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## A molecular gauge for nitrogen economy in plants

Timothy R. Fallon<sup>1,2</sup> and Jing-Ke Weng<sup>1,2,\*</sup>

<sup>1</sup>Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA 02142, USA

<sup>2</sup>Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

\*Correspondence: wengj@wi.mit.edu

### Abstract:

Understanding nitrogen metabolism in plants holds promise for future agricultural improvements. Chellamuthu et al. now identify a feedback regulation in plant nitrogen metabolism through glutamine sensing. This mechanism appears to be conserved from algae to flowering plants with a few surprising exceptions.

### Text:

At the turn of the 19<sup>th</sup> century, newly elected president of the British Academy of Science William Crookes gave a dire prediction to the assembled academy. He stated bluntly, "...all civilized nations stand in deadly peril of not having enough to eat" (Crookes, 1898). Crookes argued that a lack of fertilizer, in particular fixed nitrogen, would fatally limit food production and bring about global starvation. Thankfully the development of the Haber-Bosch process in the early decades of the 20<sup>th</sup> century allowed for the catalyzed fixation of atmospheric nitrogen into active species suitable for use in fertilizer at an industrial scale (Hager 2008). Combined with the advances in plant breeding and agricultural technology from the "Green Revolution" of the mid 20<sup>th</sup> century, we continue today to stay ahead of Crookes' dire predictions. As the human population continues to expand, further understanding and optimization of plant nitrogen utilization remains a pressing task. Towards this end, Chellamuthu et al. (2014) describe in this issue of *Cell* a significant advance in the understanding of the molecular feedback control of nitrogen metabolism across the plant kingdom.

Beyond using nitrogen for protein or nucleotide synthesis alone, many plants also produce large quantities of nitrogen containing specialized metabolites known as alkaloids, caffeine being a well-known example. Plants have also evolved to store excess nitrogen in both seeds and vegetative tissues in the form of specialized storage proteins. These proteins store nitrogen both in the amide backbone of the polypeptide, as well as by having a high proportion of amino acids enriched with nitrogen containing R groups, such as arginine, asparagine, and glutamine (Staswick, 1994). Unlike vertebrate nitrogen metabolism, which particularly in the case of carnivores often operates in a state of nitrogen excess tuned towards excretion, plants, algae, and bacteria must assimilate inorganic nitrogen from the environment, and therefore have to carefully balance nitrogen utilization. Perhaps unsurprisingly, studies of nitrogen metabolism in these organisms have revealed elegant regulatory mechanisms.

In the case of bacteria, previous studies indicated that nitrogen metabolism was

regulated through a key conserved protein, known as P<sub>II</sub> (Forchhammer, 2007). P<sub>II</sub> integrates concentration information of key metabolites, including ATP/ADP, 2-oxoglutarate, and glutamine, in order to regulate downstream proteins in nitrogen metabolism. Providing a chemical logic for this feedback, 2-oxoglutarate, ATP, and glutamine all participate in the dominant metabolic pathway for nitrogen assimilation, the glutamine synthase/glutamine oxoglutarate aminotransferase (GS/GOGAT) pathway (Forchhammer, 2007). While P<sub>II</sub> senses ATP and 2-oxoglutarate levels through a direct binding interaction, glutamine sensing is transduced through a phylogenetically divergent array of secondary sensors, which post-translationally modify P<sub>II</sub> in response to glutamine levels. P<sub>II</sub> then binds and regulates downstream enzymes in nitrogen metabolism, most notably the committed step in arginine biosynthesis, N-acetylglutamate kinase (NAGK) (Heinrich et al., 2004). NAGK is feedback inhibited by arginine, its ultimate downstream product. P<sub>II</sub> binding activates NAGK by relieving this inhibitory feedback of arginine.

The chloroplasts of plants share ancestry with modern cyanobacteria, and plant P<sub>II</sub> has been conserved throughout the long evolutionary divergence between plants and bacteria (Chellamuthu et al., 2013). Genetic studies in the model flowering plant *Arabidopsis thaliana* indicated that loss of P<sub>II</sub> produced a number of nitrogen metabolism related phenotypes (Ferrario-Méry et al., 2006). Furthermore, in vitro biochemical and structural analyses demonstrated remarkable similarity of P<sub>II</sub> with its bacterial counterparts, including the interaction with NAGK (Mizuno et al., 2007). However, the question remained if the divergent mechanisms of secondary sensing of glutamine through post-translational modifications of P<sub>II</sub> were conserved.

To better define the regulatory role of P<sub>II</sub> in plant nitrogen metabolism, Chellamuthu et al. (2014) now undertake a biochemical study on NAGKs and their corresponding P<sub>II</sub> partners from both *A. thaliana* and the model green alga *Chlamydomonas reinhardtii*. Like the P<sub>II</sub>-NAGK interaction in bacteria, previous studies had shown that *A. thaliana* P<sub>II</sub> constitutively relieves arginine feedback inhibition of NAGK (Beez et al., 2009). However, the experiments of Chellamuthu et al. with *C. reinhardtii* P<sub>II</sub> and NAGK produce a surprising finding. In contrast to *A. thaliana* and bacterial P<sub>II</sub>, *C. reinhardtii* P<sub>II</sub> does not relieve arginine feedback inhibition of NAGK. Chellamuthu et al. surmise there might be a missing small molecule in their assays that P<sub>II</sub> could be sensing. After assaying a number of primary metabolites, they identify that addition of glutamine rescues the *C. reinhardtii* P<sub>II</sub>-NAGK interaction. Glutamine, the metabolite previously known only to be sensed by P<sub>II</sub> through secondary proteins, appears to be sensed directly, revealing a dramatic mechanistic divergence in P<sub>II</sub> between plants and bacteria.

Curiously, plant P<sub>II</sub> proteins have a functionally uncharacterized but conserved C-terminal extension, which is absent in bacteria. Through X-ray crystallography, it is clear that the C-terminal extension in *C. reinhardtii* P<sub>II</sub> forms a so-called Q-loop region, which constitutes a large proportion of the glutamine-binding site. Interestingly the Q-loop of *A. thaliana* and other *Brassicaceae* contains a three-amino-acid deletion, rendering their P<sub>II</sub> unable to directly sense glutamine. In *C. reinhardtii*, NAGK activation through P<sub>II</sub> binding is glutamine-dependent, whereas deletion of the Q-loop region in P<sub>II</sub> completely

abolishes its ability to bind to NAGK. Measured in vitro, the EC<sub>50</sub> of glutamine for NAGK activation by P<sub>II</sub> is in the millimolar range, which, as noted by the authors, is comparable to reported intracellular concentrations of glutamine. Future research is required to demonstrate the physiological relevance of this sensing mechanism in vivo.

Based on phylogenetic sequence analysis and in vitro studies of representative members of diverse lineages, the P<sub>II</sub> Q-loop and direct glutamine sensing appears to be widely conserved in the plant kingdom (Figure 1). However, many interesting questions arise when considering the functional relevance of the unexpected divergence of P<sub>II</sub> within *Brassicaceae*. For instance, are the observed Q-loop deletions an adaptive change, or a deleterious mutation that became fixed in the common ancestor of *Brassicaceae*? Could *Brassicaceae* contain alternative glutamine sensing mechanisms to compensate for the loss of glutamine-sensing capability in its P<sub>II</sub>? In vivo studies of these phenomena would be important to reveal if P<sub>II</sub> divergence represents an exploitable trait for genetic engineering of crops.

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## Figure legend

P<sub>II</sub> proteins directly sense glutamine for NAGK activation and arginine biosynthesis in plants, except in *Brassicaceae*.

P<sub>II</sub> activates arginine biosynthesis by antagonizing the feedback inhibition of arginine upon the committed step of arginine biosynthesis, NAGK. In every plant taxa except *Brassicaceae*, the family that contains the model flowering plant *Arabidopsis thaliana*, glutamine is directly sensed by binding to a conserved C-terminal extension, termed the Q-loop, of P<sub>II</sub>. In the case of *Brassicaceae*, glutamine is not required for activation of NAGK by P<sub>II</sub>. In both cases, bound ATP appears to be required for P<sub>II</sub> to antagonize NAGK feedback inhibition.