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Functional analysis of various conserved domains of NPH3 involved in phototropism in Arabidopsis thaliana

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Phototropism – the directional growth (curvature) for a plant towards light, is a very important adaptive response in plants in order for them to maximize photosynthesis. Blue light triggers phototropic response in Arabidopsis thaliana via the dominant photoreceptor phototropin 1 (phot1). Genetic studies have identified several genes that encode phot1 interacting proteins. Of these, currently, only NON PHOTOTROPIC HYPOCOTYL 3 (NPH3) is known to be absolutely required for phototropism. nph3 mutants are completely aphototropic, resembling phot1 null mutants. NPH3 is a phosphoprotein containing a BTB and a coiled coil domain, and the dephosphorylation of this protein into its active state in light is known to be entirely phot1 dependant. Yet little is known of the role of NPH3 as a mediator in the phototropic signal-response pathway. To better understand the role of conserved domains of NPH3 in phototropism, a range of serial deletions and mutants of NPH3 were generated, driven by its own native NPH3 promoter and the constitutive CaMV 35S promoter in nph3-6 and wild-type Col-0 backgrounds respectively. All truncated and mutant NPH3 proteins were also translationally fused with a green fluorescent protein (GFP). Multiple T3 homozygous transgenic lines were evaluated by comparing average angles of hypocotyl curvature with those of aphotoropic nph3-6 and wild-type Col-0. Over-expression of NPH3 or different portions of NPH3 in Col-0 resulted in reduced phototropism. Selective expression of the NPH3 domains under the native promoter could not complement the null nph3-6 phenotype. The alterations in the subcellular localization of these transgenic lines were also investigated using confocal fluorescence microscopy.