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

Datta, S., Hettiarachchi, G.H.C.M., Deng, X.W., and Holm, M. (2006).
Arabidopsis CONSTANS-LIKE3 is a positive regulator of red light signaling and root growth.
Plant Cell 18**,** 70-84.

Paper II

Datta, S., Hettiarachchi, C., Johansson, H., and Holm, M. (2007).
SALT TOLERANCE HOMOLOG2, a B-box protein in *Arabidopsis* that activates transcription and positively regulates light-mediated development.
Plant Cell 19**,** 3242-3255.

Paper III

Datta, S., Johansson, H., Hettiarachchi, C., Irigoyen, M.L., Desai, M., Rubio, V., and Holm, M. (2008).
LZF1/SALT TOLERANCE HOMOLOG 3, an *Arabidopsis* B-box protein involved in light-dependent development and gene expression undergoes COP1-mediated ubiquitination.
Plant Cell (in press)

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