# STEM CELL SIGNALLING IN ARABIDOPSIS, ROLE OF MAPKS IN MERISTEM FUNCTION

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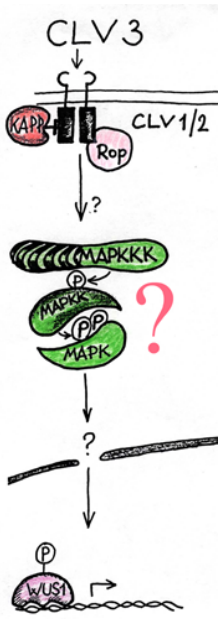
Background

Plant development relies on the maintenance and function of stem cells over their entire lifespan, a source of cells at the two apices, called root and shoot meristems. Both the renewal of a precise number of stem cells and the differentiation of many mature cell types from these cells depend on cell-cell communication. Some components in this signalling within the shoot meristem have been uncovered by genetic analysis, such as the involvement of a small peptide (Clv3), receptor like kinases (Clv1/2) or transcription factors (e.g. Wus1), and interaction screens uncovered the involvement of a G-protein (Rop) and a protein phosphatase (a negative regulator), but intracellular signalling mechanisms connecting receptors to the activation of specific genes are unknown (Figure 1). The mitogen-activated protein kinase (MAPK) pathways composed of at least three interlinked protein kinases provide versatile modules in eukaryotes to connect sensors (receptors) to intrinsic responses. There are 23 MAPKs, 10 MAPKKs, 80 MAPKKKs and 10 MAPKKKKs in Arabidopsis, providing evidence for the extensive use of this signalling mechanism in plants, but their functions in developmental processes is completely unknown.




Objectives

Expression analysis of all the MAPK components (123 genes) by using custom-designed microarrays during plant development and by in situ hybridisation of selected genes, specifically expressed or present in apical meristem. Phenotypic analysis of Arabidopsis plants in which MAPK signalling pathways are genetically altered by targeted expression of dominant-negative MAPKK constructs into specific domains within the meristem.

Experimental design

A custom-designed microarray having all 123 MAPK gene family members will be used to analyze the expression of MAPK genes during Arabidopsis development using RNA prepared from different developmental stages and from carefully dissected organs. With selected genes, expressed or specific to apical meristem, in situ hybridistaion experiments will be performed at John Doonan`s lab. We have already amplified all the 10 MAPKKs as Gateway cassettes and mutated them at a specific site to prepare kinase negative versions. These constructs are presently used to connect MAPK-signalling pathways in a cell culture system in a Marie Curie fellowship project in my lab. Within this studentship, selected kinase-negative MAPKK constructs (based on expression patterns) will be transferred to two-component expression systems allowing targeted expression at specific meristem domains. For this, we will use both the ethanol-inducible system (Roslan et al. Plant J 28:225-235, 2001), as well as the lacR based expression system (Moore et al., PNAS 95:376-381, 1998). In both systems a large selection of meristem-specific activator lines are available including domain-specific promoters such as stm, leafy, wus, ant etc. Meristem-specific phenotypes will be analyzed using stereo- and laser-scanning electron microscopes as well as by in situ localisation of selected meristem-domain-specific marker genes (Clv1, Clv3, Wus, Stm, As1, UFO) and cell cycle genes (B- and D-type cyclins). The observed phenotypes will be related to known mutants in Arabidopsis shoot meristem development.

Applicants should have or expect to gain a First or Upper Second Class Degree in Biochemistry or Biology and must be UK resident.