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Photomorphogenesis, B-Box Transcription Factors, and the Legacy of Magnus Holm

How plants perceive and respond to light has fascinated us for millennia and was first noted by Aristotle in *De Anima*. Erasmus Darwin must also have reflected on it in his garden, as evidenced by several lines in *The Botanic Garden*, his epic poem about plant life published in 1789. Almost 100 years later, the first rigorous research on phototropism was performed by his grandson Charles Darwin, who reported his experiments in *The Power of Movement in Plants*, published in 1880. Subsequently, at the beginning of the 20th century, the importance of the daily duration of light in the control of flowering time, a process now known as photoperiodism, was recognized by Julien Tournois at the Ecole Normale Supérieure in Paris and was subsequently studied in depth by Harry Allard and Wightman Garner in Arlington, Virginia in the 1920s. The key roles of red and far-red light wavelengths were slowly revealed and led to the identification of phytochrome, the most famous of all plant photoreceptors, in the 1950s (Furuya, 1993). Since then, phytochrome research has advanced enormously and achieved a major milestone in 2005 with the first three-dimensional structure (Wagner et al., 2005). The dawn of molecular genetics in *Arabidopsis thaliana* has also permitted enormous progress, leading to discovery of the key intermediates COP1 and HY5. How they work together within the intricate machinery of photomorphogenesis has been an active research area for 25 years (Franklin and Quail, 2010; Lau and Deng, 2012). One of the proponents in this research area, Magnus Holm (Figure 1), passed away tragically in 2012, but some of the last research originating from his laboratory, in collaboration with Javier Botto, reported in this issue **(Gangappa et al., pages 1243–1257)**. The work fills in another piece of the photomorphogenesis jigsaw.

In nature, a seed is likely to end up under a layer of soil or litter, where there is little or no light available. Upon germination, the seedling will undergo etiolation, resulting in an elongated hypocotyl with an apical hook protecting the undeveloped cotyledons and shoot apical meristem, in pursuit of more favorable light conditions. If this strategy is successful, light activation of the photoreceptors will result in de-etiolation and the start of photomorphogenesis, during which the elongation of the hypocotyl is inhibited, the apical hook unfolds, and the cotyledons develop in order to harvest energy from light and to fix carbon from CO_2 (Sullivan and Deng, 2003). Photoprotective pigments, such as flavonoids and anthocyanins, are synthesized, which protect the seedling from the more damaging effects of light. The dramatic transition from an etiolated to a photomorphogenic developmental mode is initiated by blue (cryptochromes [CRY]), UV (UVR8), and red/far-red (phytochromes [phyA to phyE]) photoreceptors, acting to promote photomorphogenesis through a massive transcriptional reprogramming involving transcription factors working alongside both large-scale and finely tuned changes in chromatin structure (Charron et al., 2009; Bourbousse et al., 2012).

Figure 1. Magnus Holm (1968–2012).

Forward genetic screens first performed in *Arabidopsis* in the 1980s identified a group of nine genes referred to as the *COP/DET/FUS* genes (Franklin and Quail, 2010; Lau and Deng, 2012). Mutants in these genes display photomorphogenic growth in darkness, and their recessive nature indicated that they act to suppress deetiolation in the dark. Two of the encoded proteins, DET1 and COP10, are part of an E2 ubiquitin conjugating enzyme-like complex (Yanagawa et al., 2004), whereas six exist in another complex, the COP9 signalosome (Schwechheimer and Deng, 2000), which can regulate a subset of E3 ubiquitin ligases (Lyapina et al., 2001). The last, COP1, is an E3 ubiquitin ligase acting as a master regulator of the transition from etiolated to photomorphogenic development (Sullivan and Deng, 2003). COP1 is also part of a multimeric ~700-kD protein complex that includes SPA proteins, identified as suppressors of far-red light signaling (Saijo et al., 2008). Null *cop1* mutants are adult lethal, but weak alleles show pleiotropic effects throughout the plant life cycle, including high anthocyanin accumulation, dwarf stature, and early flowering. Moreover, the weak *cop1-6* allele is able to flower in constant darkness, suggesting that later developmental transitions are derepressed in the *cop1* mutant (McNellis et al., 1994).

Consistent with the key role that COP1 plays as a repressor of photomorphogenic development, transcriptome analyses have revealed a remarkable overlap between dark-grown *cop1* seedlings and light-grown wild-type seedlings (Ma et al., 2002). Thus, photoreceptor-dependent inactivation of COP1 is sufficient and absolutely necessary to initiate photomorphogenic growth. COP1 is localized to the nucleus in the dark (von Arnim and Deng, 1994), where it

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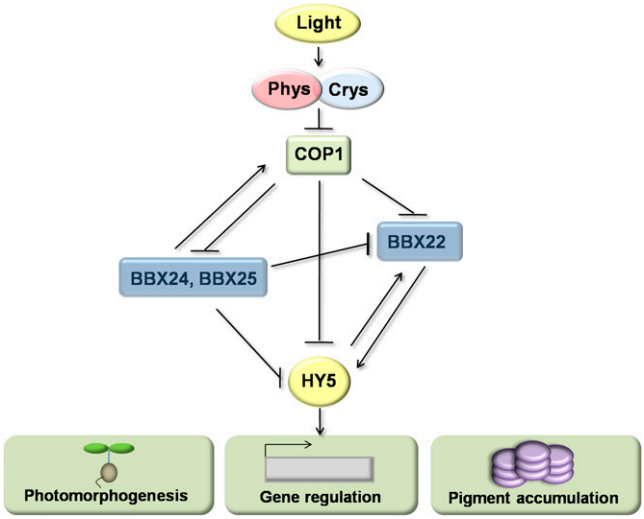

Figure 2. Proposed mode of action of BBX25 and BBX24 during seedling de-etiolation. COP1 negatively regulates HY5 and BBX22 proteins by targeting them to the 26S proteasome. HY5 induces the expression of *BBX22*, which in turn enhances the function of HY5. BBX25 and BBX24 negatively regulate *BBX22* expression by reducing HY5, interacting through the bZIP domain. COP1 may attenuate BBX25 and BBX24 function by targeting them for degradation in the light. (*Figure provided by Sreeramaiah Gangappa and Silvia Ibarra Figure based on Gangappa et al. [2013], Figure 10.*)

acts to target a range of positive regulators of photomorphogenesis for degradation via the 26S proteasome. Upon transfer to light, COP1 activity is inhibited by photoreceptor-dependent exclusion from the nucleus (Osterlund et al., 2000). In addition, CRY blue light photoreceptors interact with COP1, and light activation of CRY proteins was recently shown to directly inhibit COP1 activity in the nucleus (Liu et al., 2011; Zuo et al., 2011).

The resulting inhibition of COP1 leads to accumulation of factors that promote photomorphogenic development, most notably HY5 (Osterlund et al., 2000) and HYH, which was identified by Magnus in his first major contribution to the plant field during post-doctoral research with one of the authors (Deng) at Yale University (Holm et al., 2002).

Magnus joined the Deng laboratory at Yale University in 1998 after completing his doctorate entitled "Function and Calcium Regulation of AML1 and Basic-Helix-Loop-Helix Transcription Factors" from Umeå University, Sweden. Fascinated by plants after a PhD working with animals, Magnus wanted to switch to plant research and learn to use the plant genetic model system *Arabidopsis*. At Yale, Magnus started working on *Arabidopsis* COP1 and focused on how COP1 orchestrates downstream actions at the molecular level. Ironically, after leaving the animal field, Magnus made a discovery in *Arabidopsis* that ended up with a greater impact to the animal field. Magnus found a "consensus COP1 binding motif" on COP1 target proteins, such as HY5, STO, and HYH, which directly contacts residues in the WD40 domain of COP1 (Holm et al., 2001). This COP1 binding consensus turned out to be highly conserved, not only in plants, but also in animals. Although COP1 was first known to have homologs in mammals in 1999 (Wang et al., 1999), the biology of COP1 in animals was not understood until several years later. Guided by the COP1 binding consensus motif that Magnus discovered in *Arabidopsis*, several important targets of human COP1 were identified. These studies have so far linked human COP1 to lipid metabolism and hematopoiesis (through tribbles proteins; Qi et al., 2006; Keeshan et al., 2010), gluconeogenesis (through TORC2; Dentin et al., 2007), and stress and mitogenic pathways (through c-Jun and the ETS family of transcription factors; Bianchi et al., 2003; Baert et al., 2010; Vitari et al., 2011) and have subsequently led to the revelation that human COP1 is a tumor suppressor (Migliorini et al., 2011; Vitari et al., 2011).

In *Arabidopsis*, after HYH, Magnus identified six other COP1- and/or HY5-interacting proteins through yeast two-hybrid screens. Five of these proteins contain tandem repeated B-boxes (Zn^{2+} binding domains). The finding that COP1 interacts with B-box proteins is quite interesting since the COP1 protein contains three protein interaction domains: a RING finger, a coiled-coil, and a WD40 domain. COP1 interacts through the WD40 domain with the COP1 binding consensus motif in the B-box proteins (Holm et al., 2001), thus leaving the RING, coiled-coil, and B-box domains free to interact with other proteins.

Magnus brought the COP1-interacting proteins with him when he started his own laboratory at Gothenburg University in 2002, and since then he showed that three of them act as positive regulators of light-dependent development (Datta et al., 2006, 2007, 2008) and that some are involved in crosstalk between light and the phytohormone auxin (Sibout et al., 2006). In the last few years, evidence has also emerged that BBX proteins are additionally involved in the molecular mechanisms regulating the shade avoidance syndrome that allows plants to compete efficiently in shaded environments. Magnus also contributed to this with an article on the role of BBX21 in shade avoidance (Crocco et al., 2010), which was selected for an addendum in *Plant Signaling and Behavior* (Crocco et al., 2011).

In the new article, Gangappa et al. (2013) describe the functional characterization of BBX25 together with BBX24 in photomorphogenesis and hypocotyl shade avoidance response. In contrast with the previously published BBX4, BBX21, and BBX22 factors (Datta et al., 2006, 2007, 2008; Sibout et al., 2006; Crocco et al., 2010), BBX24 and BBX25 are negative regulators of light signaling and they act additively in the control of de-etiolation. Using a range of in vitro and in vivo methods, it is shown that BBX25 physically interacts with the bZIP domain of HY5 through its B-boxes, as previously shown for BBX24, and that they both regulate

anthocyanin gene expression in a HY5-dependent manner (Figure 2). Then, to examine the genetic relationship with COP1, a range of double and triple mutants were generated and analyzed. It was found that *bbx25* and *bbx24* mutations can additively enhance *cop1* mutant hypocotyl phenotypes irrespective of light (or dark) conditions.

To further understand how BBX24 and BBX25 affect photomorphogenesis, the authors then searched for transcription factor targets, and it was found that together they can negatively regulate the expression of *BBX22*. It was shown previously that *BBX22* expression is regulated directly by HY5 binding to its promoter (Chang et al., 2008) and that COP1 degrades BBX22 in the dark (Datta et al., 2008; Chang et al., 2011). Based on the combinatorial mutant analysis, it is therefore possible that they regulate *BBX22* expression either by altering HY5 or COP1 activity. Both hypotheses were examined, and it was found that the mechanism of action of BBX24 and BBX25 is most likely mediated by reducing the function of HY5 by forming inactive heterodimers.

In a final twist, the function of BBX24 and BBX25 in the hypocotyl shade avoidance response was examined. In shaded environments, different members of the group IV BBX factors (double B-box without a CCT domain; Khanna et al., 2009) have opposite functions (Crocco et al., 2010). Specifically, BBX21 and BBX22 act as negative regulators, whereas BBX24 and BBX25 promote hypocotyl elongation in shade (Crocco et al., 2010). In contrast with their role during de-etiolation, Gangappa et al. found that BBX24 and BBX25 can switch roles and become independent of HY5 during shade avoidance. This is not the first example of an inversion of function in the regulation of specific aspects of photomorphogenesis. Most notably, while COP1 is a negative regulator of cryptochrome- and phytochrome-activated responses, it acts positively to regulate processes activated by UVR8 photoreceptors (Oravecz et al., 2006).

Magnus was promoted in spring 2012 to Professor of Plant Molecular Biology in the Department of Biological and Environmental Sciences at Gothenburg University in Sweden. His untimely death in the summer of the same year has saddened many. As stated by Cornelia Spetea Wiklund (a professor in the same department) at the Minnestund (memorial service) for him in Gothenburg, "He was a great group leader, an excellent scientist, a good colleague and a glad teacher. He is not among us any longer and we will miss him very much." So will the photomorphogenesis research community.

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