B-box Proteins in Light-regulated Development in Arabidopsis

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Abstract

COP1 and HY5 are two key regulators of light signaling in plants. Proteins interacting with either could therefore be important regulators of light-dependent development. Previous yeast two-hybrid screens, using COP1 or HY5 as bait, identified several putative regulators of light signaling. We isolated T-DNA insertion mutants in three of these genes COL3, STH2 and STH3. Phenotypic characterization of these mutants revealed pigmentation, hypocotyl and root phenotypes suggesting that the genes have a positive role in light-regulated processes. Moreover, study of double mutants with hy5 and cop1 confirmed that all of them genetically interact with both HY5 and COP1.

COL3, STH2 and STH3 encode proteins containing N-terminal B-boxes. B-boxes are zinc-ligating domains consisting of conserved cysteine and histidine residues. In animals, B-boxes are often found together with a RING finger domain (originally termed A-box) and a coiled-coil domain forming RBCC or tripartite motif (TRIM) proteins. Although RBCC proteins are absent in Arabidopsis, there are 32 proteins with N-terminal B-boxes. This thesis deals with the characterization of the B-box containing proteins, COL3, STH2 and STH3 and the study of their role in light-regulated development of plants.

Our results show that the B-boxes play multiple roles in plant development. We found that the B-boxes in COL3 were required for localization of the protein into nuclear speckles. In STH2 and STH3, the B-box domain was found to be important for interaction with HY5, providing evidence for the role of the B-box domain in protein-protein interaction. Transient transfection assays in protoplasts indicated that functional B-box domains in STH2 and STH3 are required for transcriptional activation. We hypothesize that the B-box proteins might act as co-factors for the transcription factor HY5, regulating light-mediated transcription and development.

COP1 acts as an E3 ubiquitin ligase that targets positive regulators of photomorphogenesis for degradation in the dark. We found that COP1 could ubiquitinate STH3 in vitro suggesting that STH3 might be regulated by COP1. Our results show that COL3 co-localizes with COP1 in nuclear speckles and the two proteins interact physically. Moreover, our genetic studies show that col3, sth2 and sth3 partially suppress cop1 in the dark. All these interactions allow us to place COL3, STH2 and STH3 in the light-signaling network. Thus, starting from preliminary yeast interaction data, my doctoral work provides genetic, physiological and functional evidence for the role of B-box containing proteins in light-signaling.
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AKADEMISK AVHANDLING

för filosofie doktorsexamen vid Göteborgs Universitet, kommer att försvaras offentligt
i sal ‘Inge Schiöler’, Medicinaregatan 11, Göteborg, Fredagen den 17 oktober, 2008,
klockan 13.00

Faculty opponent: **Dr. Chris Bowler,**
CNRS UMR 8186 - Biologie Moléculaire des Organismes Photosynthétiques
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Avhandlingen basera på följande arbeten:

**Paper I**


**Paper II**


**Paper III**