about 12% of the total Rca pool (Law & Crafts-Brandner 2001). Using the genome database for Chinese Spring wheat (URGI, Unité de Recherche Génomique Info, Versailles, France; IWGSC 2014), two *TaRca* sequences were identified, in tandem, on chromosome 4 (long arm for A genome and short arm for B and D genomes). *TaRca1* has two exons, and the intron occurs within the chloroplast targeting sequence (TS), such that the mature protein (TaRca1-β) is encoded solely by exon 2 (Fig. 1). *TaRca2* has a total of six exons; the mature protein coding sequence starts in exon 2 and alternative splicing at the end of exon 5 results in either a stop codon (*TaRca2-*β) or splicing five bases before this point, extending the intron and allowing translation through exon 6 (*TaRca2-*α). The latter variant encodes a TaRca2-α isoform that is 37 amino acid residues longer than TaRca2-β (Fig. 2). Translation of *TaRca1* produces only a short TaRca1-β isoform.

By comparison with the predicted mature protein Rca sequences from a number of species (e.g. Werneke *et al*. 1989; Salvucci *et al*. 2003), TaRca1-β is predicted to have a chloroplast TS of 48 amino acids, resulting in a mature polypeptide of 42.7 kDa. TaRca2-β is predicted (by ChloroP; Emanuelsson *et al*. 1999) to have a chloroplast TS of 47 amino acids and to encode a mature protein of 42.2 kDa. These two isoforms cannot be separated visually on protein gels or Western blots, but are distinguishable from the longer TaRca2-α isoform, which has a predicted molecular weight of 46.0 kDa. Coding sequence for the mature forms of TaRca1-β, TaRca2-β and TaRca2-α from the B genome [the most highly expressed, as evidenced by expressed sequence tags (EST) data; Alison Huttly, personal communication] were cloned from total RNA extracted from *T. aestivum* cv. Cadenza and sequenced. [The wheat B genome *TaRca1-*β, *TaRca2-*β and *Tarca2-*α nucleotide sequences from cDNA

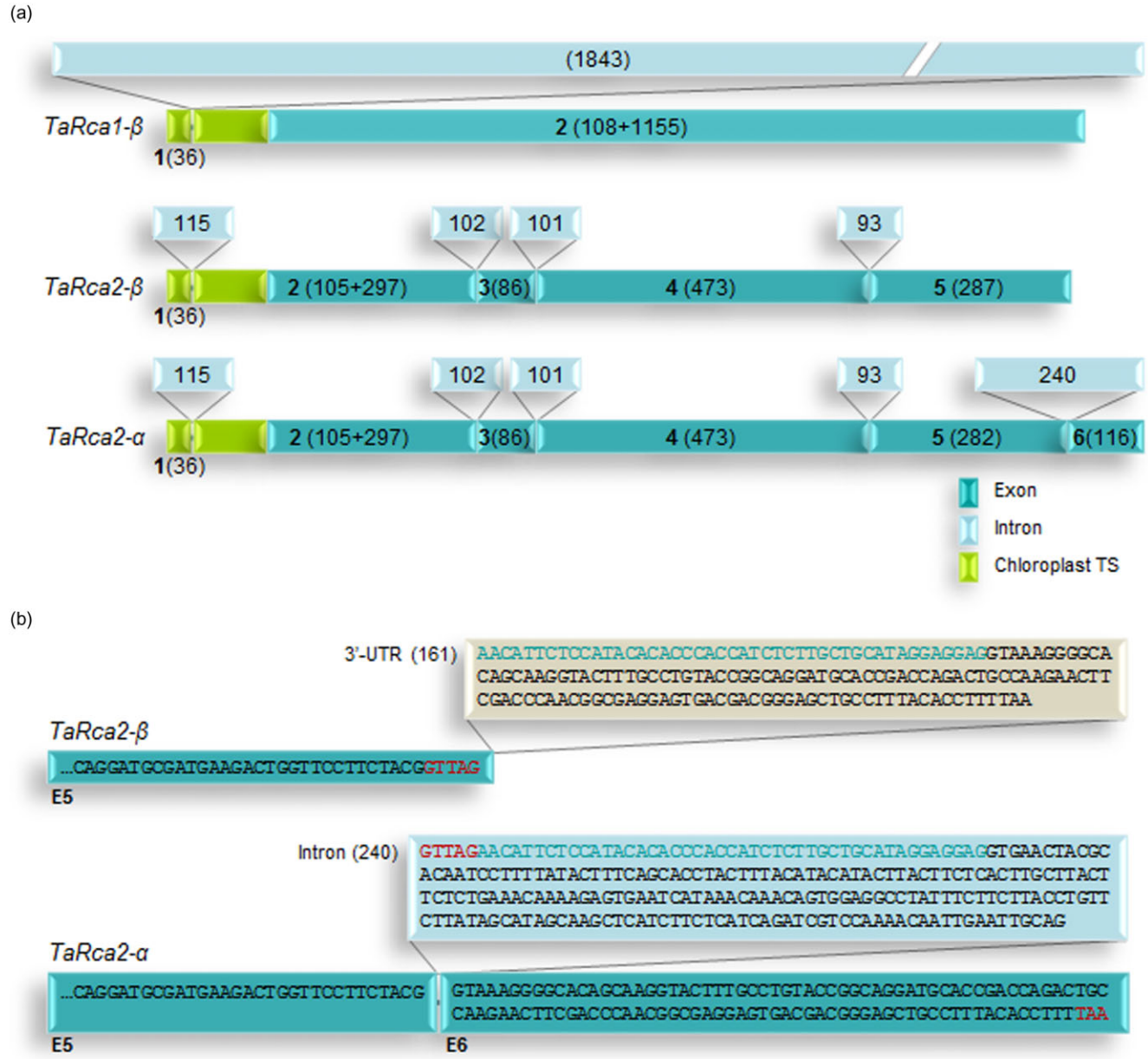

Figure 1. Wheat Rubisco activase gene structure (a) and splicing site (b). Wheat *TaRca1* encodes the short isoform TaRca1-*β*; alternative splicing of wheat *TaRca2* produces either the short TaRca2-*β* or the long TaRca2-*α* isoforms. The two genes, *TaRca1* and *TaRca2*, are consecutive in wheat chromosome 4 (long arm in genome A and short arm in genomes B and D). Wheat *TaRca1* is formed by two exons and *TaRca2* is formed by five or six exons, with the alternative splicing site at the end of exon 5. For both *TaRca1* and *TaRca2*, the first intron is in the chloroplast targeting sequence (Chloroplast TS). Exon numbers are in bold in figure (a) and annotated as E5 and E6 in (b). The number of base pairs is given in parenthesis.

cloning have been submitted to EMBL (http://www.ebi.ac.uk/ena/) and are publicly available under the accession numbers LM992844 (*TaRca1-β*), LM992845 (*TaRca2-β*) and LM992846 (*TaRca2-α*)]. The two *TaRca* genes share 83% identity in nucleotide sequence and encode *β* proteins that are 88% identical in their amino acid sequences (Fig. 2). Both coding sequences are identical to those identified within the published Chinese Spring wheat genome sequences [URGI; IWGSC (The International Wheat Genome Sequencing Consortium) 2014]. Comparison of the three wheat genomes showed a high homology (97–98%) between the nucleotide sequences encoding mature TaRca protein (Table 3). Most of these nucleotide differences are silent and the resulting amino acid sequences are 99% identical, with a maximum of four amino acids differing between the isoforms encoded by the three wheat genomes.

Given its role in the modulation of Rubisco activation and photosynthetic carbon assimilation, the gene expression, relative isoform abundance and activity of Rca must be finely regulated. Rca amounts are frequently reported to change in

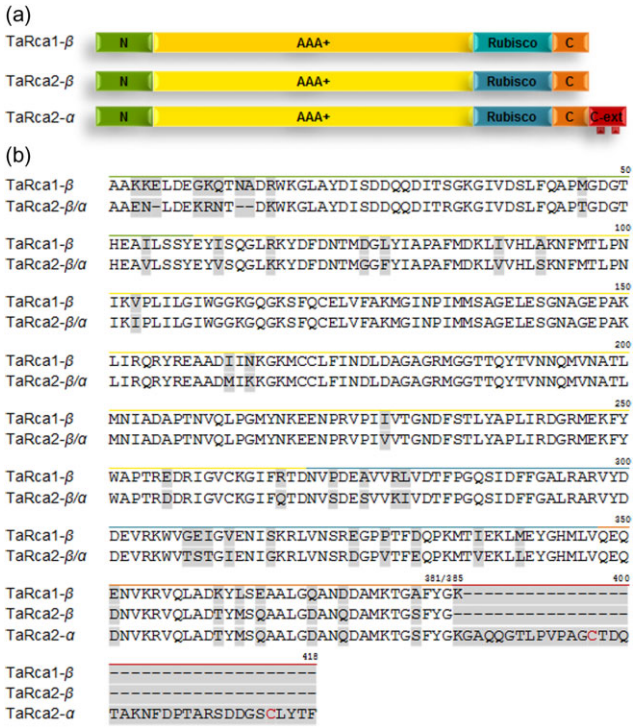

Figure 2. Protein structure (a) and amino acid sequence (b) of wheat Rubisco activase. The isoform TaRca1-β is encoded by *TaRca1*; TaRca2-β and TaRca2-α are produced by alternative splicing of *TaRca2*. Rca protein domains: N-terminal (N), AAA+ ATPase (AAA+), Rubisco recognition (Rubisco), C-terminal (C) and a 37 residue C-terminal extension (C-ext) that is present only in TaRca2-α. Coloured lines above the sequences correspond to the domains as identified in (a); shaded residues differ in TaRca2-β/α compared with TaRca1-β, red residues indicate the two C-ext cysteines that confer redox regulation.

response to a variety of stress conditions (e.g. Law & Crafts-Brandner 2001; Vassileva *et al.* 2012). Factors such as alternative splicing are likely to play a role in the post-transcriptional regulation of Rca in response to the circadian clock and growth conditions, including stress (DeRidder *et al.* 2012; Carvalho *et al.* 2013; Perez-Santángelo *et al.* 2013; Staiger & Brown 2013). Clearly, many questions on the regulation of Rca and the mechanism of interaction with Rubisco are still unanswered. Further research into the functional differences and significance of the diverse Rca isoforms is warranted and will provide valuable information for improving the efficiency and climate resilience of photosynthesis.

Table 3. Comparison of the mature coding sequence of wheat Rubisco activase (*TaRca*) genes and resulting proteins

Gene	*TaRca1-β*	*TaRca2-β*	*TaRca1-α*
Total number of nucleotides	1155	1143	1254
Nucleotide differences			
Genomes A and B	26	15	17
Genomes B and D	29	16	17
Genomes A and D	19	12	13
Protein	**TaRca1-β**	**TaRca2-β**	**TaRca1-α**
Total number of amino acids	384	380	417
Amino acid differences			
Genomes A and B	4	2	2
Genomes B and D	4	3	3
Genomes A and D	0	1	1

IMPROVEMENT OF RUBISCO AND AGRICULTURAL RESOURCE USE EFFICIENCY

Strategies to optimize Rubisco function and regulation must consider the improvement of plant resource use efficiency in current and predicted (more variable) climates. In this section, we consider not only the optimization of nitrogen and water use efficiencies but also the efficiency of using the prevailing light, as well as the overall response of carboxylation rates to fluctuations in irradiance levels and temperature. As noted earlier, optimal solutions will vary and depend upon the target crop and its growth environment. In the context of improving Rubisco function and regulation, canopy architecture also needs to be considered (Zhu *et al.* 2004; Long *et al.* 2006; Song *et al.* 2013). In light-saturated leaves, photosynthesis tends to be limited by the activity of Rubisco (A_c), whereas in light-limited leaves, photosynthesis tends to be limited by the electron transport rate (A_j). Increasing Rubisco k_{cat} in sunlit leaves would lead to an increase in CO_2 assimilation, but in shaded leaves, this would be of limited value. An increase in $S_{C/O}$ would affect the CO_2 compensation point and have an impact on A_j, thus being most favourable in leaves when RuBP regeneration is limiting (Zhu *et al.* 2004). Figure 3 shows the modelled effect on the photosynthetic response to the intercellular CO_2 concentration when wheat Rubisco is replaced by a Rubisco with superior catalytic properties. Assuming that all else would be the same, including the amount and activation state of Rubisco, the catalytic properties of the Rubisco from *Limonium gibertii* (Galmés *et al.* 2005) could significantly increase net assimilation at the CO_2 concentrations typically observed in wheat leaves. Further improvements would be achieved by a hypothetical further improvement of the $S_{C/O}$ (Fig. 3).

More efficient carboxylation by Rubisco would require less nitrogen to be invested in producing sufficient amounts of the enzyme to support photosynthesis and plant growth. Rubisco typically accounts for 30–50% of the total soluble protein (TSP) and 10–30% of the total nitrogen (N) in the leaves of C_3 species (Table 4). In the leaves of C_4 plants, the amount of Rubisco is considerably lower, with typical values ranging from 10 to 25% of the TSP and from 5 to 10% of leaf N. The percentage of leaf N allocated to Rubisco (Rubisco/N) increases in plants grown at high light compared with low light (Evans 1989; Evans & Poorter 2001). Increased Rubisco/N has also been reported in plants grown under sub- or supra-optimal temperatures (Yamori *et al.* 2005; Nagai & Makino 2009). Conversely, decreased Rubisco amounts have been observed in some species under water deficit (Parry *et al.* 2002; Tezara *et al.* 2002). In some circumstances, an

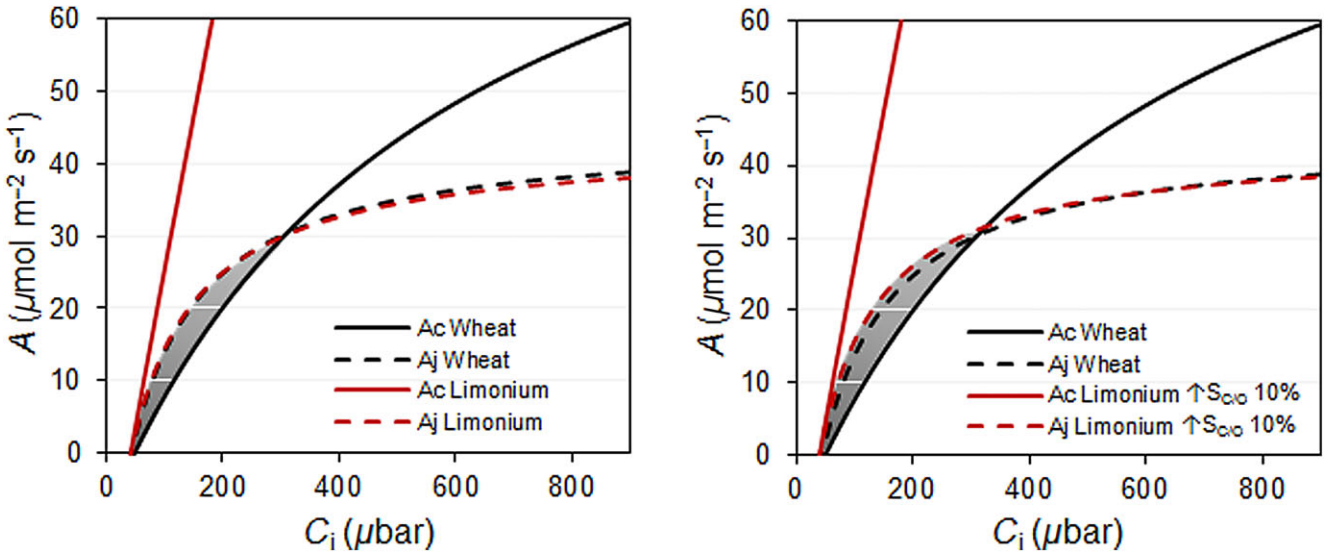

Figure 3. Modelling photosynthesis to demonstrate the benefit of replacing wheat Rubisco with Rubisco from *Limonium gibertii* at saturating irradiance. The Rubisco-limited (A_c, solid lines) and RuBP-regeneration-limited (A_j, dashed lines) rates of net CO_2 assimilation (A) were derived using the model of Farquhar *et al*. (1980) and the Rubisco kinetic constants determined for wheat (black lines; Carmo-Silva *et al*. 2010) or those determined for *L. gibertii* (red lines; Galmés *et al*. 2005). The graph on the left demonstrates the benefit of a further 10% increase in Rubisco specificity factor ($S_{C/O}$). The shaded grey area corresponds to the resulting improvement in photosynthesis. For reference, an intercellular CO_2 concentration (C_i) of about 210 μbar would be typical in leaves of field-grown wheat in the UK.

increased activation state may compensate for lower Rubisco amounts. Galmés *et al*. (2011) showed that variation exists in species adaptation to low intercellular CO_2 concentrations such that evergreen sclerophyll species maintain higher activation states, and consequently higher photosynthesis, than herbs and semi-deciduous species when exposed to low water availability. Optimizing the efficiency of RuBP carboxylation by Rubisco therefore has the potential to improve plant water use efficiency by decreasing the concentration of CO_2 required to achieve high photosynthetic rates (Parry *et al*. 2007).

Under certain environmental conditions, maximizing Rubisco activity may not be the ideal strategy to optimize photosynthesis. At low light and at high CO_2 concentrations, photosynthesis is limited by the regeneration of RuBP (von Caemmerer 2000). Plants grown at high CO_2, in free-air CO_2 enrichment experiments, typically show a decrease in Rubisco amount alongside decreased leaf N, resulting in increased photosynthetic nitrogen and water use efficiencies (PNUE & PWUE; Long *et al*. 2004; Ainsworth & Long 2005; Leakey *et al*. 2009). In plants grown at low irradiance levels, N tends to be preferentially allocated to light-harvesting components, resulting in relatively low carboxylation and photosynthetic capacities (Evans 1989). Importantly, plant species characterized by high specific leaf area (SLA) have higher PNUE at both high and low growth irradiances, and tend to allocate proportionally more N to Rubisco and have higher catalytic activity when grown at high irradiance (Poorter & Evans 1998).

In general, the amount of Rubisco in leaves increases with N supply and with leaf N content (Sage *et al*. 1987; Kumar *et al*. 2002; Table 4). When N availability is not limiting, Rubisco amounts can exceed the requirements to support photosynthesis, especially in plants exposed to low irradiance levels. However, the apparent over-investment in Rubisco is likely to provide a means of storing N (Millard 1988; Millard & Grelet 2010), which can be remobilized upon stress conditions and during senescence (Feller *et al*. 2008). Hence, Rubisco plays a central role in PNUE, affecting N storage and remobilization to seeds (Feller *et al*. 2008; Millard & Grelet 2010). Feller *et al*. (2008) provided a good schematic overview of N remobilization from vegetative to reproductive organs in maize and wheat. Strategies to optimize PNUE in crops should therefore also consider the required quality of the end product (e.g. grain protein content).

The amount and activation state of Rubisco tend to correlate negatively, as evidenced by genetic modifications to the amounts of Rubisco and Rca (Table 5). Studies with reduced Rca amounts further suggest that Rubisco activation is generally not limiting for Rubisco catalysis *in vivo* because photosynthesis is only affected when Rca amounts are reduced by more than 60%. However, most of these studies have only analysed plants grown under controlled environment conditions and the findings may not hold true for field-grown plants. The research focus is now shifting towards gaining a better understanding of plant responses to rapidly changing conditions, such as those experienced by plants in natural environments (Lawson *et al*. 2012). Rca determines the rate of photosynthetic induction following an increase in irradiance levels (Mott & Woodrow 2000). Rice plants overexpressing Rca had higher Rubisco activation states and CO_2 assimilation rates in response to varying light levels when compared to the wild type (Fukayama *et al*. 2012; Yamori *et al*. 2012). Importantly, when grown under ideal

Table 4. Rubisco amount in the leaves of flowering plants, expressed as a percentage of the leaf total soluble protein amount (Rubisco/TSP) and the leaf total nitrogen amount (Rubisco/N)

Species	Rubisco/TSP	Rubisco/N	A	g_s	Growth	Observations	Reference
10 C_3 dicots	25–42%	16–26%	17–27	–	CE	↑ Rubisco/N with growth at high light	Evans & Poorter (2001)
Soybean	*ca*. 55%	–	15–20	–	GH	Rubisco/TSP unaffected by growth [CO_2]	Campbell *et al*. (1988)
Sunflower	*ca*. 42%	–	*ca*. 20	–	GH	Rubisco/TSP unaffected by water deficit	Gimenez *et al*. (1992)
Wheat	40–58%	–	–	–	Field	–	Carmo-Silva & Andralojc (unpublished)
Arabidopsis	40%	–	14	–	CE	–	Eckardt *et al*. (1997)
Sunflower	32%	–	*ca*. 20	*ca*. 0.75	GH	↓ Rubisco/TSP under water deficit	Tezara *et al*. (2002)
Tobacco	24%	–	–	–	CE	–	Sharkey *et al*. (2001)
Tobacco	23%	–	–	–	CE	↓ Rubisco/TSP under water deficit	Parry *et al*. (2002)
Wheat	21–35%	–	–	–	GH	–	Galmés *et al*. (2014)
Barley	65–74%	–	–	–	CE	–	Ecochard *et al*. (1991)
Several C_3 species	–	9.5–28%	–	–	–	↑ Rubisco/N with N supply and light availability	Evans (1989)
Chenopodium album	–	10–27%	–	–	–	↑ Rubisco/N with N supply	Sage *et al*. (1987)
Rice	–	20–30%	10–37	–	GH	↑ Rubisco/N with N supply	Makino *et al*. (1997)
Rice	–	25–28%	15–30	–	GH	↑ Rubisco/N with N supply	Suzuki *et al*. (2007)
Rice	–	*ca*. 30%	25–30	–	CE	↑ Rubisco amount with low or high growth temperature	Nagai & Makino (2009)
Rice	–	29%	*ca*. 32	*ca*. 0.6	CE	↑ Rubisco/N with N supply	Yamori *et al*. (2011)
Rice	–	17%	–	–	GH	–	Suzuki & Makino (2012)
Wheat	–	21%	–	–	GH	–	Evans & Seemann (1984)
Wheat	–	25–30%	25–30	–	CE	↑ Rubisco amount with low or high growth temperature	Nagai & Makino (2009)
Wheat	–	21%	*ca*. 30	*ca*. 0.5	CE	↑ Rubisco/N with N supply	Yamori *et al*. (2011)
Spinach	–	14–17%	*ca*. 20	–	CE	↑ Rubisco/N with low growth temperature	Yamori *et al*. (2005)
Spinach	–	24%	*ca*. 35	*ca*. 0.45	CE	↑ Rubisco/N with N supply	Yamori *et al*. (2011)
Tobacco	–	24%	*ca*. 28	*ca*. 0.45	CE	↑ Rubisco/N with N supply	Yamori *et al*. (2011)
27 C_4 grasses	*ca*. 14%	4–8%	25–35	0.16–0.23	CE	↓ Rubisco/N with N supply	Ghannoum *et al*. (2005)
Three C_4 grasses	15–25%[a]	–	25–30	0.15–0.3	GH	Rubisco amount unaffected by water deficit[a]	Carmo-Silva *et al*. (2008)
Maize (C_4)	–	8.5%	20–50	–	–	–	Makino *et al*. (2003)
Amaranthus retroflexus (C_4)	–	5–9%	–	–	–	↑ Rubisco/N with N supply	Sage *et al*. (1987)

Reported Rubisco amounts were typically in the range of 1–5 g m^{-2} in C_3 leaves (and about 25% lower for C_4 plants). Net CO_2 assimilation (A, μmol m^{-2} s^{-1}) and stomatal conductance (g_s, mol m^{-2} s^{-1}), and observed response to growth conditions or treatments, when available, are provided for reference.

[a]Unpublished data.

CE, controlled environment; GH, glasshouse; TSP, total soluble protein.

conditions, rice plants overexpressing barley Rca had slightly lower Rubisco amounts and lower net CO_2 assimilation rates than wild-type plants, resulting in no overall net benefit for plant growth (Fukayama *et al*. 2012). Conversely, rice plants overexpressing maize Rca exhibited a higher activation state of Rubisco (and higher net CO_2 assimilation rates) at low light levels and more rapid photosynthetic induction following increases in light intensity (Yamori *et al*. 2012). Thus, Rubisco activation appears not to limit photosynthesis under optimal, steady-state conditions but, as suggested by Yamori *et al*. (2012), the enhancement of Rca capacity will improve Rubisco function and CO_2 assimilation in environments where light levels fluctuate. Results obtained with transgenic *Arabidopsis* expressing a β-isoform of Rca that is largely insensitive to the ADP/ATP ratio suggest that manipulating Rca regulatory properties has the potential to confer improved Rubisco function, photosynthetic CO_2 assimilation and plant growth under fluctuating light conditions (Carmo-Silva & Salvucci 2013).

In addition to its central role in adjusting CO_2 assimilation to the prevailing light level, Rca is very sensitive to elevated temperatures and has been shown to limit photosynthesis in some species under relatively moderate heat stress conditions (Crafts-Brandner & Salvucci 2000; Salvucci & Crafts-Brandner 2004b; Sage *et al*. 2008). Studies with *Arabidopsis* have shown that increasing the thermal stability

Table 5. Genetic engineering of Rubisco activity and regulation

Reference	Protein	Organism	Modification	Observed response
Rodermel *et al.* (1988)	rbcS	Tobacco	rbcS antisense	↓ Rubisco amount with consequent effects on plant growth
Quick *et al.* (1991)	rbcS	Tobacco	rbcS antisense	Low light: ↓ Rubisco amount 40%; ↑ Rubisco activation 65%; ↓ *A* 6%
Hudson *et al.* (1992)	rbcS	Tobacco	rbcS antisense	↓ V_i up to 82%; ↓ *A* up to 63%
Masle *et al.* (1993)	rbcS	Tobacco	rbcS antisense	↓ V_i up to 87%; ↓ growth at ambient CO_2
Furbank *et al.* (1996)	rbcS	*Flaveria bidentis*	Decreased rbcS expression (antisense)	↓ Rubisco amount correlated with photosynthesis at high light and ambient CO_2
Larson *et al.* (1997)	rbcL	*Chlamydomonas*	P89R	Altered species-specificity of Rubisco–Rca interaction
Makino *et al.* (1997)	rbcS	Rice	Decreased rbcS expression (antisense)	↓ Rubisco amount 35%; ↓ *A* 20% at ambient CO_2 but ↑ *A* 5–15% at high CO_2
Ott *et al.* (2000)	rbcL	*Chlamydomonas*	D94K	Altered species-specificity of Rubisco–Rca interaction
Mitchell *et al.* (2004)	rbcS	Wheat	Decreased rbcS expression (antisense)	↓ Rubisco amount unstable! (recovered to wild-type levels by T2 generation)
Suzuki *et al.* (2007)	rbcS	Rice	rbcS OE	↑ Rubisco amount 30%; ↓ Rubisco activation 8%; V_i unaffected
Suzuki *et al.* (2009)	rbcS	Rice	rbcS OE	Uppermost leaves: ↑ Rubisco amount; ↓ Rubisco activation; V_i unaffected
Ishikawa *et al.* (2011)	rbcS	Rice and sorghum	Chimeric enzyme: rice with some rbcS from sorghum	↑ Rubisco amount 24%; ↓ Rubisco activation slightly
Zhang *et al.* (2011)	rbcL and rbcS	Tomato and tobacco	Hybrid enzyme: rbcL replacement	↓ Rubisco amount; ↑ Rubisco activation
Wachter *et al.* (2013)	rbcL and rbcS	Chlamydomonas and tobacco/ Spinach/ Arabidopsis	Hybrid enzyme: rbcS replacement	No effect on species specificity of Rubisco activation by Rca: hybrid Rubisco still efficiently activated by Arabidopsis but not tobacco Rca
Morita *et al.* (2014)	rbcS	Rice	rbcS OE: isoform absent from leaf blades	High light: ↑ Rubisco amount; ↓ Rubisco activation
Shen *et al.* (1991)	Rca	Spinach	K169R, K169L, K169T	No Rca activity (ATP binding site)
Shen & Ogren (1992)	Rca-α/Rca-β	Spinach	K107M	↓ Rubisco activation and ATP hydrolysis in both isoforms
Shen & Ogren (1992)	Rca-α/Rca-β	Spinach	Q109E	↑ Rubisco activation in Rca-β (not in Rca-α) and ↑ Rubisco activation/ATP hydrolysis in both
Shen & Ogren (1992)	Rca-α/Rca-β	Spinach	Q109K, S112P	No Rca activity
Shen & Ogren (1992)	Rca-β	Spinach	C256S	↓ Rubisco activation and ATP hydrolysis
Mate *et al.* (1993)	Rca	Tobacco	Decreased Rca expression (antisense)	↓ Rca content 75%; ↑ Rubisco/TSP 65%; ↓ Rubisco activation and *A* ~ 50%; ↓ plant growth
Jiang *et al.* (1994)	Rca	Tobacco	Decreased Rca expression (antisense)	High light: ↓ Rca 90% resulted in modestly ↓ *A* and Rubisco activation
Salvucci & Klein (1994)	Rca	Tobacco	K247R, K247C, K247Q	↓ ATP hydrolysis 97–98% and abolished Rubisco activation
Esau *et al.* (1996)	Rca	Spinach	N-terminal truncated (12 amino acids)	↓ Rubisco activation activity by almost 100% and ↓ ATP hydrolysis by about 50%
Esau *et al.* (1996)	Rca	Spinach	C-terminal truncated (19 amino acids)	↑ Rubisco activation activity with little effect on ATP hydrolysis
Esau *et al.* (1996)	Rca	Spinach	C-terminal truncated (19 amino acids) + Q109E	↑ Rubisco activation activity/ATP hydrolysis
Mate *et al.* (1996)	Rca	Tobacco	Decreased Rca expression (antisense)	↓ Rca 95% resulted in ↓ Rubisco activation and *A*
van de Loo & Salvucci (1996)	Rca	Tobacco	N-terminal truncated (50 amino acids)	↓ Rubisco activation activity by almost 100% with no effect on ATP hydrolysis
van de Loo & Salvucci (1996)	Rca	Tobacco	W16A, W16C	↓ Rubisco activation 90% with no effect on ATP hydrolysis
van de Loo & Salvucci (1996)	Rca	Tobacco	W16F, W16Y	↓ Rubisco activation 30–50% with no effect on ATP hydrolysis
Eckardt *et al.* (1997)	Rca	Arabidopsis	Decreased Rca expression (antisense)	↓ Rca 60–70% resulted in moderately ↓ Rubisco activation, *A* and growth rates
He *et al.* (1997)	Rca	Tobacco	Decreased Rca expression (antisense)	↓ Rca 96% resulted in ↓ *A* and growth rates and delayed senescence
Esau *et al.* (1998)	Rca	Spinach/Tobacco	Chimeric enzymes: 1/4 and 3/4 of each species	Species-specificity of Rubisco–Rca interaction associated with Rca C-terminal region
Hammond *et al.* (1998)	Rca	Tobacco	Decreased Rca expression (antisense)	↓ Rca 80% resulted in ↓ rate of Rubisco activation following an increase in light intensity
Zhang & Portis (1999)	Rca-α	Arabidopsis	C-terminal deletion and site-directed mutants	C-terminal extension Cys residues (C392 and C411) lessen ADP sensitivity of Rca-α
Kallis *et al.* (2000)	Rca-β	Arabidopsis	Q111E, Q111D	↑ Rubisco activation; ↓ sensitivity to inhibition by ADP; no effect on thermostability
Sharkey *et al.* (2001)	Rca	Tobacco	Decreased Rca expression (antisense)	↓ Rca up to 100%: severely ↓ *A*, ↑ heat sensitivity and compromised recovery post-stress

Table 5. *Cont.*

Reference	Protein	Organism	Modification	Observed response
Zhang *et al.* (2002)	Rca	Arabidopsis	rwt43: *rca* transformant expressing only Rca-β	Eliminated the decrease of Rubisco activation in darkness
Zhang *et al.* (2002)	Rca	Arabidopsis	rwt46: *rca* transformant expressing only Rca-α	Similar to wild-type in response to darkness, $\downarrow A$
Kim & Portis (2005)	Rca-β and Rca-α	Arabidopsis	rwt43 and rwt46: *rca* transformants expressing only Rca-β or only Rca-α	rwt46: $\downarrow A$, $\uparrow$ heat sensitivity and compromised recovery post-stress
Li *et al.* (2005)	Rca	Tobacco	D311K, D311K/L314V	Altered species-specificity of Rubisco–Rca interaction
von Caemmerer *et al.* (2005)	Rca	*Flaveria bidentis*	Decreased Rca expression (antisense)	$\downarrow$ Rca >70% resulted in $\downarrow$ Rubisco activation & A
Jin *et al.* (2006)	Rca	Rice	Decreased Rca expression (antisense)	$\downarrow$ Rca 70%; $\uparrow$ Rubisco amount 77%; $\downarrow V_i$ 47%, $\downarrow$ A 49%
Li *et al.* (2006)	Rca	Tobacco	R241A, R244A, R296A	Eliminated Rubisco activation activity and $\downarrow$ ATP hydrolysis 90–98%
Salvucci *et al.* (2006)	Rca-β and Rca-α	Arabidopsis	rwt43 & rwt46: *rca* transformants expressing only Rca-β or only Rca-α	Rca-α more sensitive to heat stress than Rca-β ($\downarrow A$ and Rubisco activation)
Wang & Portis (2006)	Rca-α	Arabidopsis	D390A, E394A, D401A	Reduced sensitivity to inhibition of Rca activity by ADP
Kurek *et al.* (2007)	Rca-β	Arabidopsis	Thermostable isoform (gene shuffling)	$\uparrow$ Rubisco activation, A and plant growth at high temperature
Hendrickson *et al.* (2008)	Rca	*Flaveria bidentis*	Decreased Rca expression (antisense)	$\downarrow$ Rca >75% resulted in $\downarrow$ Rubisco activation & A (at both 25 and 40 °C)
Salvucci (2008)	Rca-β and Rca-α	Arabidopsis	Plants containing low amounts of Rca-β only (Δ43)	$\downarrow A$ and $\uparrow$ heat sensitivity and compromised recovery post-stress compared to wild type; association with cpn-60β
Kumar *et al.* (2009)	Rca	Arabidopsis/Tobacco	Chimeric Rca in Arabidopsis (tobacco enzyme with Rubisco-recognition domain from Arabidopsis)	$\uparrow$ Rubisco activation, A and plant growth at high temperature
Yamori & von Caemmerer (2009)	Rca	Tobacco	Decreased Rca expression (antisense)	$\downarrow$ Rca 90–95% resulted in $\downarrow$ Rubisco activation 32% and A 59% at 25 °C ($\downarrow$ Rubisco activation 41% and A 86% at 40 °C)
Barta *et al.* (2010)	Rca-β	Arabidopsis	8 amino acid S-Tag added to C-terminal	$\downarrow$ heat stability
Cai *et al.* (2010)	Rca	Tobacco	Decreased Rca expression (RNAi)	$\downarrow$ Rca 75–95% resulted in $\downarrow A$ and growth
Stotz *et al.* (2011)	Rca	Tobacco	Y361A, C-terminal truncation (ΔC360)	Eliminated Rubisco activation and ATP hydrolysis activities
Stotz *et al.* (2011)	Rca	Tobacco	W16A, N-terminal truncation (ΔN68)	Eliminated Rubisco activation activity with no effect on ATP hydrolysis
Fukayama *et al.* (2012)	Rca	Rice/Barley	OE of barley Rca in rice	$\downarrow$ Rubisco amount; $\downarrow A$; $\uparrow$ rate of photosynthetic induction by light
Yamori *et al.* (2012)	Rca	Rice/Maize	OE of maize Rca in rice	$\uparrow$ rate of photosynthetic induction and Rubisco activation by light; $\uparrow$ Rubisco activation at high temperature
Carmo-Silva & Salvucci (2013)	Rca	Arabidopsis	rwt43: *rca* transformant expressing only Rca-β	$\downarrow$ Rca sensitivity to inhibition by ADP, $\uparrow$ Rubisco activation at low light, $\uparrow$ rate of photosynthetic induction by light
Carmo-Silva & Salvucci (2013)	Rca	Arabidopsis/Tobacco	Chimeric Rca-β with tobacco sensor-2 domain in Arabidopsis background	No effect neither on Rca sensitivity to inhibition by ADP nor on Rubisco activation
Carmo-Silva & Salvucci (2013)	Rca	Tobacco	17 amino acids in sensor-2 replaced by those in Arabidopsis	$\downarrow$ Rca sensitivity to inhibition by ADP

Modifications to Rubisco large and small subunits (rbcL, rbcS) and to Rubisco activase (Rca) resulting in alteration of the respective protein amounts and isoforms, effects on the Rubisco–Rca interaction or changes in the balance between Rubisco activation and ATP hydrolysis activity of Rca. When both rbcL and rbcS were targeted and a hybrid enzyme produced, the respective organisms corresponding to rbcL and rbcS are identified, respectively. Modifications including a change of more than one residue are identified by a '/'. The observed response is relative to the wild-type proteins of the respective photosynthetic organism.
A, net CO_2 assimilation; OE, overexpression; *rca*, mutant expressing no Rca; V_i, Rubisco carboxylation activity.

of Rca improves photosynthesis and plant growth under moderate heat stress (Kurek *et al.* 2007; Kumar *et al.* 2009). The Rca from species native to warm environments was shown to be less thermally sensitive than the Rca from species native to cool environments (Crafts-Brandner & Salvucci 2000; Carmo-Silva & Salvucci 2011; Carmo-Silva *et al.* 2012). In a wild relative of rice, higher Rubisco activation state was associated with improved photosynthetic thermotolerance (Scafaro *et al.* 2012). Efforts are underway to engineer wheat plants to express cotton Rca to test the hypothesis that expression of more thermally stable forms of Rca, in combination with the native less thermally stable

forms of Rca, will broaden the temperature response of Rubisco activation and photosynthesis. Photorespiration increases with temperature, as a consequence of altered solubilities of CO_2 and O_2 and decreased $S_{C/O}$ (Keys 1999), and therefore, it is possible that increasing Rubisco activation at high temperatures may have a negative impact on photosynthetic efficiency. However, the promising results with *Arabidopsis* and rice described earlier suggest that maintaining Rubisco activity at moderately high temperatures will be beneficial. We consider that optimizing Rubisco function and regulation for a range of environmental conditions will both improve photosynthetic performance under current conditions as well as contribute to mitigating the effects of climate change on CO_2 assimilation and biomass production.

CONCLUSION

Rubisco is central to plant productivity and much research has been directed towards improving its properties. The lack of viable chloroplast transformation protocols for many crops still hinders direct manipulation of the chloroplast-encoded large subunit of Rubisco. However, the advanced technologies now available and the comprehensive knowledge on Rubisco properties, function, regulation and interactions make the challenge of improving Rubisco activity in crops an attainable goal. Improving the activation of Rubisco by Rca has the potential to enhance Rubisco activity and CO_2 assimilation under fluctuating light levels (promoted by cloud coverage, leaf shadowing, leaf angle and sunflecks) and moderately high temperatures (predicted to occur more frequently by climate change scenarios). The structural characterization of Rca (Henderson *et al*. 2011; Mueller-Cajar *et al*. 2011; Stotz *et al*. 2011) has enabled modelling of the interaction between Rubisco and Rca (Wachter *et al*. 2013). A clear understanding of this interaction is essential to fully exploit the benefits of maintaining Rubisco activity to improve photosynthesis and crop productivity. Importantly, there is no single solution for optimizing CO_2 assimilation in crops and useful solutions will need to be tailored to the intended growth environment. The delicate balance between RuBP consumption (Rubisco activity) and regeneration (Calvin cycle) (Salvucci 1989; Raines 2003) needs to be considered in attempts to optimize Rubisco function and regulation to enable greater photosynthetic resource use efficiency in current and projected climates.

ACKNOWLEDGMENTS

E.C.S. dedicates this manuscript to Mike Salvucci, a colleague and friend, on the occasion of his retirement. We thank Alison Huttly for EST data, Alfred Keys and two reviewers for their critical comments to earlier versions of the manuscript, and Doug Orr for introducing the term *Rubiscologist*. The authors' research was funded by the Biotechnology and Biological Sciences Research Council (BBSRC, UK) through the 20:20 Wheat® Institute Strategic Programme (BBSRC BB/J/00426X/1).

REFERENCES

Ainsworth E.A. & Long S.P. (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.

Andersson I. (2008) Catalysis and regulation in Rubisco. *Journal of Experimental Botany* **59,** 1555–1568.

Andralojc P.J., Madgwick P.J., Tao Y., Keys A., Ward J.L., Beale M.H., . . . Parry M.A.J. (2012) 2-Carboxy-D-arabinitol 1-phosphate (CA1P) phosphatase: evidence for a wider role in plant Rubisco regulation. *Biochemical Journal* **442,** 733–742.

Andrews T.J. & Whitney S.M. (2003) Manipulating ribulose bisphosphate carboxylase/oxygenase in the chloroplasts of higher plants. *Archives of Biochemistry and Biophysics* **414,** 159–169.

Ayala-Ochoa A., Vargas-Suárez M., Loza-Tavera H., León P., Jiménez-García L.F. & Sánchez-de-Jiménez E. (2004) In maize, two distinct ribulose 1,5-bisphosphate carboxylase/oxygenase activase transcripts have different day/night patterns of expression. *Biochimie* **86,** 439–449.

Badger M.R. & Lorimer G.H. (1981) Interaction of sugar phosphates with the catalytic site of ribulose-1,5-bisphosphate carboxylase. *Biochemistry* **20,** 2219–2225.

Bar-Even A., Noor E., Lewis N.E. & Milo R. (2010) Design and analysis of synthetic carbon fixation pathways. *Proceedings of the National Academy of Sciences of the United States of America* **107,** 8889–8894.

Barta C., Dunkle A.M., Wachter R.M. & Salvucci M.E. (2010) Structural changes associated with the acute thermal instability of Rubisco activase. *Archives of Biochemistry and Biophysics* **499,** 17–25.

Berry J.A., Lorimer G.H., Pierce J., Seemann J.R., 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 of the United States of America* **84,** 734–738.

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

von Caemmerer S. (2000) *Biochemical Models of Leaf Photosynthesis*, CSIRO Publishing, Collingwood, Australia.

von Caemmerer S., Hendrickson L., Quinn V., Vella N., Millgate A.G. & Furbank R.T. (2005) Reductions of Rubisco activase by antisense RNA in the C_4 plant *Flaveria bidentis* reduces Rubisco carbamylation and leaf photosynthesis. *Plant Physiology* **137,** 747–755.

Cai B., Zhang A., Yang Z., Lu Q., Wen X. & Lu C. (2010) Characterization of photosystem II photochemistry in transgenic tobacco plants with lowered Rubisco activase content. *Journal of Plant Physiology* **167,** 1457–1465.

Campbell W.J., Allen L.H. & Bowes G. (1988) Effects of CO_2 concentration on Rubisco activity, amount, and photosynthesis in soybean leaves. *Plant Physiology* **88,** 1310–1316.

Carmo-Silva A.E. & Salvucci M.E. (2011) The activity of Rubisco's molecular chaperone, Rubisco activase, in leaf extracts. *Photosynthesis Research* **108,** 143–155.

Carmo-Silva A.E. & Salvucci M.E. (2013) The regulatory properties of Rubisco activase differ among species and affect photosynthetic induction during light transitions. *Plant Physiology* **161,** 1645–1655.

Carmo-Silva A.E., Powers S.J., Keys A.J., Arrabaça M.C. & Parry M.A.J. (2008) Photorespiration in C_4 grasses remains slow under drought conditions. *Plant, Cell & Environment* **31,** 925–940.

Carmo-Silva A.E., Keys A.J., Andralojc P.J., Powers S.J., Arrabaça M.C. & Parry M.A.J. (2010) Rubisco activities, properties, and regulation in three different C_4 grasses under drought. *Journal of Experimental Botany* **61,** 2355–2366.

Carmo-Silva A.E., Gore M.A., Andrade-Sanchez P., French A.N., Hunsaker D.J. & Salvucci M.E. (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.

Carvalho R., Feijão C. & Duque P. (2013) On the physiological significance of alternative splicing events in higher plants. *Protoplasma* **250,** 639–650.

Crafts-Brandner S.J. & Salvucci M.E. (2000) Rubisco activase constrains the photosynthetic potential of leaves at high temperature and CO_2. *Proceedings of the National Academy of Sciences of the United States of America* **97,** 13430–13435.

DeRidder B., Shybut M., Dyle M., Kremling K.G. & Shapiro M. (2012) Changes at the 3′-untranslated region stabilize Rubisco activase transcript levels during heat stress in Arabidopsis. *Planta* **236,** 463–476.

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

Eckardt N.A., Snyder G.W., Portis A.R. Jr. & Ogren W.L. (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.

Ecochard R., Cavalie G., Nicco C., Piquemal M. & Sarrafi A. (1991) Rubisco content and specific activity in barley (*Hordeum vulgare* L.): I. Genetic variability. *Journal of Experimental Botany* **42,** 39–43.

Edmondson D.L., Badger M.R. & Andrews T.J. (1990a) A kinetic characterization of slow inactivation of ribulosebisphosphate carboxylase during catalysis. *Plant Physiology* **93,** 1376–1382.

Edmondson D.L., Kane H.J. & Andrews T.J. (1990b) Substrate isomerization inhibits ribulosebisphosphate carboxylase-oxygenase during catalysis. *FEBS Letters* **260,** 62–66.

Edwards G.E., Ku M.S.B. & Monson R.K. (1985) C_4 photosynthesis and its regulation. In *Photosynthetic Mechanisms and the Environment* (eds J. Barber & N.R. Baker), pp. 287–327. Elsevier Science Publishers B.V. (Biomedical Division), Amsterdam, the Netherlands.

Ellis R.J. (1979) The most abundant protein in the world. *Trends in Biochemical Sciences* **4,** 241–244.

Emanuelsson O., Nielsen H. & Heijne G.V. (1999) ChloroP, a neural network-based method for predicting chloroplast transit peptides and their cleavage sites. *Protein Science* **8,** 978–984.

Esau B.D., Snyder G.W. & Portis A.R. Jr. (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.D., Snyder G.W. & Portis A.R. Jr. (1998) Activation of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) with chimeric activase proteins. *Photosynthesis Research* **58,** 175–181.

Evans J. (1989) Photosynthesis and nitrogen relationships in leaves of C_3 plants. *Oecologia* **78,** 9–19.

Evans J.R. & Poorter H. (2001) Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. *Plant, Cell & Environment* **24,** 755–767.

Evans J.R. & Seemann J.R. (1984) Differences between wheat genotypes in specific activity of ribulose-1,5-bisphosphate carboxylase and the relationship to photosynthesis. *Plant Physiology* **74,** 759–765.

Farquhar G.D., von Caemmerer S. & Berry J.A. (1980) A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. *Planta* **149,** 78–90.

Feiz L., Williams-Carrier R., Wostrikoff K., Belcher S., Barkan A. & Stern D.B. (2012) Ribulose-1,5-bis-phosphate carboxylase/oxygenase accumulation factor1 is required for holoenzyme assembly in maize. *The Plant Cell* **24,** 3435–3446.

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

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,** 1615–1624.

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

Furbank R.T., Chitty J.A., von Caemmerer S. & Jenkins C.L.D. (1996) Antisense RNA inhibition of *Rbc*S gene expression reduces Rubisco level and photosynthesis in the C_4 plant *Flaveria bidentis*. *Plant Physiology* **111,** 725–734.

Galmés J., Flexas J., Keys A.J., Cifre J., Mitchell R.A.C., Madgwick P.J., . . . Parry M.A.J. (2005) Rubisco specificity factor tends to be larger in plant species from drier habitats and in species with persistent leaves. *Plant, Cell & Environment* **28,** 571–579.

Galmés J., Ribas-Carbó M., Medrano H. & Flexas J. (2011) Rubisco activity in Mediterranean species is regulated by the chloroplastic CO_2 concentration under water stress. *Journal of Experimental Botany* **62,** 653–665.

Galmés J., Kapralov M.V., Andralojc P.J., Conesa M.À., Keys A.J., Parry M.A.J. & Flexas J. (2014) Expanding knowledge of the Rubisco kinetics variability in plant species: environmental and evolutionary trends. *Plant, Cell & Environment* **37,** 1989–2001.

Ghannoum O., Evans J.R., Chow W.S., Andrews T.J., Conroy J.P. & von Caemmerer S. (2005) Faster Rubisco is the key to superior nitrogen-use efficiency in NADP-malic enzyme relative to NAD-malic enzyme C_4 grasses. *Plant Physiology* **137,** 638–650.

Gimenez C., Mitchell V.J. & Lawlor D.W. (1992) Regulation of photosynthetic rate of two sunflower hybrids under water stress. *Plant Physiology* **98,** 516–524.

Gutteridge S., Parry M.A.J., Burton S., Keys A.J., Mudd A., Feeney J., . . . Pierce J. (1986) A nocturnal inhibitor of carboxylation in leaves. *Nature* **324,** 274–276.

Hagemann M., Fernie A.R., Espie G.S., Kern R., Eisenhut M., Reumann S., . . . Weber A.P.M. (2013) Evolution of the biochemistry of the photorespiratory C_2 cycle. *Plant Biology* **15,** 639–647.

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

Hanson M.R., Gray B.N. & Ahner B.A. (2013) Chloroplast transformation for engineering of photosynthesis. *Journal of Experimental Botany* **64,** 731–742.

Harpel M.R., Serpersu E.H., Lamerdin J.A., Huang Z.-H., Gage D.A. & Hartman F.C. (1995) 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 A.L. & Jensen R.G. (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.L., von Caemmerer S., Hudson G.S., Price G.D., Badger M.R. & Andrews T.J. (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 J.N., Kuriata A.M., Fromme R., Salvucci M.E. & Wachter R.M. (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.

Hendrickson L., Sharwood R., Ludwig M., Whitney S.M., Badger M.R. & von Caemmerer S. (2008) The effects of Rubisco activase on C_4 photosynthesis and metabolism at high temperature. *Journal of Experimental Botany* **59,** 1789–1798.

Holbrook G.P., Turner J.A. & Polans N.O. (1992) Dark inhibition of ribulose-1,5-bisphosphate carboxylase oxygenase in legumes – a biosystematic study. *Photosynthesis Research* **32,** 37–44.

Hozain M.I., Salvucci M.E., Fokar M. & Holaday A.S. (2010) The differential response of photosynthesis to high temperature for a boreal and temperate *Populus* species relates to differences in Rubisco activation and Rubisco activase properties. *Tree Physiology* **30,** 32–44.

Hudson G.S., Evans J.R., von Caemmerer S., Arvidsson Y.B.C. & Andrews T.J. (1992) Reduction of ribulose-1,5-bisphosphate carboxylase/oxygenase content by antisense RNA reduces photosynthesis in transgenic tobacco plants. *Plant Physiology* **98,** 294–302.

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.

IWGSC (The International Wheat Genome Sequencing Consortium) (2014) A chromosome-based draft sequence of the hexaploid bread wheat (*Triticum aestivum*) genome. *Science* **345,** 1251788.

Jiang C.Z., Quick W.P., Alred R., Kliebenstein D. & Rodermel S.R. (1994) Antisense RNA inhibition of Rubisco activase expression. *The Plant Journal* **5,** 787–798.

Jiang Y., Wang J., Tao X. & Zhang Y. (2013) Characterization and expression of Rubisco activase genes in *Ipomoea batatas*. *Molecular Biology Reports* **40,** 6309–6321.

Jin S.-H., Hong J., Li X.-Q. & Jiang D.-A. (2006) Antisense inhibition of Rubisco activase increases Rubisco content and alters the proportion of Rubisco activase in stroma and thylakoids in chloroplasts of rice leaves. *Annals of Botany* **97,** 739–744.

Jordan D.B. & Chollet R. (1983) Inhibition of ribulose bisphosphate carboxylase by substrate ribulose 1,5-bisphosphate. *Journal of Biological Chemistry* **258,** 13752–13758.

Jordan D.B. & Ogren W.L. (1983) Species variation in the kinetic properties of ribulose-1,5-bisphosphate carboxylase oxygenase. *Archives of Biochemistry and Biophysics* **227,** 425–433.

Jordan D.B., Chollet R. & Ogren W.L. (1983) Binding of phosphorylated effectors by active and inactive forms of ribulose-1,5-bisphosphate carboxylase. *Biochemistry* **22,** 3410–3418.

Kallis R.P., Ewy R.G. & Portis A.R. Jr. (2000) Alteration of the adenine nucleotide response and increased Rubisco activation activity of Arabidopsis Rubisco activase by site-directed mutagenesis. *Plant Physiology* **123,** 1077–1086.

Kane H.J., Wilkin J.M., Portis A.R. Jr. & Andrews T.J. (1998) Potent inhibition of ribulose-bisphosphate carboxylase by an oxidized impurity in ribulose-1,5-bisphosphate. *Plant Physiology* **117,** 1059–1069.

Kanevski I. & Maliga P. (1994) Relocation of the plastid *rbcL* gene to the nucleus yields functional ribulose-1,5-bisphosphate carboxylase in tobacco chloroplasts. *Proceedings of the National Academy of Sciences of the United States of America* **91,** 1969–1973.

Kapralov M. & Filatov D. (2007) Widespread positive selection in the photosynthetic Rubisco enzyme. *BMC Evolutionary Biology* **7,** 1–10.

Kapralov M.V., Smith J.A.C. & Filatov D.A. (2012) Rubisco evolution in C_4 eudicots: an analysis of Amaranthaceae *sensu lato*. *PLoS ONE* **7,** e52974.

Keys A.J. (1986) Rubisco – its role in photorespiration. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **313,** 325–336.

Keys A.J. (1999) Biochemistry of photorespiration and the consequences for plant performance. In *Plant Carbohydrate Biochemistry* (eds J.A. Bryant, M.M. Burrell & N.J. Kruger), pp. 147–162. BIOS Scientific Publishers, Oxford, UK.

Keys A.J., Major I. & Parry M.A.J. (1995) Is there another player in the game of Rubisco regulation? *Journal of Experimental Botany* **46,** 1245–1251.

Khan S., Andralojc P.J., Lea P.J. & Parry M.A.J. (1999) 2-Carboxy-D-arabitinol 1-phosphate protects ribulose 1,5-bisphosphate carboxylase/oxygenase against proteolytic breakdown. *European Journal of Biochemistry* **266,** 840–847.

Kim K. & Portis A.R. Jr. (2004) Oxygen-dependent H_2O_2 production by Rubisco. *FEBS Letters* **571,** 124–128.

Kim K. & Portis A.R. Jr. (2005) Temperature dependence of photosynthesis in Arabidopsis plants with modifications in Rubisco activase and membrane fluidity. *Plant & Cell Physiology* **46,** 522–530.

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

Kubien D.S., Whitney S.M., Moore P.V. & Jesson L.K. (2008) The biochemistry of Rubisco in *Flaveria*. *Journal of Experimental Botany* **59,** 1767–1777.

Kumar A., Li C. & Portis A.R. 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.

Kumar P.A., Parry M.J., Mitchell R.C., Ahmad A. & Abrol Y. (2002) Photosynthesis and nitrogen-use efficiency. In *Photosynthetic Nitrogen Assimilation and Associated Carbon and Respiratory Metabolism* (eds C.H. Foyer & G. Noctor), pp. 23–34. Kluwer Academic Publishers, Dordrecht, the Netherlands.

Kurek I., Thom K.C., Bertain S.M., Madrigal A., Liu L., Lassner M.W. & 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 W.A., Ogren W.L. & Hageman R.H. (1974) Regulation of soybean net photosynthetic CO_2 fixation by interaction of CO_2, O_2 and ribulose 1,5-diphosphate carboxylase. *Plant Physiology* **54,** 678–685.

Larson E.M., O'Brien C.M., Zhu G., Spreitzer R.J. & Portis A.R. Jr. (1997) Specificity for activase is changed by a Pro-89 to Arg substitution in the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase. *Journal of Biological Chemistry* **272,** 17033–17037.

Law R.D. & Crafts-Brandner S.J. (2001) High temperature stress increases the expression of wheat leaf ribulose-1,5-bisphosphate carboxylase/oxygenase activase protein. *Archives of Biochemistry and Biophysics* **386,** 261–267.

Lawson T., Kramer D.M. & Raines C.A. (2012) Improving yield by exploiting mechanisms underlying natural variation of photosynthesis. *Current Opinion in Biotechnology* **23,** 215–220.

Leakey A.D.B., Ainsworth E.A., Bernacchi C.J., Rogers A., Long S.P. & Ort D.R. (2009) Elevated CO_2 effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. *Journal of Experimental Botany* **60,** 2859–2876.

Li C., Wang D. & Portis A.R. Jr. (2006) Identification of critical arginine residues in the functioning of Rubisco activase. *Archives of Biochemistry and Biophysics* **450,** 176–182.

Li C.H., Salvucci M.E. & Portis A.R. Jr. (2005) Two residues of Rubisco activase involved in recognition of the Rubisco substrate. *Journal of Biological Chemistry* **280,** 24864–24869.

Long S.P., Ainsworth E.A., Rogers A. & Ort D.R. (2004) Rising atmospheric carbon dioxide: plants FACE the future. *Annual Review of Plant Biology* **55,** 591–628.

Long S.P., Zhu X.G., Naidu S.L. & Ort D.R. (2006) Can improvement in photosynthesis increase crop yields? *Plant, Cell & Environment* **29,** 315–330.

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

McNevin D., von Caemmerer S. & Farquhar G.D. (2006) Determining Rubisco activation kinetics and other rate and equilibrium constants by simultaneous multiple non-linear regression of a kinetic model. *Journal of Experimental Botany* **57,** 3883–3900.

Makino A., Shimada T., Takumi S., Kaneko K., Matsuoka M., Shimamoto K., ... Yamamoto N. (1997) Does decrease in ribulose-1,5-bisphosphate carboxylase by antisense *Rbc*S lead to a higher N-use efficiency of photosynthesis under conditions of saturating CO_2 and light in rice plants? *Plant Physiology* **114,** 483–491.

Makino A., Sakuma H., Sudo E. & Mae T. (2003) Differences between maize and rice in N-use efficiency for photosynthesis and protein allocation. *Plant & Cell Physiology* **44,** 952–956.

Masle J., Hudson G.S. & Badger M.R. (1993) Effects of ambient CO_2 concentration on growth and nitrogen use in tobacco (*Nicotiana tabacum*) plants transformed with an antisense gene to the small subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase. *Plant Physiology* **103,** 1075–1088.

Mate C.J., Hudson G.S., von Caemmerer S., Evans J.R. & Andrews T.J. (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.

Mate C.J., von Caemmerer S., Evans J.R., Hudson G.S. & Andrews T. (1996) The relationship between CO_2-assimilation rate, Rubisco carbamylation and Rubisco activase content in activase-deficient transgenic tobacco suggests a simple model of activase action. *Planta* **198,** 604–613.

Middleton C.P., Senerchia N., Stein N., Akhunov E.D., Keller B., Wicker T. & Kilian B. (2014) Sequencing of chloroplast genomes from wheat, barley, rye and their relatives provides a detailed insight into the evolution of the Triticeae tribe. *PLoS ONE* **9,** e85761.

Millard P. (1988) The accumulation and storage of nitrogen by herbaceous plants. *Plant, Cell & Environment* **11,** 1–8.

Millard P. & Grelet G.-A. (2010) Nitrogen storage and remobilization by trees: ecophysiological relevance in a changing world. *Tree Physiology* **30,** 1083–1095.

Mitchell R.A.C., Joyce P.A., Rong H., Evans V.J., Madgwick P.J. & Parry M.A.J. (2004) Loss of decreased-Rubisco phenotype between generations of wheat transformed with antisense and sense *rbc*S. *Annals of Applied Biology* **145,** 209–216.

Moore B.D., Sharkey T.D., Kobza J. & Seemann J.R. (1992) Identification and levels of 2′-carboxyarabinitol in leaves. *Plant Physiology* **99,** 1546–1550.

Moreno J., García-Murria M.J. & Marín-Navarro J. (2008) Redox modulation of Rubisco conformation and activity through its cysteine residues. *Journal of Experimental Botany* **59,** 1605–1614.

Morita K., Hatanaka T., Misoo S. & Fukayama H. (2014) Unusual small subunit that is not expressed in photosynthetic cells alters the catalytic properties of Rubisco in rice. *Plant Physiology* **164,** 69–79.

Moroney J., Jungnick N., DiMario R. & Longstreth D. (2013) Photorespiration and carbon concentrating mechanisms: two adaptations to high O_2, low CO_2 conditions. *Photosynthesis Research* **117,** 121–131.

Mott K.A. & Woodrow I.E. (2000) Modelling the role of Rubisco activase in limiting non-steady-state photosynthesis. *Journal of Experimental Botany* **51,** 399–406.

Mueller-Cajar O. & Whitney S.M. (2008) 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., Stotz M., Wendler P., Hartl F.U., 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., Stotz M. & Bracher A. (2014) Maintaining photosynthetic CO_2 fixation via protein remodelling: the Rubisco activases. *Photosynthesis Research* **119,** 191–201.

Nagai T. & Makino A. (2009) Differences between rice and wheat in temperature responses of photosynthesis and plant growth. *Plant & Cell Physiology* **50,** 744–755.

Neuwald A.F., Aravind L., Spouge J.L. & Koonin E.V. (1999) AAA^+: a class of chaperone-like ATPases associated with the assembly, operation, and disassembly of protein complexes. *Genome Research* **9,** 27–43.

Nobel P.S. (1991) Achievable productivities of certain CAM plants: basis for high values compared with C_3 and C_4 plants. *New Phytologist* **119,** 183–205.

Ott C.M., Smith B.D., Portis A.R. Jr. & Spreitzer R.J. (2000) Activase region on chloroplast ribulose-1,5-bisphosphate carboxylase/oxygenase: nonconservative substitution in the large subunit alters species specificity of protein interaction. *Journal of Biological Chemistry* **275,** 26241–26244.

Paech C., Pierce J., McCurry S.D. & Tolbert N.E. (1978) Inhibition of ribulose-1,5-bisphosphate carboxylase/oxygenase by ribulose-1,5-bisphosphate epimerization and degradation products. *Biochemical and Biophysical Research Communications* **83,** 1084–1092.

Parry M.A.J., Andralojc P.J., Parmar S., Keys A.J., Habash D., Paul M.J., . . . Servaites J.C. (1997) Regulation of Rubisco by inhibitors in the light. *Plant, Cell & Environment* **20,** 528–534.

Parry M.A.J., Andralojc P.J., Khan S., Lea P.J. & Keys A.J. (2002) Rubisco activity: effects of drought stress. *Annals of Botany* **89,** 833–839.

Parry M.A.J., Madgwick P.J., Carvalho J.F.C. & Andralojc P.J. (2007) Prospects for increasing photosynthesis by overcoming the limitations of Rubisco. *Journal of Agricultural Science* **145,** 31–43.

Parry M.A.J., Keys A.J., Madgwick P.J., Carmo-Silva A.E. & Andralojc P.J. (2008) Rubisco regulation: a role for inhibitors. *Journal of Experimental Botany* **59,** 1569–1580.

Parry M.A.J., Andralojc P.J., Scales J.C., Salvucci M.E., Carmo-Silva A.E., Alonso H. & Whitney S.M. (2013) Rubisco activity and regulation as targets for crop improvement. *Journal of Experimental Botany* **64,** 717–730.

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

Pearce F.G. & Andrews T.J. (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.

Perez-Santángelo S., Schlaen R.G. & Yanovsky M.J. (2013) Genomic analysis reveals novel connections between alternative splicing and circadian regulatory networks. *Briefings in Functional Genomics* **12,** 13–24.

Poorter H. & Evans J.R. (1998) Photosynthetic nitrogen-use efficiency of species that differ inherently in specific leaf area. *Oecologia* **116,** 26–37.

Portis A.R. Jr., Lilley R.M. & Andrews T.J. (1995) Subsaturating ribulose-1,5-bisphosphate concentration promotes inactivation of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) – studies using continuous substrate addition in the presence and absence of Rubisco activase. *Plant Physiology* **109,** 1441–1451.

Qian J. & Rodermel S.R. (1993) Ribulose-1,5-bisphosphate carboxylase/oxygenase activase cDNAs from *Nicotiana tabacum*. *Plant Physiology* **102,** 683–684.

Quick W.P., Schurr U., Scheibe R., Schulze E.D., Rodermel S.R., Bogorad L. & Stitt M. (1991) Decreased ribulose-1,5-bisphosphate carboxylase-oxygenase in transgenic tobacco transformed with 'antisense' *rbc*S. *Planta* **183,** 542–554.

Raines C.A. (2003) The Calvin cycle revisited. *Photosynthesis Research* **75,** 1–10.

Raven J.A. (2013) Rubisco: still the most abundant protein of Earth? *New Phytologist* **198,** 1–3.

Robinson S.P. & Portis A.R. Jr. (1988) 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 S.P. & Portis A.R. Jr. (1989) 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.

Rodermel S.R., Abbott M.S. & Bogorad L. (1988) Nuclear-organelle interactions: nuclear antisense gene inhibits ribulose bisphosphate carboxylase enzyme levels in transformed tobacco plants. *Cell* **55,** 673–681.

Rundle S.J. & Zielinski R.E. (1991) Organization and expression of two tandemly oriented genes encoding ribulosebisphosphate carboxylase/oxygenase activase in barley. *Journal of Biological Chemistry* **266,** 4677–4685.

Sage R.F. (2002) Variation in the k_{cat} of Rubisco in C_3 and C_4 plants and some implications for photosynthetic performance at high and low temperature. *Journal of Experimental Botany* **53,** 609–620.

Sage R.F. & Seemann J.R. (1993) Regulation of ribulose-1,5-bisphosphate carboxylase oxygenase activity in response to reduced light-intensity in C_4 plants. *Plant Physiology* **102,** 21–28.

Sage R.F., Pearcy R.W. & Seemann J.R. (1987) The nitrogen use efficiency of C_3 and C_4 plants: III. Leaf nitrogen effects on the activity of carboxylating enzymes in *Chenopodium album* (L.) and *Amaranthus retroflexus* (L.). *Plant Physiology* **85,** 355–359.

Sage R.F., Way D.A. & Kubien D.S. (2008) Rubisco, Rubisco activase, and global climate change. *Journal of Experimental Botany* **59,** 1581–1595.

Salvucci M.E. (1989) Regulation of Rubisco activity *in vivo*. *Physiologia Plantarum* **77,** 164–171.

Salvucci M.E. (2008) Association of Rubisco activase with chaperonin-60β: a possible mechanism for protecting photosynthesis during heat stress. *Journal of Experimental Botany* **59,** 1923–1933.

Salvucci M.E. & Crafts-Brandner S.J. (2004a) Mechanism for deactivation of Rubisco under moderate heat stress. *Physiologia Plantarum* **122,** 513–519.

Salvucci M.E. & Crafts-Brandner S.J. (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.

Salvucci M.E. & Klein R.R. (1994) Site-directed mutagenesis of a reactive lysyl residue (Lys-247) of Rubisco activase. *Archives of Biochemistry and Biophysics* **314,** 178–185.

Salvucci M.E., Portis A.R. Jr. & Ogren W.L. (1985) A soluble chloroplast protein catalyzes ribulose-bisphosphate carboxylase/oxygenase activation *in vivo*. *Photosynthesis Research* **7,** 193–201.

Salvucci M.E., Werneke J.M., Ogren W.L. & Portis A.R. Jr. (1987) Purification and species distribution of Rubisco activase. *Plant Physiology* **84,** 930–936.

Salvucci M.E., Osteryoung K.W., Crafts-Brandner S.J. & Vierling E. (2001) Exceptional sensitivity of Rubisco activase to thermal denaturation *in vitro* and *in vivo*. *Plant Physiology* **127,** 1053–1064.

Salvucci M.E., van de Loo F.J. & Stecher D. (2003) Two isoforms of Rubisco activase in cotton, the products of separate genes not alternative splicing. *Planta* **216,** 736–744.

Salvucci M.E., DeRidder B.P. & Portis A.R. Jr. (2006) Effect of activase level and isoform on the thermotolerance of photosynthesis in Arabidopsis. *Journal of Experimental Botany* **57,** 3793–3799.

Savir Y., Noor E., Milo R. & Tlusty T. (2010) Cross-species analysis traces adaptation of Rubisco toward optimality in a low-dimensional landscape. *Proceedings of the National Academy of Sciences of the United States of America* **107,** 3475–3480.

Sawchuk M.G., Donner T.J., Head P. & Scarpella E. (2008) Unique and overlapping expression patterns among members of photosynthesis-associated nuclear gene families in Arabidopsis. *Plant Physiology* **148,** 1908–1924.

Scafaro A.P., Yamori W., Carmo-Silva A.E., Salvucci M.E., von Caemmerer S. & Atwell B.J. (2012) Rubisco activity is associated with photosynthetic thermotolerance in a wild rice (*Oryza meridionalis*). *Physiologia Plantarum* **146,** 99–109.

Scales J.C., Parry M.A.J. & Salvucci M.E. (2014) A non-radioactive method for measuring Rubisco activase activity in the presence of variable ATP : ADP ratios, including modifications for measuring the activity and activation state of Rubisco. *Photosynthesis Research* **119,** 355–365.

Schrader S.M., Kane H.J., Sharkey T.D. & von Caemmerer S. (2006) High temperature enhances inhibitor production but reduces fallover in tobacco Rubisco. *Functional Plant Biology* **33,** 921–929.

Seemann J.R., Badger M.R. & Berry J.A. (1984) Variations in the specific activity of ribulose-1,5-bisphosphate carboxylase between species utilizing differing photosynthetic pathways. *Plant Physiology* **74,** 791–794.

Seemann J.R., Berry J.A., Freas S.M. & Krump M.A. (1985) Regulation of ribulose bisphosphate carboxylase activity *in vivo* by a light-modulated

inhibitor of catalysis. *Proceedings of the National Academy of Sciences of the United States of America* **82,** 8024–8028.

Servaites J.C. & Geiger D.R. (1995) Regulation of ribulose 1,5-bisphosphate carboxylase/oxygenase by metabolites. *Journal of Experimental Botany* **46,** 1277–1283.

Servaites J.C., Parry M.A.J., Gutteridge S. & Keys A.J. (1986) Species variation in the predawn inhibition of ribulose-1,5-bisphosphate carboxylase oxygenase. *Plant Physiology* **82,** 1161–1163.

Sharkey T.D., Badger M.R., von Caemmerer S. & Andrews T.J. (2001) Increased heat sensitivity of photosynthesis in tobacco plants with reduced Rubisco activase. *Photosynthesis Research* **67,** 147–156.

Sharwood R.E. & Whitney S.M. (2014) Correlating Rubisco catalytic and sequence diversity within C_3 plants with changes in atmospheric CO_2 concentrations. *Plant, Cell & Environment* **37,** 1981–1984.

Shen J.B. & Ogren W.L. (1992) Alteration of spinach ribulose-1,5-bisphosphate carboxylase/oxygenase activase activities by site-directed mutagenesis. *Plant Physiology* **99,** 1201–1207.

Shen J.B., Orozco E.M. & Ogren W.L. (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.

Song Q., Zhang G. & Zhu X.-G. (2013) Optimal crop canopy architecture to maximise canopy photosynthetic CO_2 uptake under elevated CO_2 – a theoretical study using a mechanistic model of canopy photosynthesis. *Functional Plant Biology* **40,** 108–124.

Spreitzer R.J. (2003) Role of the small subunit in ribulose-1,5-bisphosphate carboxylase/oxygenase. *Archives of Biochemistry and Biophysics* **414,** 141–149.

Spreitzer R.J. & Salvucci M.E. (2002) Rubisco: structure, regulatory interactions, and possibilities for a better enzyme. *Annual Review of Plant Biology* **53,** 449–475.

Staiger D. & Brown J.W.S. (2013) Alternative splicing at the intersection of biological timing, development, and stress responses. *The Plant Cell* **25,** 3640–3656.

Stotz M., Mueller-Cajar O., Ciniawsky S., Wendler P., Hartl F.U., Bracher A. & Hayer-Hartl M. (2011) Structure of green-type Rubisco activase from tobacco. *Nature Structural & Molecular Biology* **18,** 1366–1378.

Suzuki Y. & Makino A. (2012) Availability of Rubisco small subunit up-regulates the transcript levels of large subunit for stoichiometric assembly of its holoenzyme in rice. *Plant Physiology* **160,** 533–540.

Suzuki Y., Ohkubo M., Hatakeyama H., Ohashi K., Yoshizawa R., Kojima S., ... Makino A. (2007) Increased Rubisco content in transgenic rice transformed with the 'Sense' *rbc*S gene. *Plant & Cell Physiology* **48,** 626–637.

Suzuki Y., Miyamoto T., Yoshizawa R., Mae T. & Makino A. (2009) Rubisco content and photosynthesis of leaves at different positions in transgenic rice with an overexpression of *RBCS*. *Plant, Cell & Environment* **32,** 417–427.

Tcherkez G. (2013) Modelling the reaction mechanism of ribulose-1,5-bisphosphate carboxylase/oxygenase and consequences for kinetic parameters. *Plant, Cell & Environment* **36,** 1586–1596.

Tcherkez G., Farquhar G.D. & Andrews T.J. (2006) Despite slow catalysis and confused substrate specificity, all ribulose bisphosphate carboxylases may be nearly perfectly optimized. *Proceedings of the National Academy of Sciences of the United States of America* **103,** 7246–7251.

Tezara W., Mitchell V., Driscoll S.P. & Lawlor D.W. (2002) Effects of water deficit and its interaction with CO_2 supply on the biochemistry and physiology of photosynthesis in sunflower. *Journal of Experimental Botany* **53,** 1781–1791.

To K.-Y., Suen D.-F. & Chen S.-C.G. (1999) Molecular characterization of ribulose-1,5-bisphosphate carboxylase/oxygenase activase in rice leaves. *Planta* **209,** 66–76.

Vassileva V., Demirevska K., Simova-Stoilova L., Petrova T., Tsenov N. & Feller U. (2012) Long-term field drought affects leaf protein pattern and chloroplast ultrastructure of winter wheat in a cultivar-specific manner. *Journal of Agronomy and Crop Science* **198,** 104–117.

Vu C.V., Allen L.H. Jr. & Bowes G. (1984) Dark-light modulation of Ribulose bisphosphate carboxylase activity in plants from different photosynthetic categories. *Plant Physiology* **76,** 843–845.

Wachter R.M., Salvucci M.E., Carmo-Silva A.E., Barta C., Genkov T. & Spreitzer R.J. (2013) Activation of interspecies-hybrid Rubisco enzymes to assess different models for the Rubisco–Rubisco activase interaction. *Photosynthesis Research* **117,** 557–566.

Wang D. & Portis A.R. Jr. (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 D., Xie S.Z., Yang J. & Wang Q.F. (2014) Molecular characteristics and expression patterns of Rubisco activase, novel alternative splicing variants in a heterophyllous aquatic plant, *Sagittaria graminea*. *Photosynthetica* **52,** 83–95.

Wang Z.Y., Snyder G.W., Esau B.D., Portis A.R. Jr. & Ogren W.L. (1992) Species-dependent variation in the interaction of substrate-bound ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and Rubisco activase. *Plant Physiology* **100,** 1858–1862.

Watillon B., Kettmann R., Boxus P. & Burny A. (1993) Developmental and circadian pattern of Rubisco activase mRNA accumulation in apple plants. *Plant Molecular Biology* **23,** 501–509.

Werneke J.M., Chatfield J.M. & Ogren W.L. (1989) Alternative mRNA splicing generates the two ribulosebisphosphate carboxylase/oxygenase activase polypeptides in spinach and Arabidopsis. *The Plant Cell* **1,** 815–825.

Weston D.J., Bauerle W.L., Swire-Clark G.A., Moore B.D. & Baird W.M.V. (2007) Characterization of Rubisco activase from thermally contrasting genotypes of *Acer rubrum* (Aceraceae). *American Journal of Botany* **94,** 926–934.

Whitney S.M. & Sharwood R.E. (2008) Construction of a tobacco master line to improve Rubisco engineering in chloroplasts. *Journal of Experimental Botany* **59,** 1909–1921.

Whitney S.M., von Caemmerer S., Hudson G.S. & Andrews T.J. (1999) Directed mutation of the Rubisco large subunit of tobacco influences photorespiration and growth. *Plant Physiology* **121,** 579–588.

Whitney S.M., Houtz R.L. & Alonso H. (2011a) Advancing our understanding and capacity to engineer nature's CO_2-sequestering enzyme, Rubisco. *Plant Physiology* **155,** 27–35.

Whitney S.M., Sharwood R.E., Orr D., White S.J., 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 of the United States of America* **108,** 14688–14693.

Wingler A., Lea P.J., Quick W.P. & Leegood R.C. (2000) Photorespiration: metabolic pathways and their role in stress protection. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **355,** 1517–1529.

Woodrow I.E., Kelly M.E. & Mott K.A. (1996) Limitation of the rate of ribulosebisphosphate carboxylase activation by carbamylation and ribulosebisphosphate carboxylase activase activity: development and tests of a mechanistic model. *Australian Journal of Plant Physiology* **23,** 141–149.

Xu K., He B., Zhou S., Li Y. & Zhang Y. (2010) Cloning and characterization of the Rubisco activase gene from *Ipomoea batatas* (L.) Lam. *Molecular Biology Reports* **37,** 661–668.

Yamori W. & von Caemmerer S. (2009) Effect of Rubisco activase deficiency on the temperature response of CO_2 assimilation rate and Rubisco activation state: insights from transgenic tobacco with reduced amounts of Rubisco activase. *Plant Physiology* **151,** 2073–2082.

Yamori W., Noguchi K. & Terashima I. (2005) Temperature acclimation of photosynthesis in spinach leaves: analyses of photosynthetic components and temperature dependencies of photosynthetic partial reactions. *Plant, Cell & Environment* **28,** 536–547.

Yamori W., Nagai T. & Makino A. (2011) The rate-limiting step for CO_2 assimilation at different temperatures is influenced by the leaf nitrogen content in several C_3 crop species. *Plant, Cell & Environment* **34,** 764–777.

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,** 871–880.

Yin Z., Meng F., Song H., Wang X., Xu X. & Yu D. (2010) Expression quantitative trait loci analysis of two genes encoding Rubisco activase in soybean. *Plant Physiology* **152,** 1625–1637.

Yin Z., Zhang Z., Deng D., Chao M., Gao Q., Wang Y., ... Xu C. (2014) Characterization of Rubisco activase genes in maize: an α-isoform gene functions alongside a β-isoform gene. *Plant Physiology* **164,** 2096–2106.

Yoon M., Putterill J.J., Ross G.S. & Laing W.A. (2001) Determination of the relative expression levels of Rubisco small subunit genes in Arabidopsis by rapid amplification of cDNA ends. *Analytical Biochemistry* **291,** 237–244.

Zhang N. & Portis A.R. Jr. (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 of the United States of America* **96,** 9438–9443.

Zhang N., Kallis R.P., Ewy R.G. & Portis A.R. Jr. (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 of the United States of America* **99,** 3330–3334.

Zhang X.-H., 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 R.G. (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., Bohnert H., Jensen R. & Wildner G. (1998) Formation of the tight-binding inhibitor, 3-ketoarabinitol-1,5-bisphosphate by ribulose-1,5-bisphosphate carboxylase/oxygenase is O_2-dependent. *Photosynthesis Research* **55,** 67–74.

Zhu X.G., Portis A.R. Jr. & Long S.P. (2004) Would transformation of C_3 crop plants with foreign Rubisco increase productivity? A computational analysis extrapolating from kinetic properties to canopy photosynthesis. *Plant, Cell & Environment* **27,** 155–165.

Received 16 May 2014; received in revised form 1 August 2014; accepted for publication 3 August 2014

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Reviews on Rubisco. A non-exhaustive list (!) of review manuscripts on diverse aspects of 'Rubiscology'.

Table S2. Genetic engineering of Rubisco catalysis. Modifications to the Rubisco large and small subunits (rbcL, rbcS) resulting in changes to the catalytic properties of Rubisco.