Regulation of sodium-dependent bicarbonate transporter, SbtA

LAURA WEY

Extended abstract

Predictions warn that food production must double by 2050 to meet the needs of predicted population growth (Figure 1) (Tilman et al. 2011). While food crop yields have increased due to breeding for increased harvest index (proportion of biomass that is grain), they are now plateauing (Parry et al. 2011). In addition to better agronomic practices, genetic improvements are needed to increase crop biomass, and therefore yield (Leegood et al. 2010).

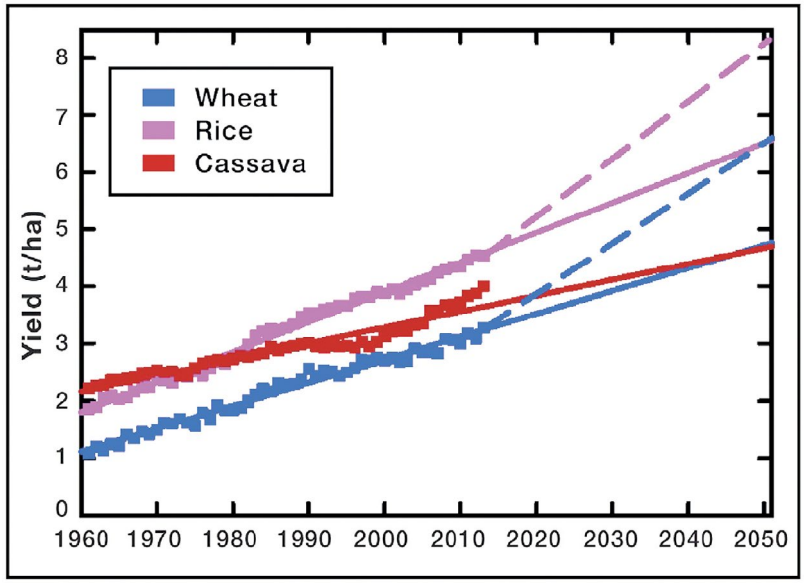

Figure 1: The annual average global yields of crops from 1961 to 2013.

The solid lines are the annual average global yields from 1961 to 2013 then projected forward to 2050; the broken lines are the projected demand for cassava (red), rice (pink), and wheat (blue).

Source: Long et al. 2015.

One genetic engineering strategy to increase crop yields, towards which my research contributes, aims to improve the efficiency of photosynthesis in C_3 plants, a number of which are important crops, such as rice and wheat. C_3 plants have a suboptimal photosynthetic CO_2 fixation rate. This is primarily due to the inefficiencies of the major CO_2-fixing enzyme, Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) (Whitney et al. 2011; Raven et al. 2012). In addition, C_3 plants suffer a CO_2 drawdown from the ambient air (380 ppm CO_2, measured in 2009) to the chloroplast stroma (typically ~190 ppm CO_2), because of diffusive resistance through stomata, cell wall, cytoplasm and chloroplast membrane to chloroplast stroma (Evans et al. 2009).

It has been proposed that C_3 photosynthesis could be improved by integrating components of the CO_2-concentrating mechanism (CCM) (Figure 2) from photosynthetic cyanobacteria (also known as blue-green algae) into the chloroplasts in mesophyll cells (Price et al. 2013). The cyanobacterial CCM is a collection of adaptations that enable cyanobacteria to photosynthesise efficiently.

The first step in the proposed genetic engineering strategy to improve the efficiency of photosynthesis in C_3 crops would be to add sodium-dependent bicarbonate transporter A (SbtA) from the cyanobacterial CCM into the chloroplast inner envelope in C_3 plants (Figure 3) (Price et al. 2011). The primary objective of this step is to reduce the diffusive CO_2 drawdown from the sub-stomatal space to the chloroplast stroma. Modelling indicates that a 5–15 per cent improvement in photosynthetic CO_2 fixation rate at constant sub-stomatal levels is theoretically feasible from this modification (McGrath & Long 2014). A Na^+/H^+ antiporter, such as NhaS3 from the cyanobacterial CCM or NHD1 from *Arabidopsis thaliana*, would need to accompany SbtA to the chloroplast to generate a driving Na^+-gradient.

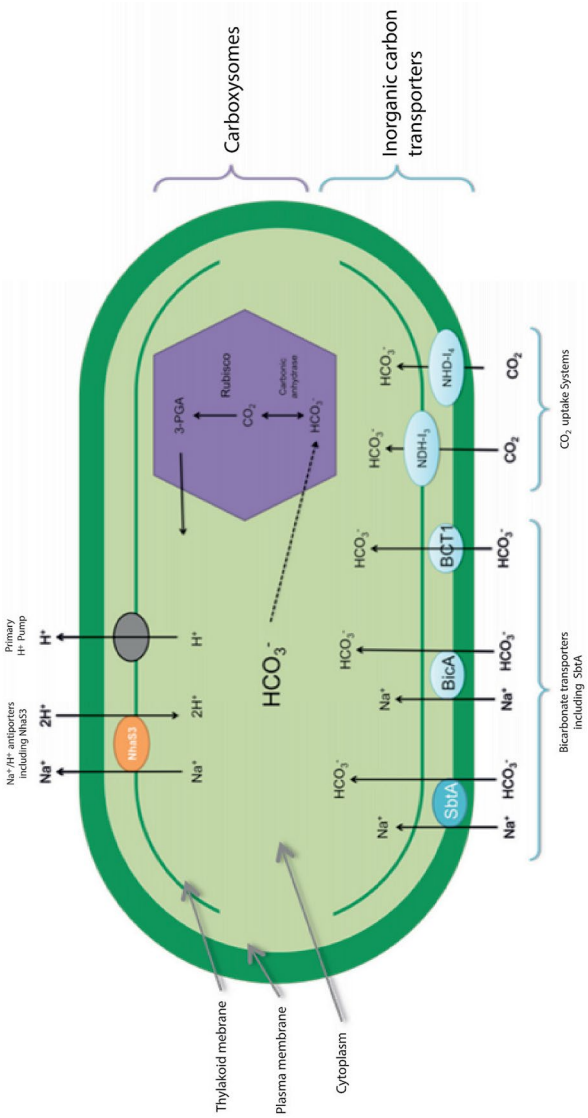

Figure 2: The main components of the cyanobacterial CO_2-concentrating mechanism (CCM) from photosynthetic cyanobacteria (also known as blue-green algae) to be integrated into the chloroplasts of C_3 plants.

(1) A series of active C_i uptake systems, including three HCO_3^- transporters and two active CO_2 uptake systems. SbtA is a high-affinity, low-flux Na^+/ HCO_3^- symporter (Price et al. 2004). BicA is a medium-to-low-affinity, high-flux Na^+-dependent HCO_3^- transporter (Price and Howitt 2011). BCT1 is a complex, high-affinity HCO_3^- transporter (Omata et al. 1999). NDH-I 3 and NDH-I 4 are active CO_2 systems (Price et al. 2002). There are also Na^+/H^+ antiporters and primary proton pumps in the plasma membrane. (2) The carboxysomes, which are bacterial microcompartments that contain carbonic anhydrase (CA) and Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) (Rae et al. 2013).

Source: Author.

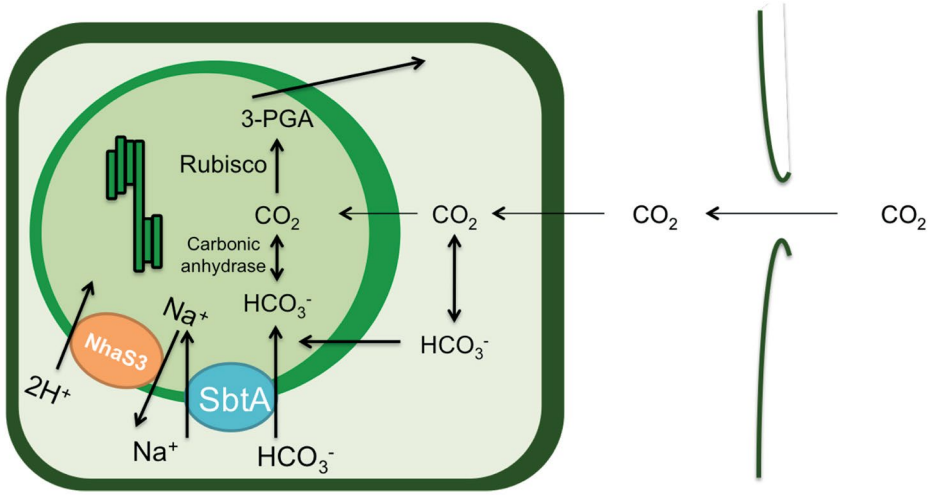

Figure 3: The first phase of the proposed genetic engineering strategy to improve photosynthesis in C_3 crops is to add SbtA HCO_3^- transporters (blue) and NhaS3 Na^+/H^+ antiporters (orange) from the cyanobacterial CCM into the chloroplast inner envelope membrane.

Source: Author.

For the first phase of the proposed genetic engineering strategy to be successful, SbtA not only needs to be active in chloroplasts, but also ideally able to be controlled by manipulation of its regulatory systems. Therefore, an understanding of the regulation of SbtA is essential. SbtB, a small, soluble protein, is an inhibitor of SbtA via their direct interaction (SbtA-SbtB) (Du et al. 2014). It is hypothesised that SbtB acts as a 'curfew' protein to inactivate SbtA in the dark, to prevent wasteful uptake and toxic leakage of Na^+ into the cyanobacterial cell. It is unknown precisely how SbtA and SbtB interact or whether post-translational modifications or allosteric regulations mediate the regulatory activity of SbtB.

My research aimed to elucidate a greater understanding of the regulation of SbtA by SbtB inhibitor protein. I aimed to determine the potential role of small effector molecules and potential phosphorylation sites of SbtB residues in mediating the regulatory activity of SbtB in SbtA-SbtB. Specifically, I investigated SbtA and SbtB from *Cyanobium sp.* 7001. The potential role of effectors in mediating the regulatory activity of SbtB was investigated by conducting in vitro SbtA-SbtB binding assays using immobilised metal affinity chromatography (IMAC) with effectors and immunological detection. From a list of several possible effectors, we are currently building up evidence that a key effector regulates SbtA-SbtB (pers. comm. Bratati Mukherjee). The potential role of putative phosphorylation of SbtB residues in mediating the regulatory activity of SbtB was investigated by conducting ^{14}C-labelled HCO_3^- uptake assays and in vitro SbtA-SbtB binding

assays using SbtB phosphorylation mimic mutants. It was found that substituting a glutamic acid at a key threonine residue in SbtB reduced the SbtA-SbtB interaction.

Further research is currently underway to further elucidate the regulation of SbtA so that SbtA can be controlled by manipulation of its regulatory systems when transplanted into key crop plants to improve photosynthesis efficiency and, in turn, yield.

This extended abstract is based on Laura's Honours research submitted in 2015 as partial fulfilment of the requirements for the Bachelor of Philosophy (Honours) in Science.

Bibliography

Du, J., B. Förster, L. Rourke, S.M. Howitt & G.D. Price. 2014. Characterisation of Cyanobacterial Bicarbonate Transporters in *E. coli* Shows that SbtA Homologs Are Functional in This Heterologous Expression System. *PloS one* 9: e115905.

Evans, J.R., R. Kaldenhoff, B. Genty & I. Terashima. 2009. Resistances along the CO_2 diffusion pathway inside leaves. *Journal of Experimental Botany* 60: 2235–2248.

Leegood, R.C., J.R. Evans & R.T. Furbank. 2010. Food security requires genetic advances to increase farm yields. *Nature* 464: 831.

Long, S.P., A. Marshall-Colon & X.-G. Zhu. 2015. Meeting the Global Food Demand of the Future by Engineering Crop Photosynthesis and Yield Potential. *Cell* 161: 5666.

Mcgrath, J.M. & S.P. Long. 2014. Can the Cyanobacterial Carbon-Concentrating Mechanism Increase Photosynthesis in Crop Species? A Theoretical Analysis. *Plant Physiology* 164: 2247–2261.

Omata, T., G.D. Price, M.R. Badger, M. Okamura, S. Gohta & T. Ogawa. 1999. Identification of an ATP-binding cassette transporter involved in bicarbonate uptake in the cyanobacterium *Synechococcus* sp strain PCC 7942. *Proceedings of the National Academy of Sciences of the United States of America* 96: 13571–13576.

Parry, M.A.J., M. Reynolds, M.E. Salvucci, C. Raines, P.J. Andralojc, X.G. Zhu, G.D. Price, A.G. Condon & R.T. Furbank. 2011. Raising yield potential of wheat. II. Increasing photosynthetic capacity and efficiency. *Journal of Experimental Botany* 62: 453–467.

Price, G.D. & S.M. Howitt. 2011. The cyanobacterial bicarbonate transporter BicA: its physiological role and the implications of structural similarities with human SLC26 transporters. *Biochemistry and Cell Biology* 89: 178–188.

Price, G.D., F.J. Woodger, M.R. Badger, S.M. Howitt & L. Tucker. 2004. Identification of a SulP-type bicarbonate transporter in marine cyanobacteria. *Proceedings of the National Academy of Sciences of the United States of America* 101: 18228–18233.

Price, G.D., J.J.L. Pengelly, B. Forster, J.H. Du, S.M. Whitney, S. Von Caemmerer, M.R. Badger, S.M. Howitt & J.R. Evans. 2013. The cyanobacterial CCM as a source of genes for improving photosynthetic CO_2 fixation in crop species. *Journal of Experimental Botany* 64: 753–768.

Price, G.D., M.R. Badger & S. Von Caemmerer. 2011. The prospect of using cyanobacterial bicarbonate transporters to improve leaf photosynthesis in C_3 crop plants. *Plant Physiology* 155: 20–26.

Price, G.D., S. Maeda, T. Omata & M.R. Badger. 2002. Modes of active inorganic carbon uptake in the cyanobacterium, *Synechococcus* sp PCC7942. *Functional Plant Biology* 29: 131–149.

Rae, B.D., B.M. Long, M.R. Badger & G.D. Price. 2013. Functions, Compositions, and Evolution of the Two Types of Carboxysomes: Polyhedral Microcompartments That Facilitate CO_2 Fixation in Cyanobacteria and Some Proteobacteria. *Microbiology and Molecular Biology Reviews* 77: 357–379.

Raven, J.A., M. Giordano, J. Beardall & S.C. Maberly. 2012. Algal evolution in relation to atmospheric CO_2: Carboxylases, carbon-concentrating mechanisms and carbon oxidation cycles. *Philosophical Transactions of the Royal Society Biological Sciences* 367: 493–507.

Tilman, D., C. Balzer, J. Hill & B.L. Befort. 2011. Global food demand and the sustainable intensification of agriculture. *Proceedings of the National Academy of Sciences of the United States of America* 108: 20260–20264.

Whitney, S.M., R.L. Houtz & H. Alonso. 2011. Advancing Our Understanding and Capacity to Engineer Nature's CO_2-Sequestering Enzyme, Rubisco. *Plant Physiology* 155: 27–35.

This text is taken from *The ANU Undergraduate Research Journal, Volume Seven, 2015*, edited by Daniel McKay, published 2016 by ANU eView, The Australian National University, Canberra, Australia.