EDITORIAL: REFLECTIONS ON THE PLANT CELL CLASSICS

LOF and GOF Alleles Shed Light on the Molecular Basis of phyB Signaling in Plants

The birth of The Plant Cell in 1989 coincided with the rise of the era of plant molecular genetics enabled by the growing community of Arabidopsis researchers. Advances in sequencing and molecular genetic technologies at that time, rudimentary by today's standards, already had begun to identify many of the master regulatory genes important for plant growth and developmental processes by leveraging mutants identified by forward genetic approaches years earlier. Among the most eagerly anticipated discoveries in the plant photobiology field was the isolation and characterization of phytochrome genes - five in Arabidopsis thaliana - first reported in the landmark paper in Genes and Development by Sharrock and Quail (1989).

This seminal discovery set in motion a race to secure and characterize loss-of-function (LOF) mutants in the five Arabidopsis phytochromes by multiple labs that focused on the long hypocotyl (hy1-hy5) mutants reported by Koomneef et al. (1980). Looking back on these studies, the report by Reed et al. (1993) in The Plant Cell stands out as a milestone in our field, revealing the molecular basis of multiple hy3 LOF alleles of phytochrome B. PhyB mutants all display elongated hypocotyls under continuous white or red light and, among other LOF phenotypes, also exhibit constitutive shade avoidance behavior, consistent with the known role of phyB as the dominant red/far red reversible phytochrome regulator in light-grown Arabidopsis plants. These observations are also consistent with the evidence that, aside from hy3, none of the original long hypocotyl mutant loci had lesions in other phytochromes.

Through RFLP mapping, sequencing, and phenotypic characterization of multiple hy3 mutants in Ler, Col and Ws ecotypes, Reed et al. (1993) identified both strong nonsense and weak missense alleles of PHYB from EMS mutagenesis screens as well as an insertional mutant from screens of T-DNA insertion libraries. Our field and the greater plant biology community have significantly benefited from these landmark studies, as these alleles have been used for countless genetic interaction studies, for structure-function studies, comparative analyses of functional role of conserved residues in PHYBs from other plant species, and for construction of mutants lines deficient in all five phytochromes (Strasser et al. 2010; Hu et al. 2013). The latter are invaluable tools for investigating other light-dependent processes, e.g. photosynthesis, phototropism, photoacclimation, chlorophyll synthesis, etc., owing to the absence of the phytochrome elephant in the room. It is no surprise that the paper by Reed et al. was featured with a commentary in The Plant Cell when it appeared in 1993, and that it has been one of the very few highly cited phytochrome papers (over 900 times by Google Scholar; close to 700 by Web of Science).

From a biochemist's perspective, LOF mutants represent a treasure trove of potential insight into the structural basis of protein function(s). The number of LOF alleles of PHYB has expanded considerably since the work of Reed et al. (1993) and even further since the last time they were catalogued by Rockwell et al. in 2006. Identification of new LOF alleles of phyB have benefited from intragenic suppressor studies of over-expressed PHYB transgenes, from investigations addressing the functional consequence of truncated, chimeric and/or novel missense alleles by phenotypic complementation, and from molecular analysis of genetic variation in the PHYB locus. These studies are best exemplified by the tour de force study by Oka et al. (2008).

Strong LOF missense alleles that target residues scattered throughout the PHYB sequence clearly indicate the importance of nearly every domain of this large multidomain photoreceptor. For example, mutations within the photosensory domain of phyB have revealed insight into residues critical for binding of its linear tetrapyrrole (bilin) chromophore, the wavelength sensing range and thermal dark reversion of phyB. Recent studies underscore the importance of thermal dark reversion for the temperature sensing role of phyB (Jung et al., 2016; Legris et al., 2016). Based on mutations that affect the light dependent interaction of phyB with downstream regulators such as PIFs, COP1 and numerous components of the circadian clock, heterodimerization with other phytochromes, nuclear import and photobody formation that correlate with phyB function, many researchers in this field have identified unique and overlapping sites of interaction within both its photosensory and regulatory regions.

Forward genetic approaches also have proven effective for identifying gain-of-function (GOF) mutants in many plant genes. Surprisingly, such alleles of PHYB were not recovered in screens for mutants that could develop photomorphogenetically in the absence of light. While some weak GOF alleles that are hypersensitive to red light had previously been identified, these mostly stabilized the photoactivated state of phyB by inhibiting thermal dark reversion and were not active in the dark. The first strong dominant GOF PHYB allele identified, i.e. YHB, contained a missense mutation in a universally conserved Tyr residue, whose expression not only triggered constitutive photomorphogenesis, but also fully suppressed shade avoidance responses (Su & Lagarias, 2007). Also highlighted in The Plant Cell at that time, this breakthrough arose serendipitously from studies identifying the same Tyr-to-His variant in a cyanobacterial phytochrome that was fluorescent and poorly photoactive (Fischer et al. 2004). The same substitution also confers GOF activity to phyA as well (Su & Lagarias, 2007). Only very recently was another strong dominant GOF missense allele of phyB identified by Jeong et al. (2016), which also affects a conserved Tyr residue near the bilin chromophore.
Dominant light-insensitive alleles of PHYB like YHB are powerful tools for investigating other light-dependent processes, such as photosynthesis, chlorophyll synthesis, photodamage, and processes regulated by the flavin-based blue-light photoreceptors in the cryptochrome, phototropin and light–oxygen–voltage-domain F-box protein families—again without the complication of differential activation of phytochrome signaling. For example, red light was shown to attenuate the YHB activity in Arabidopsis seedlings, unmasking a red-mediated, phytochrome-independent pathway that globally influence gene expression (Hu et al. 2009). It will be interesting to identify the nature of this light-mediated negative signaling pathway in the future.

Ongoing studies to fully understand the molecular basis of the activities of LOF and GOF variants of phyB will benefit from recent advances in structural biology studies. The crystal structure of the photosensory region of Arabidopsis phyB reported by Burgie et al. (2014) is a significant first step. The field looks forward to the successful determination of the structure of the full length phyB photoreceptor. Structural comparisons of LOF and GOF PHYB alleles will assuredly play a key role therein and are anticipated to illuminate the molecular basis of phyB signaling in plants.

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