# ELEXIGNAL

## Modelling electrical signalling in plants in relation to the polarisation of development and morphogenesis, and to the adaptive response to stress

## ABSTRACT

With the support of Agropolis Foundation, the research project ELEXIGNAL, new in the UMR (BPMP), has been established and has motivated the creation of a new research team by the projectmanager Jean-Baptiste THIBAUD [J-BT]. Many contacts were made with foreign research teams (mainly European). Internally at BPMP, the ELEXIGNAL project-team was joined by newly recruited staff (Tou Cheu Xiong [TCX] CR2 INRA and Claire CORRATGÉ-FAILLIE [CC-F] IR CNRS) and the strong support of both INRA and CNRS resulting in recruitment of more staff (permanent or contract) to join the team (Tracey Ann CUIN post-doc CNRS / Marie Curie [TAC], Elsa Ronzier PhD CNRS / INRA, Frédéric Sanchez [FS] INRA Research Technician, CC-F CNRS Research Engineer of whom the contact was renewed). At its inception (March 2009, shortly before arriving at Montpellier of Erwan MICHARD [EM], the holder of the "Junior Chair", which was the subject of the contract with Agropolis Fondation), the ELEXIGNAL project-team chose expanding the scope of its search field (i) to the regulation of calcium transport systems involved in electrical signalling and (ii) to the calcium signalling. In this way, the project was gaining scientific consistency (electrical signalling and calcium signalling are closely related), it valued TCX's personal investment in establishing calcium imaging at BPMP and it incorporated in the strategic framework of the UMR BPMP, strengthening interactions between electrophysiology and imaging platforms, and overcoming some methodological limitations in favour of several UMR teams. After the first year devoted (i) to obtaining experimental data in planta (patch-clamp recording of protoplasts), which aimed to clarify the electrophysiological properties of ion transport systems involved in electrical and calcium signalling and (ii) to start mathematical modelling of a plant cell excitability, EM made a first extended stay in the Canadian partner, Prof. Anne-Gaëlle ROLLAND-LAGAN, in charge of promoting the start of the 'applied mathematics and computer modelling" part of the project.
After his return from Canada, EM was able to fully concentrate on the development of a mathematical model of the electrical excitability in Arabidopsis. In fact, since her appointment in October 2010, TAC was responsible for starting the acquisition of electrical signals among different genotypes of Arabidopsis studied in the project, aided by CC-F and in collaboration of our partners Universities Geneva and Lublin. Two types of Shaker potassium channels have been identified, one as regulating excitability, the other as significantly affecting the amplitude and duration of the action potential. In parallel, EM has worked on two mathematical models: the first describing a typical Arabidopsis leaf cell, built on the basis of original experimental data (obtained in this project) and data from the literature, the second describing (i) a cellular network consisting of entities described by the first model, but with sets of parameters corresponding to the histological differences observed in the leaves of Arabidopsis and (ii) the exchangesof electrical and chemical signals between cells . The first model works well, seems more stable and consistent with experimental reality (signals recorded in the leaves of Arabidopsis by TAC) than the "reference" model (Sukhov & J Membr Biol 2009 Vodeneev 232:59). We co published with one of our partners (Dr Ingo Dreyer) two articles making use of the modelling of the control of membrane potential by Shaker channels studied in our project (Gajdanowicz et al. 2011 Proc Natl Acad Sci U.S. A. 108:864; Sandmann et al., 2011, Plant Signal Behav &, 6:558). On the other hand, data from TAC and the first model of EM are subject to a publication being currently evaluated. Note that this
will be the first publication demonstrating experimentally the involvement of channels identified at the molecular level in the plant cell excitability along with a simple model satisfactorily simulating action potentials (triggered by either an electrical or a mechanical stimulus) and consequently allowing to predict underlying events as, for example calcium signals co-ordinated with the action potentials. The second model, more ambitious and far more complex (developed in interaction with our partners in Ottawa at the beginning, then in Bern from 2011), has yet to get completed before its publication. For their part, ER, FS and TCX started the identification of calcium-dependent kinases that regulate Shaker potassium channels (functional screening by co-expression in Xenopus oocytes), used biochemistry

techniques for the identification of phosphorylation sites of these channels by these kinases ("on-chip" phosphorylation of synthetic peptides exhibiting all the putative phosphorylation sites of channels tested), and innovative imaging techniques for the visualisation in planta of calcium signals (new bioluminescent probes). The results of this part of the program is also very promising. We have identified several kinases of the CPK family ("Calcium-dependent Protein Kinases") that selectively target different Shaker-type channels and do alter their operation. Through the analysis of peptide-arrays, we are close to identifying sites involved in the regulation of these channels through their phosphorylation state. A publication is being prepared with a first example of a Shaker-CPK pair for which we have characterised the molecular interaction in planta (including "FRET-FLIM") and showed a strong developmental and stomatal phenotype. Finally, we have just submitted an article introducing a new technique (5 times more sensitive than the reference technique) for in situ visualisation of calcium waves that propagate in photosynthetic tissues.

**Keywords :** Operation, Model, Protein/proteomic

**Year :** 2008
**Project number :** 0803-022
**Type of funding :** AAP INRIA
**Project type :** AAP
**Research units in the network :**
**Start date :** 2009-01-01
**End date :** 2012-12-30
**Flagship project :** no

**Project leader :** Jean-Baptiste Thibaud Anne-Gaelle Rolland-Lagan
**Project leader's institution :** CNRS
**Project leader's RU :** BPMP

**Budget allocated :** 194157.8737 €
**Total budget allocated ( including co-financing) :** 265969.69 €
**Funding :** RTRA

## PERSPECTIVES

The work described above will be pursued within the team-project ELEXIGNAL, which will remain within BPMP during the next contract of this UMR. The multi-cellular model will be finalised and published. Recordings of action potentials will continue on other mutant genotypes available in the team and will be analysed in relation with the associated calcium imaging events. Finally, phenotyping, in terms of electrical signalling and calcium, of plant lines carrying mutations on CPKs or their target-channels will be undertaken. This work will produce an integrative view of the molecular mechanisms of calcium and electrical signalling in plants in relation to adaptive responses to environmental stress of these plants.