eXtra Botany

Insight

Big progress for small subunits: new Rubisco mutants in Arabidopsis

Amanda P. Cavanagh*

School of Life Sciences, University of Essex, Wivenhoe Park, Colchester, UK
*Correspondence: a.cavanagh@essex.ac.uk

Nearly 75 years after Rubisco was first isolated from spinach leaves, the enzyme’s function and regulation remain an important research topic and longstanding target to improve plant productivity. In plants, Rubisco is a complex made up of eight large subunits (LSUs) encoded in the chloroplast genome (rbcL), while eight small subunits (SSUs) are encoded in the nucleus as a multi-rbcS gene family. Despite decades of investigation, the function and interaction of the different small subunits in plant Rubisco remain enigmatic. Khumsupan et al. (2020) have used CRISPR/Cas9 [clustered regularly interspaced palindromic repeats (CRISPR)/CRISPR-associated protein 9] and T-DNA insertion lines within Arabidopsis thaliana to generate a suite of single and multiple gene knockout mutants for the four rbcS members. This enabled characterization of the homogenous Rubisco pool consisting of a single SSU isoform in planta. In doing so, they provide a powerful toolkit to expand our understanding of Rubisco structure–function relationships inside the leaf.

Engineering increased photosynthetic capacity has shown improvements in both model and food crops under field conditions, reigniting strategies to optimize or redesign photosynthesis to increase crop yields sustainably (Kromdijk et al., 2016; Ermakova et al., 2019; South et al., 2019). A persistent target for improvement aims to overcome the inefficiencies of the central carbon-fixing enzyme in photosynthesis, Rubisco. Rubisco has a slow catalytic rate and also catalyses a competing oxygenation reaction, promoting the energetically intensive photorespiration cycle. Accordingly, optimizing the regulation and amount of Rubisco can result in improved crop growth and yield (Parry et al., 2012; Salesse-Smith et al., 2018; Yoon et al., 2020). In recent years, surveys of both plant species and other photosynthetic organisms indicate that there is also exploitable variation in Rubisco performance that could be an important target for crop improvement (Sharwood et al., 2016; Young et al., 2016; Orr et al., 2016; Galmés et al., 2019), but our understanding of the structure–function relationships underpinning this variation remains fairly cryptic, and limits our ability to mine this resource.

Evidence across plant and algal Rubiscos has demonstrated that structural changes in both the LSU, where the catalytic site resides at the interface between dimers, as well as the SSU can impact the Rubisco carboxylation rate, substrate specificity, and multimer assembly (well reviewed in the accompanying article). A long-awaited goal for the Rubisco research community was realized with the expression of a recombinant Arabidopsis Rubisco in Escherichia coli (Aigner et al., 2017). This has accelerated the ability to explore structure–function relationships across Rubisco, and has in fact been harnessed to demonstrate that the kinetic performance of recombinant tobacco Rubisco varies depending on the SSU composition (Lin et al., 2019, Preprint). However, recombinant enzymes might vary slightly in performance due to as yet unreconciled post-translational modifications that occur in vivo, and cannot inform the impact of organ-specific localization or developmental-specific expression of specific Rubisco isoforms (Laterre et al., 2017; Lin et al., 2019, Preprint). In Arabidopsis, the SSU-encoding gene family comprises four members: rbcS1A, rbcS1B, rbcS2B, and rbcS3B. Only one gene (1A) is expressed in root tips, while another (1B) is exclusively expressed in the lower side of the leaf, is unresponsive to light pulses, and lacks the light regulatory elements found in the promoters of the other three (Dedonder et al., 1993; Sawchuk et al., 2008). Since rbcS1B also shows the smallest contribution to overall SSU expression and has been replaced with a duplicate copy of rbcS2B in several global accessions, it is easy to discount any functional importance of this isoform (Schwarte and Tiedemann, 2011). However, the crystal structure of Arabidopsis Rubisco captured a homogenous SSU
composition of only this supposed low abundant isoform (Valegård et al., 2018). Khumsupan et al. were unable to isolate a 1B mutant in conjunction with any other SSU isoform in Arabidopsis, presenting compelling evidence that there is a key contributory role for this least expressed 1B isoform during early development.

**Measuring Rubisco in the leaf**

Perhaps the most exciting aspect of the plants generated by Khumsupan et al. (2020) is their potential to be used to examine the impact of homogenous SSU populations on Rubisco activity in vivo. This bypasses not only issues of recombinant enzyme performance, but also the assumptions that in vitro assays make about in vivo conditions, such as pH, CO₂ concentration, and molecular crowding environment at the site of carboxylation. The widely used parameterization of C₃ photosynthesis in response to temperature has been determined using both antisense tobacco and Arabidopsis plants containing ~15% and 40% Rubisco content, respectively (Bernacchi et al., 2001; Walker et al., 2013). However, apart from reductions in Rubisco content, do all small subunit knockdowns have the same impact on enzyme performance? Although a full kinetic parameterization requires measurements of CO₂ response curves over a range of oxygen partial pressures, the in vivo rate of Rubisco carbon assimilation (kcatCO₂) may be estimated if both the maximum rate of carboxylation and the Rubisco content of the leaf are known (Box 1). This representation normalizes the carboxylation activities calculated by Khumsupan et al. to account for the amount of Rubisco in the leaves and suggests that, in the absence of differences in Rubisco activation, Rubisco carboxylation activity could be increased by 6–13% over the wild-type activity in 2b3b, 1a2b,
and 1a3b plants (Box 1). As previously shown (von Caemmerer et al., 1994; Walker et al., 2013), rates of Rubisco carboxylation estimated in vivo are greater than the in vitro measurements from similar T-DNA insertion lines (Atkinson et al., 2017), and probably drastically overestimate the in vivo activity of the 1a2b3b line containing only the 1B isoform. These differences could reflect unreconciled changes in activation state in the mutant lines, but this cannot explain the much higher estimate from line 1a2b3b. The Rubisco turnover rate required to maintain the leaf carboxylation rate ($V_{\text{Cmax}}$) observed for the 1a2b3b line is more than triple that of any other line. Though estimates of in vitro $V_{\text{Cmax}}$ (i.e. Rubisco content×in vitro kcat CO2) are much lower than observed $V_{\text{Cmax}}$ suggesting that the in vitro kcat CO2 may be different for the 1B only isoform, they are unlikely to be as different as predicted here (Box 1). The discrepancy could reflect slight differences between the leaves sampled for biochemical analysis and those used for physiological measurements, and may be further compounded by the estimation method Khumsupan et al. (2020) used to derive $V_{\text{Cmax}}$ from their $A/C_{\text{i}}$ curves.

Estimated increases in the Rubisco carboxylation rate among the double mutants are small relative to the wild type and are obscured by the accompanying 37–61% reductions seen in leaf Rubisco content. However, when their impact is modelled based on similar Rubisco concentrations in the leaves, as could be achieved through simultaneous overexpression of specific Rubisco SSUs and accessory proteins (Salesse-Smith et al., 2018; Yoon et al., 2020), then the impact of these small differences in Rubisco activity on leaf carbon gain is apparent over a broad temperature range (Box 2). Apart from the triple mutant, this limited analysis suggests that Arabidopsis Rubiscos lacking the SSU 1A isoform may provide the most beneficial impact on modelled leaf assimilation, which is notable as this is the SSU isoform targeted via the antisense construct in plants used to derive our current in vivo Rubisco temperature responses in Arabidopsis (Walker et al., 2013).

Future perspectives

In using CRISPR/Cas9 to establish plants with a homogenous Rubisco composition, Khumsupan et al. (2020) have presented additional evidence that 1B has a greater impact on Rubisco than previously considered, which suggest as yet unexplored roles for Rubisco isoforms in plant development. Importantly, the generation of novel mutants described here offers a compelling new tool to address the longstanding question of the impact on Rubisco SSU composition on holoenzyme activity in vivo.

Keywords: Arabidopsis, photosynthesis, Rubisco.

References

Aigner H, Wilson RH, Bracher A, Calisse L, Bhat JY, Hartl FU, Hayer-Hartl M. 2017. Plant RuBiSoCo assembly in E. coli with five chloroplast chaperones including BSD2. Science 358, 1272–1278.

Atkinson N, Leitão N, Orr DJ, Meyer MT, Carmo-Silva E, Griffiths H, Smith AM, McCormick AJ. 2017. Rubisco small subunits from the unicellular green alga Chlamydomonas complement Rubisco-deficient mutants of Arabidopsis. New Phytologist 214, 655–667.
Bernacchi CJ, Singsaas EL, Pimentel C, PortisJr AR, Long SP. 2001. Improved temperature response functions for models of Rubisco-limited photosynthesis. Plant, Cell & Environment 24, 263–259.

Boyd RA, Cavanagh AP, Kubien DS, Cousins AB. 2018. Temperature response of Rubisco kinetics in Arabidopsis thaliana: thermal breakpoints and implications for reaction mechanisms. Journal of Experimental Botany 70, 231–242.

Dedonder A, Rethy R, Fredericq H, Van Montagu M, Krebbers E. 1993. Arabidopsis rbcS genes are differentially regulated by light. Plant Physiology 101, 801–808.

Ermakova M, Lopez-Calacagno PE, Raines CA, Furbank RT, von Caemmerer S. 2019. Overexpression of the Rieske FeS protein of the cytochrome b6f complex increases C4 photosynthesis in Setaria viridis. Communications Biology 2, 314.

Galmés J, Capó-Bauçà S, Niinemets Ü, Iñiguez C. 2019. Potential improvement of photosynthetic CO2 assimilation in crops by exploiting the natural variation in the temperature response of Rubisco catalytic traits. Current Opinion in Plant Biology 49, 60–67.

Khumsupan P, Kozlowska MA, Orr DJ, Andreou AI, Nakayama N, Patron N, Carmo-Silva E, McCormick AJ. 2020. Generating and characterizing single- and multigene mutants of the Rubisco small subunit family in Arabidopsis. Journal of Experimental Botany 71, 5963–5975.

Kromdijk J, Głowacka K, Leonelli L, Gabilly ST, Iwai M, Niyogi KK, Long SP. 2016. Improving photosynthesis and crop productivity by accelerating recovery from photoprotection. Science 354, 857–861.

Laterre R, Pottier M, Remacle C, Boutry M. 2017. Photosynthetic trichomes contain a specific Rubisco with a modified pH-dependent activity. Plant Physiology 173, 2110–2120.

Lin MT, Stone WD, Chaudhari V, Hanson MR. 2019. Enzyme kinetics of tobacco Rubisco expressed in Escherichia coli varies depending on the small subunit composition. bioRxiv 562223. [Preprint].

Orr DJ, Alcântara A, Kapralov MV, Andralojc PJ, Carmo-Silva E, Parry MAJ. 2016. Surveying Rubisco diversity and temperature response to improve crop photosynthetic efficiency. Plant Physiology 172, 707–717.

Parry MAJ, Andralojc PJ, Scales JC, Salvucci ME, Carmo-Silva AE, Alonso H, Whitney SM. 2012. Rubisco activity and regulation as targets for crop improvement. Journal of Experimental Botany 64, 717–730.

Salesse-Smith CE, Sharwood RE, Busch FA, Kromdijk J, Bardal V, Stern DB. 2018. Overexpression of Rubisco subunits with RAF1 increases Rubisco content in maize. Nature Plants 4, 802–810.

Sawchuk MG, Donner TJ, 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.

Schwarte S, Tiedemann R. 2011. A gene duplication/loss event in the ribulose-1,5-bisphosphate-carboxylase/oxygenase (Rubisco) small subunit gene family among accessions of Arabidopsis thaliana. Molecular Biology and Evolution 28, 1861–1876.

Sharwood RE, Ghannoum O, Kapralov MV, Gunn LH, Whitney SM. 2016. Temperature responses of Rubisco from Paniceae grasses provide opportunities for improving C3 photosynthesis. Nature Plants 2, 16186.

South PF, Cavanagh AP, Liu HW, Ort DR. 2019. Synthetic glycolate metabolism pathways stimulate crop growth and productivity in the field. Science 363, eaat9077.

Valegård K, Hasse D, Andersson I, Gunn LH. 2018. Structure of Rubisco from Arabidopsis thaliana in complex with 2-carboxyarabinitol-1,5-bisphosphate. Acta Crystallographica. Section D, Structural Biology 74, 1–9.

von Caemmerer S, Evans JR, Hudson GS, Andrews TJ. 1994. The kinetics of ribulose-1,5-bisphosphate carboxylase/oxygenase in vivo inferred from measurements of photosynthesis in leaves of transgenic tobacco. Planta 195, 88–97.

Walker B, Ariza LS, Kaines S, Badger MR, Cousins AB. 2013. Temperature response of in vivo Rubisco kinetics and mesophyll conductance in Arabidopsis thaliana: comparisons to Nicotiana tabacum. Plant, Cell & Environment 36, 2108–2119.

Yoon D-K, Ishiyama K, Suganami M, et al. 2020. Transgenic rice overproducing Rubisco exhibits increased yields with improved nitrogen-use efficiency in an experimental paddy field. Nature Food 1, 134–139.

Young JN, Heureux AMC, Sharwood RE, Rickaby REM, Morel FMM, Whitney SM. 2016. Large variation in the Rubisco kinetics of diatoms reveals diversity among their carbon-concentrating mechanisms. Journal of Experimental Botany 67, 3445–3456.