MASTERBIOSCIENCES
ECOLE NORMALE SUPERIEURE DE LYON

Offre de stage de Master / Master Internship offer

Tuteur du stage et Laboratoire d'accueil / Internship supervisor and Host laboratory:

Laboratoire de Reproduction et Développement des Plantes. http://www.ens-lyon.fr/RDP/
Supervisor: Gwyneth INGRAM (Directrice de recherche CNRS, responsable d'équipe/group leader); Gwyneth.Ingram@ens-lyon.fr; 04-72-72-89-92

Titre du projet de recherche / Research project title:

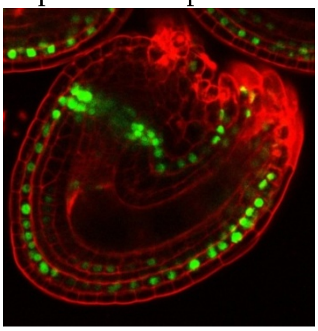
MECHANICAL SIGNALLING IN SEED GROWTH COORDINATION

Description du projet / Project description:

Introduction, contexte scientifique:

The seed of the model plant Arabidopsis thaliana is composed of three genetically distinct structures: the seed coat, the endosperm, and the embryo. The maternally derived seed coat and the endosperm protect and nourish the diploid embryo, which gives rise to the next generation. In addition, a finely controlled interplay between expansion of the endosperm, and mechanical constraints imposed by the surrounding seed coat early in seed development, determine the final size of the seed, and thus of the mature embryo (reviewed in Ingram; 2010).

Because of its physically and genetically compartmentalized structure, the Arabidopsis seed represents a uniquely powerful system in which to study the mechanisms underlying mechanical signaling in plants. Recent work in the SeedDev Group at the RDP has taken advantage of this in order to identify the seed coat cell layer most likely to respond to mechanical stresses imposed by the endosperm. We have developed micromanipulation techniques allowing the imposition and release of mechanical constraints within the seed coat. Finally, we have identified a mechanosensitive gene (*MIT1*) expressed specifically in this cell layer, and known to be involved in the control of final seed size. The project aim is to use the techniques and tools which we have developed in order to address fundamental questions regarding the molecular mechanisms via which mechanosensing is achieved in plants.



MIT1 promoter activity

Description du sujet de stage/project description:

You will combine a bottom up approach, based on analysis of the control of the expression of *MIT1*, with a top-down approach based on the functional and cellular analysis of a potential mechanosensor, to address mechanisms of mechanoperception in the developing seed coat. A functional *MIT1* promoter has been identified and tested in stably transformed *Arabidopsis* plants. This promoter is active in ovules prior to fertilization, but shows a greatly increased activity post fertilization and during seed growth, presumably due to the mechanical stretching of the testa. You will be involved in the dissection of this promoter, based on the analysis of plants harbouring deletion constructs, with the aim of identifying regulatory elements which are necessary for mechanosensitivity. You will combine this analysis with bioinformatic analysis of co-expressed genes, using publicly available data. Your results will be used as the basis for further experiments to identify mechano-regulated transcription factors in the seed coat.

In a parallel approach, you will be involved in confocal-microscope-based analysis of the subcellular regulation of a potential mechanosensory protein within the testa. This will involve applying micromanipulation techniques to various marker lines permitting the visualization of changes in protein localization in response to imposed mechanical constraints.

Techniques utilisées/Methods:

Light and confocal microscopy, micromanipulation of developing seeds, image analysis, basic cloning and molecular biology techniques, plant culture. *In silico* analysis.

Publications du laboratoire (5 max) / Lab publications (5 max):

San-Bento R, Farcot E, Galletti R, Creff A, Ingram G. 2013. Epidermal identity is maintained by cell-cell communication via a universally active feedback-loop in Arabidopsis thaliana. Plant J. 77,46–58.

Xing, Q, Creff, A., Waters, A., Tanaka, H., Goodrich, J. and Ingram, G. C. 2013. ZHOUPI controls embryonic cuticle formation via a signalling pathway involving the subtilisin protease ABNORMAL LEAF-SHAPE1 and the receptor kinases GASSHO1 and GASSHO2. Development 140(4): 770-9.

Denay G, Creff A, Moussu S, Wagnon P, Thévenin J, Gérentes MF, Chambrier P, Dubreucq B, Ingram G. 2014. Endosperm breakdown in Arabidopsis requires heterodimers of the basic helix-loop-helix proteins ZHOUPI and INDUCER OF CBP EXPRESSION Development 141(6):1222-7.

Johnson KL, Faulkner C, Jeffree CE, Ingram GC. 2008.The phytocalpain defective kernel 1 is a novel Arabidopsis growth regulator whose activity is regulated by proteolytic processing. Plant Cell 10:2619-30

Ingram, G. C. 2010. Family life at close quarters: communication and constraint in angiosperm seed development. Protoplasma *247*, 195–214.