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CHLOROPLAST BIOLOGY

An ancestral route to executing chloroplast cost/benefit analysis

The impacts of the well-known prokaryotic ancestry of chloroplasts extend to the occurrence of a bacterial alarm hormone or “alarmone” in plants, acting upon nutrient deficiency or stress. A new study shows that chloroplast development itself is reduced by alarmone, with surprising, apparently paradoxical consequences for plant growth.

Enrique López-Juez

Nucleotides are the building blocks of nucleic acids but also, in standard or modified forms, play ancestral roles as intracellular switches or signals. When the most studied bacterium, *E. coli*, experiences amino acid or fatty acid starvation, a tetra- (or penta-) phosphate nucleotide, (p)ppGpp, is enzymatically produced to trigger genetic responses protecting against such starvation. The phenomenon has been called “stringent response”, and ppGpp “alarmone”[1]. On page xxx of this issue, Maekawa *et al.*[2] show that plant enzymes which synthesise alarmone are present in chloroplasts, and that extra alarmone production results in pale leaves and impaired photosynthesis in plants as a result of making chloroplasts “slimmer”. And yet, those plants develop larger leaves with more cells and a greater biomass, and with greater resilience in the face of deficiency of the two agriculturally important elements, nitrogen and phosphorous. What could have led to such a surprising result? And what can we learn from it when faced with the challenge of growing more crops with less agronomic inputs and in less stable environments?

In *E. coli* two types of synthase (RelA and SpoT) produce alarmone by combining GTP and two phosphates of ATP. The ribosome-associated RelA detects the presence of uncharged tRNAs, an indicator of aminoacid deficiency, while SpoT detects deficiency in fatty acids. 15 years ago the study of a plant protein involved in disease resistance found that it associated, and potentially “guarded”, another protein with homology to RelA and SpoT[3]. Today such proteins have been called RSHs. Four of them occur in the genome of *Arabidopsis*, of which three are capable of synthesising alarmone *in vitro*. These proteins are of obvious prokaryotic ancestry, which is not surprising as all chloroplasts derive from one assimilation event into a eukaryotic cell of a symbiotic cyanobacterium, whose genome has since shrank to a few dozen genes and instead shaped nuclear genomes[4]. Nuclear genes originally derived from chloroplasts today play multiple functions, including photoperception, hormone action and, in this case, alarmone synthesis. A variety of stresses have been shown to elevate the levels of ppGpp[5]. But what purpose does this serve? The answer has been difficult to come by. Loss of one or more of these RSHs has little effect, probably owing to redundancy in function, while attempted overexpression has only achieved “co-suppression”, unintended reduction. Maekawa and coworkers targeted two highly similar RSHs. In their study, they first showed that these

enzymes are localised to chloroplasts (adding to the previous two already known to be located in those organelles). They then reasoned that overexpression of either of them, while avoiding cosuppression, would be possible in an *rsh*-double mutant background. They were right: overexpression was achieved, and the line with the highest level of RSH3 accumulated 4 fold the alarmone level of the wild type. Unexpectedly, the transgenic plants showed pale leaves with less chlorophyll, less lipids per leaf mass (particularly the chloroplast lipids), less protein, most noticeably the most abundant chloroplast proteins, reduced photosynthetic capacity and increased photosynthetic stress. Microscope observation revealed that, while cells contained normal numbers of chloroplasts, such organelles were much slimmer than in the controls. Indeed, the levels of expression of all chloroplast-encoded genes tested were less than half the control.

The greatest surprise, however, was the fact that those plants actually grew twice as large as the controls. And these were not bloated plants because the plant dry biomass was doubled, without a change of cell size.

Is this really possible? Is plant biomass not the result of photosynthesis? How can plants with impaired photosynthesis grow larger? Certainly all carbon in plants, loaded with energy, is fixed by photosynthesis. But there are “cheaper” forms of biomass possible to build at a lower energy cost. With both expensive total lipid and total protein substantially reduced, it is likely that extra cell walls and other polysaccharide material account for the difference.

It is interesting to note that a thorough examination of various natural strains of *Arabidopsis* of varying growth had earlier identified a negative correlation between starch at the end of each day and biomass[6]. One could argue that plants which invest much energy in immediate growth put less aside for later, and *vice versa*. When considering the action of alarmone, though, a different judgement call appears to be made: alerted to limited availability of nutrients, it is advantageous to avoid building unnecessarily costly organelles. Indeed, aside from the vacuole, chloroplasts account for the bulk of the cellular content of leaf cells, and contain much of the lipid and the most abundant proteins in plants. Hence, developing chloroplasts is costly. Even global deficiencies in the synthesis of nucleotides are first noted as reduced ability to replicate chloroplast DNA in sufficient quantities, leading to greening defects[7].

The result of such cost/benefit analysis and execution became particularly apparent when Maekawa et al. exposed their plants to actual nutrient deficiency. Growth on low nitrogen led control plants to accumulate starch (in order to store an inactive form of carbon, to maintain a carbon/nitrogen metabolic balance) and premature senescence of older leaves (to rapidly recycle nutrients). Under phosphorous deficiency the plants produced a pigment indicative of stress. Alarmone overproducers did neither; they accumulated less starch, maintained their older leaves and exhibited less stress. The implications for eventual crop production appear staggering. Here, apparently, is a counterintuitive way of maintaining or increasing growth and biomass under limiting conditions: reduce chloroplast development. But the cost is also clear: alarmone action led to Arabidopsis plants

more akin to potato or cassava tubers than they were to nutritious, protein and oil-rich soybean.

Much remains to be explored in these plants with increased alarmone. What is the means by which chloroplast development is reduced? The observed decrease in the expression of genes encoded in the chloroplast genome goes some way to explaining, but how this occurs both for genes that use a standard bacterial-type RNA polymerase (as alarmone does in *E. coli*[1]) and for those that use a different one is unclear. Apart from the modulation of gene expression, protein translation could also be a target. The global decrease in the thickness of individual chloroplasts, while probably maintaining their number and area, also suggests the continued action of a regulatory mechanism which maintains the chloroplast occupancy of cells[8]. This contrasts with reductions in the replication of the chloroplast genome leading to decreased division, and fewer, larger chloroplasts[7]. And, because RSH was originally identified as a possible interactor of a plant defence protein[3], one would wonder how these plants would respond to pathogens.

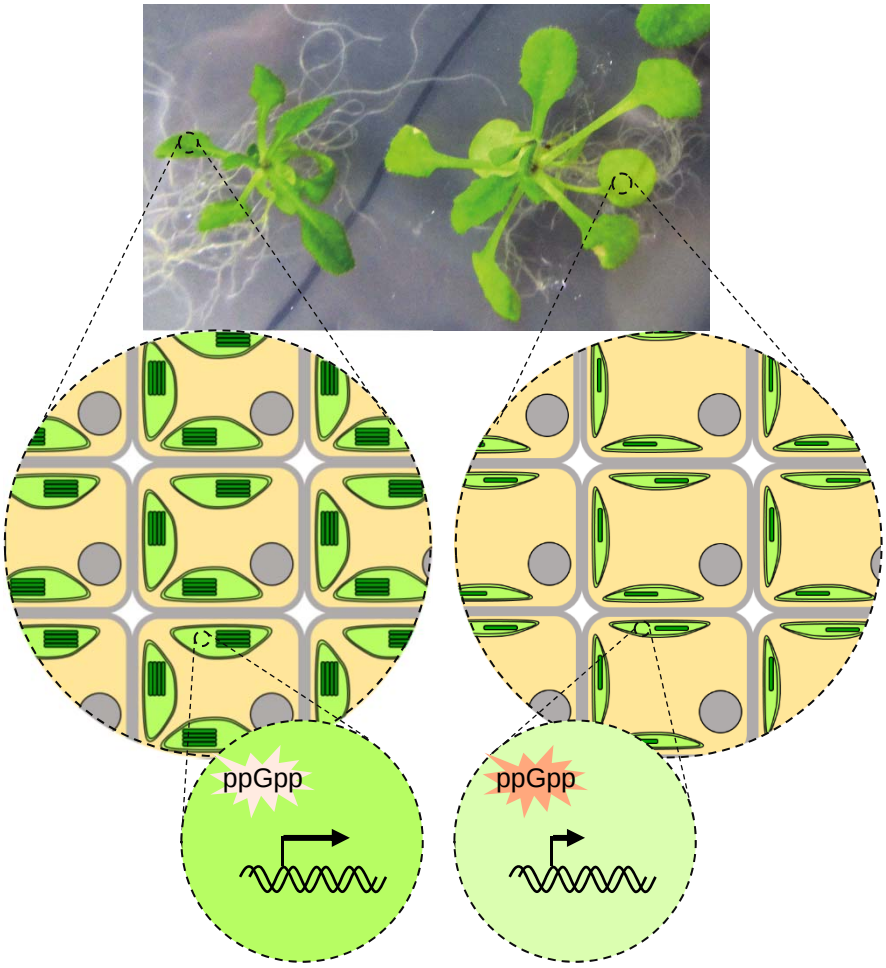
Engineering photosynthesis is one key, little explored route to increased crop yields where progress is demonstrably possible[9]. Ultimately all biomass is a product of chloroplast activity. Eons of evolutionary history may have optimised plants under multiple selection forces, for example competitive ability, not just yield, and it is likely that the optima for crops are not the same as those outside agriculture. It is nevertheless surprising how little we know about the mechanisms that determine the extent of chloroplast development (in contrast to the advances made in understanding the extent of mitochondrial development in cells of our own bodies[10]), and this limits our ability to rationally intervene. Studies like that of Maekawa and collaborators remind us how much there is to be learned, both on how to alter the chloroplast compartment, and on the circumstances, costs and benefits implicated.

Enrique López-Juez is in the School of Biological Sciences, Royal Holloway University of London, Egham, Surrey, TW20 0EX, UK.

References

1. Hauryliuk V., Atkinson G.C., Murakami K.S., Tenson T. & Gerdes K. *Nature Rev. Microbiol.* **13**, 298-309 (2015).
2. Maekawa M. *et al. Nature Plants* xxx (2015).
3. van der Biezen, E.A., Sun, J., Coleman, M.J., Bibb, M.J. & Jones, J.D. *Proc. Natl. Acad. Sci. USA* **97**, 3747-3752 (2000).
4. Deusch O. *et al. Mol. Biol. Evol.* **25**, 748-761 (2008).
5. Takahashi, K., Kasai, K. & Ochi, K. *Proc. Natl. Acad. Sci. USA* **101**, 4320-4324 (2004).
6. Sulpice R. *et al. Proc. Natl. Acad. Sci. USA* **106**, 10348–10353 (2009).
7. Garton S., Knight H., Warren G.J., Knight M.R., Thorlby G.J. *Plant J.* **50**, 118-127 (2007).
8. Osteryoung K.W., Pyke K.A. *Annu. Rev. Plant Biol.* **65**, 443-472 (2014).
9. Long S.P., Marshall-Colon A., Zhu X.G. *Cell* **161**, 56-66 (2015).
10. Scarpulla R.C., Vega R.B., Kelly D.P. *Trends Endocrinol. Metab.* **23**, 459-466.

Figure 1. Rosettes of nutrient media-grown *Arabidopsis thaliana* wild type (left) and a plant overexpressing a chloroplast-localised enzyme that synthesises ppGpp (alarmone), a compound used by bacteria as a nutrient starvation signal (right). Elevated levels of alarmone reduce the development of individual chloroplasts (without otherwise changing their number), leading to plants which are larger, composed of more cells, yet less energy/nutrient dense. The action of ppGpp is in part due to reduced accumulation of chloroplast transcripts. Image of plants courtesy of Shinji Masuda.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