

8.2.6. Exploring plastid division pathways in *Arabidopsis*

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Plastids arise by division and the control of division site placement is mediated in part by AtMinD1 and AtMinE1 in *Arabidopsis*. We have shown that AtMinE1 is a topological specificity factor and that AtMinE1 forms homodimers and heterodimers with AtMinD1. AtMinE1 and AtMinD1 show specific intraplasmic localisation patterns and a C-terminal helix deletion in AtMinD1 results in inappropriate localisation and disrupted plastid division. Using FRET we have shown that AtMinD1 is capable of forming homodimers inside chloroplasts and that this dimerisation capacity is abolished by a single C-terminal A296G mutation. Our data demonstrates that AtMinE1 and AtMinD1 act in concert and that the formation of AtMinD1 homodimers and AtMinD1/AtMinE1 heterodimers is paramount for correct intraplasmic localisation patterns and correct division machinery placement. The assembly of the different AtFtsZ proteins in chloroplasts in relation to the AtMinD1/AtMinE1 protein complex will also be discussed. GIANT CHLOROPLAST 1, a positive factor involved in plastid division will be briefly described. A working model of plastid division will be presented.

8.2.7. Friendly: a mitochondrial dynamics mutant defective in mitochondrial inheritance?

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Mitochondria are ubiquitous and vital eukaryotic organelles. Although identified over 50 years ago as the site of oxidative energy metabolism little is known about the genetic control of mitochondrial shape, size, number and distribution (collectively termed mitochondrial dynamics) in higher plants. We identified a suite of *Arabidopsis* mutants with altered mitochondrial dynamics and are using these as tools to identify the underlying genes, proteins and mechanisms. In one mutant, *friendly* (*fmt*), mitochondria form clusters of tens of organelles. The *fmt* locus was identified and the gene, *FMT*, is homologous to the *D. discoideum* *cluA* and *S. cerevisiae* *CLU1* genes that are known to be involved in the maintenance of the correct cellular distribution of mitochondria. Apart from a tetratricopeptide repeat domain, the FMT protein has no significant homology to proteins of known function. We will present data testing the hypotheses that: (i) FMT is involved in association of mitochondria with the cytoskeleton and (ii) the clustered mitochondrial phenotype in *friendly* perturbs the stochastic inheritance of mitochondria into daughter cells during cell division.

8.3.1. Specifying the site of tip growth and the plane of asymmetric division in fucoid algae

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Eggs of fucoid algae are initially apolar cells and fertilization initiates a developmental program that breaks the symmetry resulting in selection of a site of localized tip growth. Growth site selection, which is influenced by a variety of environmental vectors, involves assembly of a cortical actin network nucleated by an Arp2/3 complex at the presumptive growth site. This nascent axis is labile and perception of subsequent spatial cues results in disassembly of the existing cortical actin and reassembly at the newly established growth site. Once tip growth has been initiated, the growth axis positions the mitotic spindle, which in turn determines the plane of asymmetric division. The spindle is positioned by centrosomal microtubules that interact with the cell cortex preferentially at the growth site, perhaps mediated by the microtubule-associated protein EB1. We anticipate that identification of additional molecules involved in signal transduction, actin assembly and microtubule search and capture will be facilitated by ongoing genomics projects.

8.3.2. Immediate upright (*imm*), a mutant of the marine macroalga *Ectocarpus siliculosus* (Phaeophyceae, Ectocarpales) affected in polarity and development

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Sporophyte and gametophyte are independent generations in *Ectocarpus siliculosus*. Wild-type sporophytes show bilateral

symmetric germination resulting in the development of a prostrate, branched filament. The first germ tube is positively phototropic in response to unilateral white light. Erect thalli develop by 'mediate differentiation' i.e. they do not form before the prostrate thallus has reached a certain size. Gametophytes, in contrast, exhibit a developmentally asymmetric bilateral germination and 'immediate differentiation'. The first germ tube is negatively phototropic and develops into a thin rhizoid, whereas the second germ tube immediately develops into a broader and erect (positively phototropic) filament. We describe a spontaneous mutant *immediate upright* in which the sporophyte has a germination pattern resembling that of gametophytes, including negative phototropism of the first germ tube and immediate development of an upright filament. Crossing studies showed that the mutation is recessive compared to the wt and is located on an autosome. This is the first genetic study of a developmental mutant in *Ectocarpus*.

8.3.3. Signalling, actin, endosomes and root hair tip growth

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Root hair tip-growth is actin- and signalling-dependent process. Both endogenous and YFP-tagged SIMK, a stress-induced mitogen-activated protein kinase (MAPK), accumulate in vesicle-rich tip regions of *Medicago* and *Arabidopsis* root hairs. SIMK distribution and function correlates with the organization of the actin cytoskeleton visualized by immunolabelling and with new F-actin *in vivo* marker GFP-ABD2. MAPK inhibitor UO126 abolished tip growth by disrupting vesicular traffic but overexpression of constitutively active SIMK overcomes this growth inhibition, suggesting a role of SIMK in actin-dependent vesicular traffic. Overexpression of SIMK activator SIMKK in *Arabidopsis* resulted in overactivation of AMPK6 (SIMK homologue) and phenotype of ectopic and branched root hairs. Additionally, several MAPKs associate with endomembranes and endosomes in control and stressed cells. New endosomal molecular markers FYVE and RabF2a were developed and will be presented as useful tool for studies on actin-dependent motility of endosomes in root hairs.

8.3.4. Microtubules in *Medicago truncatula* root hairs during hair development and response to rhizobial signal molecules.

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Tip-growing legume root hairs have two populations of microtubules: cortical microtubules (CMTs), which are present in all stages of root hair development, and - differently from *Arabidopsis* root hairs - a dense array of endoplasmic microtubules (EMTs), which the hairs acquire during the initiation of tip-growth and maintain until growth stops. EMTs are crucial to the polar distribution of cytoplasm, to nuclear positioning, and to a high growth rate.

In tip-growing hairs, signal molecules of symbiotic rhizobacteria (Nod factors) cause a subtle and transient shortening of the EMT array, whereas in growth-arresting hairs this effect is more pronounced and leads to a complete disintegration of the EMT array. The latter have a short EMT array and respond to Nod factors with a distinct change in hair morphology, called root hair deformation. The Nod-factor induced disappearance of EMTs correlates with a loss of polar cytoarchitecture and straight growth directionality, whereas the reappearance of EMTs correlates with the new set up of polar cytoarchitecture and new growth potential in growth-arresting hairs. CMTs showed no obvious response to Nod factors.

8.3.5. Vacuole form and movement in growing root hairs.

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Tip-growing root hairs are useful models of polarized plant cell growth. Root hair vacuoles must expand rapidly as hair tips grow to maintain turgor pressure through water uptake. Vacuolar