Arabidopsis FHY1 and FHL function in phyA signaling

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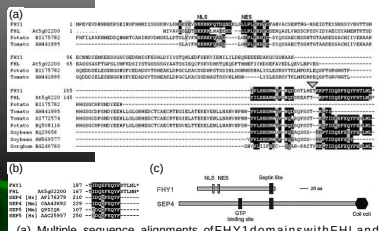
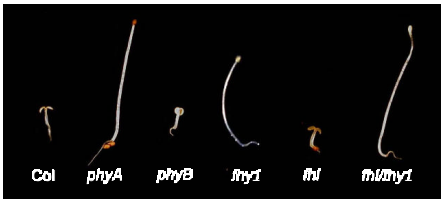
Plants use the photoreceptor family of phytochromes to sense their light environmentin thered/far-redregionof the spectrum.PhytochromeA(phyA)is the primary photoreceptor that regulates early seedling development. phyA mediated seedling de-etiolation is the critical developmental transition from heterotrophictophotoauxotrophicgrowth.Irradiationwithhighfluenceratefarred provides a w a y toassess specifically the role of phyAin this processand was used to isolatephyAsignalingintermediates. *fhy1-3* isaninsertionmutantofa geneencodinga phyAsignal transductioncomponent. FHY1 is a s mall24 kDa protein with little similaritytoknownproteins,besidesa smallconservedseptin-related domain at the C-terminus, a nuclear localization signal (NLS) and a nuclearexclusionsignal(NES).TheNLSandNESofFHY1areindeedinvolvedin itsnuclearlocalizationandexclusion(Zeidleretal.2004).Nuclearlocalizationof FHY1 is needed for theexecutionofresponsesdownstreamofphyA.FHY1has one homologous gene inArabidopsis, FHL (*FHY1 L*ike), for which overlapping functionalitywithFHY1hasbeendemonstrated(Zhouetal.2005).Wegenerated double *fhl/fhy1* mutantlinesandanalysedthephysiologicalresponseinHIR(high irradianceresponse) conditions.FHY1andFHLbotharespecificallyimpairedin phyA signaling. The double knockout lines show a very strong phenotype, comparabletothe *phyA* nullmutant.Thisphenotypeisnotduetochangesinphy A levels. *In vivo* interaction studies using BiFC showedthat FHY1 and FHL form homoandheterodimers.In CFP:FHY1andphyA:GFP cotransformedprotoplasts light dependent intracellular distribution was analysed and co-translocation as wellasco-localization ofphyAandFHY1couldbeobserved.

FHY1 has homologues inArabidopsis and other plant species



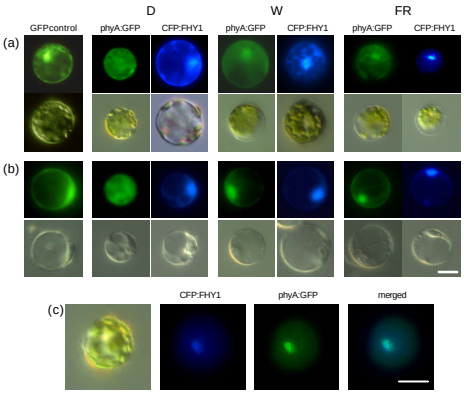
(a) Multiple sequence alignments ofFHY1domainswithFHLand predicted translation products from other species. The nuclear localization (NLS) and exclusion (NES) motifs are indicated. (b) sequencealignmentofFHY1andFHLseptinlikedomainwithseptins from *H.sapiens* and *M.musculus*. **(c)** SchematiccomparisonofFHY1 featureswiththosefoundinseptins.

Physiology



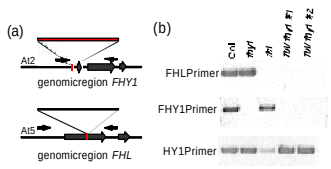
Phenotypesof4doldseedlingsgrownin5µmol/m^{2}s FR

FHY1andphyAco-localize *invivo*



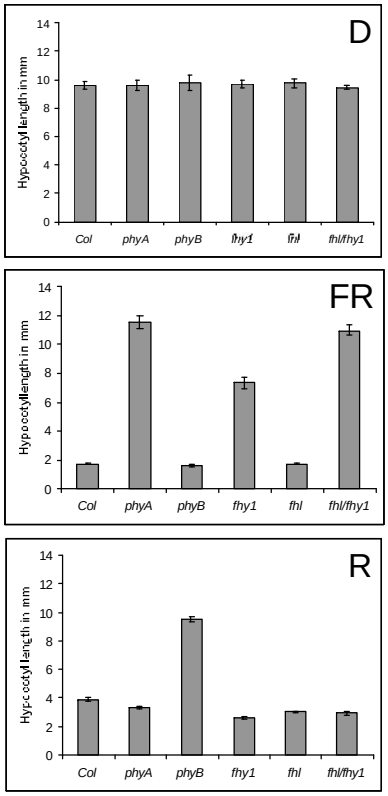
Localization of YFP, phyA:GFP and CFP:FHY1 in Arabidopsis wildtype protoplastsafter16hincubationinDorirradiationwith30µmolm^{-2}s^{-1}W or5µmol m^{-2}s^{-1}FR.(a)Localizationinlightgrownprotoplasts.(b)Localizationinprotoplasts frometiolatedseedlings.(c)Co-localizationofphyA:GFPandCFP:FHY1after14 hincubationinDand6hirradiationwith5µmolm^{-2}s^{-1} FR.Scalebarsrepresent25 µm.

Generation andanalysis o f a *fhl/fhy1* doublemutant



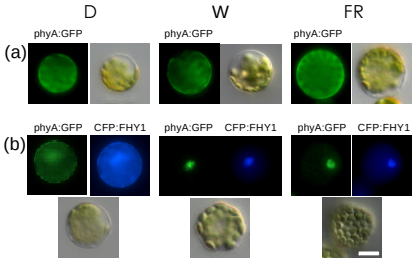
(a) Illustration of the FHY1andFHLgenomic loci with the T-DNA insertions.(b)PCRanalysisofthy1,fhlandfhl/fhy1homozygouslines withsequence-specificoligonucleotides.Sizeofamplifiedproducts:FHL 520bp;FHY11190bp;HY11600bp.

FHY1andFHLhaveaspecificphyAphenotype



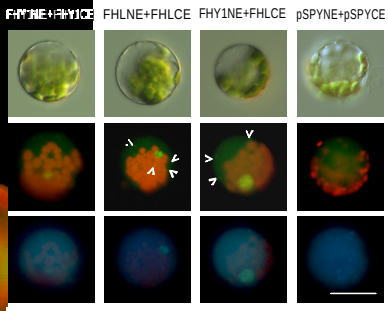
Phenotypes of 4 d old FR grown seedlings. Hypocotyl length measurementsof4doldseedlingsgrownindarkness(D),FR5µmolm^{-2}s^{-1}, andR5µmolm^{-2}s^{-1}. Columnsrepresentmeanhypocotyllengthfrom3 independentexperiments(20-30seedlingseachexperiment).Errorbars representthestandarderror.

phyA nuclearimport is impaired in fhl/fhy1



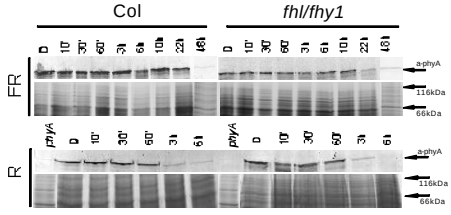
(a)LocalizationofphyA:GFPinArabidopsis *fhl/fhy1* protoplastsafterincubationin Dorirradiationwith30µmolm^{-2}s^{-1}W or5µmolm^{-2}s^{-1} FR.(b)Localisationof phyA:GFP andCFP:FHY1 inco-transformedArabidopsis *fhl/fhy1* protoplasts after16hincubationinDorirradiationwith30µmolm^{-2}s^{-1}W or5µmolm^{-2}s^{-1}FR. Scalebarrepresents25µm.

BiFC



BiFC (bimolecular fluorescence complementation) visualisation of FHY1 homodimerisation, FHL homodimerisation and FHY1-FHL heterodimerisation in Tobacco protoplasts. Protoplasts expressing transientlyFHY1orFHLC-terminalfusionwithYFPN-terminal(NE)or C-terminalhalf(CE)(Walter *etal*. 2004).Co-expressedunfusedNand C-terminal halfesofYFP(pSPYNE,pSPYCE)shownosignificant fluorescence. FHY1homodimerisationwas detectedinthenucleus, whereas FHL homodimerisation as well as FHL and FHY1 heterodimerisationcouldalsobedetectedinthecytoplasm.Scalebar represents30µm.

fhl/fhy1 does notchangephyAlevels



KineticofphyAdegradationin4doldwildtypeandfhl/fhy1seedlingsirradiatedwith5 µmolm^{-2}s^{-1}FRor5µmolm^{-2}s^{-1}R fortheindicatedtime.Theupperpanelshowsproteins immunostainedwitha-phyAantibodyinaWesternblotanalysis.Thelowerpanelshowsa similargelstainedwithCoomassie.

Walter,M.,Chaban,C.,Schutze,K.,Batistic,O.,Weckermann,K.,Nake,C.,Blazevic,D.,Grefen,C.,Schumacher,K.,Oecking,C.,Harter,K.,and Kudla,J. (2004)Visualizationofprotein interactionsinlivingplantcellsusingbimolecularfluorescencecomplementation.Plant J.**40**:428-438.

Zeidler,M.,Bolle,C.,andChua,N.H. (2001)Thephytochrome Aspecificsignalingcomponent PAT3isapositiveregulatorofArabidopsis photomorphogenesis.PlantCellPhysiol.**42**:1193-1200.