COP1/SPA ubiquitin ligase complexes repress anthocyanin accumulation under low light and high light conditions

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Abbreviations: COP1, constitutive photomorphogenic 1; CRY1, cryptochrome 1; HY5, elongated hypocotyl 5; PAP, production of anthocyanin pigment; SPA, supressor of phytochrome A-105

In Arabidopsis and many other plant species, anthocyanin pigments accumulate only after light exposure and not in darkness. Excess light of very high fluence rates leads to a further, very strong increase in anthocyanin levels. How excess light is sensed is not well understood. Here, we show that mutations in the key repressor of light signaling, the COP1/SPA complex, cause a strong hyperaccumulation of anthocyanins not only under normal light but also under excess, high light conditions. Hence, normal light signaling via COP1/SPA is required to prevent hyperaccumulation of anthocyanins under these high light conditions. However, since cop1 and spa mutants show a similar high-light responsiveness of anthocyanin accumulation as the wild type it remains to be resolved whether COP1/SPA is directly involved in the high-light response itself.

Anthocyanins are secondary metabolites belonging to the class of flavonoids. They range in color from red to blue and provide a visual cue to flowers and fruits to help attract animals for pollination or seed dispersal.1 In vegetative tissues anthocyanins are involved in processes such as protection of the photosynthetic apparatus, herbivory and free-radical scavenging.2 Anthocyanin biosynthesis increases in response to environmental stresses such as nutrient deficiency, drought, low temperature, pathogen infection and high light.3 Regarding the protection of the photosynthetic apparatus, it was proposed already decades ago that anthocyanins shade the photosynthetic apparatus from excess light and therefore reduce susceptibility to photo-inhibition.4 Excess light was shown to damage the photosynthetic apparatus at least in part by reactive oxygen species (ROS) generated by excitation energy and electrons leaking from the photochemical reactions and electron transport system.5

In Arabidopsis and many other species, anthocyanin production is a feature of light-grown plants and does not occur in darkness. The biosynthesis of anthocyanins is controlled by transcription factors which induce the expression of structural genes in the anthocyanin biosynthesis pathway. Anthocyanin biosynthesis in response to light involves the transcription factor ELONGATED HYPOCOTYL 5 (HY5) and a protein complex consisting of MYB-bHLH-WD40 transcription factors (MBW complex).6-10 Both, HY5 and the MBW transcription factors PRODUCTION OF ANTHOCYANIN PIGMENT 1 (PAP1, also called MYB75) and PAP2 (also called MYB90) as members of the MBW complex are regulated by light on the transcriptional and posttranslational levels.11-14 Posttranslationally, HY5, PAP1 and PAP2 proteins are ubiquitinated in dark-grown seedlings via the CONSTITUTIVE PHOTOMORPHOGENIC 1/ SUPPRESSOR OF PHYTOCHROME A-105 (COP1/SPA) E3 ubiquitin ligase.11,12 When seedlings or plants are exposed to light, the light-activated photoreceptors inhibit the activity of the COP1/SPA ubiquitin ligase, thereby causing stabilization of the HY5, PAP1 and PAP2 proteins. The COP1/SPA complex consists of COP1 and members of the 4-member SPA protein family (SPA1-SPA4). Defects in COP1 or SPA1 genes cause constitutive
photomorphogenesis in darkness with seedlings showing features of light-grown seedlings in complete darkness.\textsuperscript{15-18}

We have recently shown that the constitutive production of anthocyanins in dark-grown \textit{cop1} and \textit{spa} mutants involves the failure to degrade PAP1 and PAP2 proteins in darkness.\textsuperscript{12} In these experiments, we had observed that \textit{cop1–4} mutants – besides producing anthocyanin in darkness – were hyperresponsive to light, accumulating much higher anthocyanin levels in light-grown seedlings when compared to the wild type. Hence, \textit{cop1–4} mutant seedlings were not truly constitutively photomorphogenic but retained considerable light responsiveness, suggesting that light did not fully inactivate the COP1/SPA complex under the light conditions used (40 \(\mu\text{mol m}^{-2}\text{s}^{-1}\) white light). To further investigate this phenomenon we grew \textit{cop1}, \textit{spa} triple and \textit{spa} quadruple mutants under higher fluence rates of light.

\textbf{Figure 1A} shows that dark-grown \textit{cop1} and \textit{spa} mutants - here in particular \textit{cop1–6} and the \textit{spa} quadruple mutant – accumulated anthocyanins while the wild type did not. This phenotype reflects the constitutive photomorphogenesis of \textit{cop1} and \textit{spa} mutants as mentioned above. When grown in white light up to 125 \(\mu\text{mol m}^{-2}\text{s}^{-1}\), \textit{cop1} and \textit{spa} mutant seedlings accumulated higher levels of anthocyanin than the wild type. This was particularly pronounced at low fluence rates (5 and 20 \(\mu\text{mol m}^{-2}\text{s}^{-1}\)) at which wild-type seedlings accumulated only very low levels of anthocyanins.
anthocyanin when compared to cop1 and spa mutants or wild-type seedlings grown at higher fluence rates. These results confirm that cop1 and spa mutations affect anthocyanin levels not only in darkness but also in the light. However, the light-response of cop1–4 and spa mutants appeared to saturate at 40 μmol m⁻² s⁻¹, implying that there may be COP1/SPA-independent mechanisms controlling anthocyanin production under high light. On the other hand, the cop1–6 mutant showed a non-saturating further increase in anthocyanin production over the fluence rates used.

To further analyze the Arabidopsis high-light response we grew Arabidopsis wild type, cop1 and spa mutants under true high light fluence rates of 800 μmol m⁻² s⁻¹. Since germinating seedlings did not tolerate such excess light, we exposed 10-day-old rosette-stage plants grown in normal light (100 μmol m⁻² s⁻¹) to high light for 12 h. All genotypes responded to high-light treatment with a strong increase in anthocyanin accumulation (Fig. 1B). Again, cop1–6 and spa quadruple mutants showed approx. 6 or 9-fold higher anthocyanin levels, respectively, than the wild type (Fig. 1B). cop1–4 and the two spa triple mutants exhibited a less severe phenotype. Hence, defects in the COP1/SPA E3 ligase cause hyperaccumulation of anthocyanin when compared to the wild type also under these high-light conditions. When analyzing the responsiveness to high light, the wild type and all cop1 or spa mutants exhibited a similar 3-4-fold increase in anthocyanin levels in high light relative to normal light (Fig. 1C). Hence, while cop1 and spa mutants exhibited much higher absolute anthocyanin levels than the wild type in high light, the responsiveness of these mutants to high light was not significantly different from that of the wild type. Therefore, the responsiveness of anthocyanin accumulation due to high light exposure may be COP1/SPA-independent, with COP1/SPA mainly being a prerequisite for a high-light response but not being directly involved in the high light response itself. This conclusion would be consistent with the idea that photoreceptor activities are thought to saturate at fluence rates much below 800 μmol m⁻² s⁻¹. On the other hand, the photoreceptor cry1 was shown to be required for normal high-light responsiveness in Arabidopsis. Most importantly, cry1 mutants showed a strongly reduced high light-induced expression of PAP1, PAP2 as well as of structural genes in the anthocyanin biosynthesis pathway. Together, the data suggest that cryptochrome signaling via COP1/SPA is required for normal accumulation of anthocyanins under high light conditions. Consistent with this idea, a downstream component of COP1/SPA signaling, HY5, was shown to be required for the accumulation of anthocyanins under high light. Whether and, if so, how photoreceptors and other mechanisms sensing excess light, e.g. the redox state of the electron transport chain in the chloroplast, co-act in the regulation of gene expression in response to high light remains to be resolved.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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