Abstract

Light is the basis of all life on earth and does not only provide the plants with energy, it is also an important source of information. Already during the process of germination, light exerts a great influence on the further development and the continuous growth of the plant. As a central repressor of photomorphogenesis, the COP1-SPA E3 ubiquitin ligase complex is responsible for the degradation of transcription factors, which are needed for a light response. The activity of the COP1-SPA complex is regulated by the interaction with photoreceptors, which contribute to the inactivation of the COP1-SPA complex. Overall, *Arabidopsis thaliana* has four SPA proteins, which have common, but also distinct functions in light signal transduction. While SPA3 and SPA4 are required primarily for vegetative growth and flowering, SPA1 and SPA2 regulate the suppression of photomorphogenesis in the dark. In particular SPA2 shows a strong regulation to light exposure. Already a brief irradiation of the seedling with light has the consequence that SPA2 is rapidly degraded, resulting in immediate inactivation of the COP1-SPA complex. It is assumed that the instability of SPA2 is caused by the interaction with the phytochrome photoreceptors and depends on the ubiquitination activity of COP1. Since the COP1-SPA complex was shown to be part of the CULLIN4-based E3 ligase, the CUL4-DDB1$^{COP1-SPA2}$ complex, we investigated whether the degradation of SPA2 is exclusively mediated by COP1 or depends on the CULLIN4-based E3 ligase activity. In the present work, the hypothesis that SPA2 degradation is independent on CULLIN4 (CUL4) activity and depends on the auto-ubiquitination activity of the COP1-SPA2 complex could not be confirmed. Instead, it could be shown that the degradation of SPA2 is subjected to a CULLIN4-based E3 ligase mechanism. In mutants, in which the function of CUL4 was impaired, higher SPA2 protein levels in the dark or a slower SPA2 degradation was detected. Furthermore, it could be revealed that the degradation of SPA2 is regulated by another CUL4-based E3 ligase, the CUL4$^{COP10-DET1-DDB1}$ (CDD) complex. Since SPA2 interacts with Damaged DNA Binding 1 (DDB1) to form the CUL4-DDB1$^{COP1-SPA2}$ complex, targeted point mutations in the SPA2 WD-Repeat domain were generated to separate SPA2 from the CUL4-based E3 ligase complex. Based on the idea to separate SPA2 and DDB1, a putative helical motif was identified in SPA2 that could mediate the interaction between DDB1 and SPA2. Due to the fact that degradation of SPA2 is dependent on phytochrome A, new phytochrome interaction site in SPA2 was identified which may be responsible for SPA2 degradation. In independent approaches phytochrome A was shown to interact with both the N-terminus and the C-terminus, presumably the WD repeat domain of SPA2. At the same time, transgenic lines lacking functional N-terminus or the WD repeat of SPA2 exhibited no degradation of the truncated protein in the light. Interestingly, the transgenic SPA2 line lacking a functional N-terminus exhibited a skotomorphogenic phenotype both in the dark and in light and almost lost the ability to perceive light.