ENS – IISER Network / BIOSANTEXC Project

Internship Proposal Form

(Discipline/Field name): Developmental biology

Internship title: Stochasticity and robustness in gene expression patterns during flower development

Keywords related with the subject (minimum 3): Gene regulatory networks, AGAMOUS, time course experiments, fluorescence quantification

Name of the laboratory at ENS: Laboratory of Plant Reproduction & Development

Name of the internship supervisor(s): Pradeep Das

Email(s): pradeep.das@ens-lyon.fr

Prerequisites for the internship: Familiarity with dissections and confocal microscopy would help

Requested level: Ideally M2, but M1 with microscopy experience could work

Foreseen internship dates: April-June, 2024

BIOSANTEXC is a project co-funded by the French Ministry of Europe and Foreign Affairs (Ministère de l'Europe et des Affaires Etrangères).

Internship proposal (description and expected training outcomes / half page min, 1 page max):

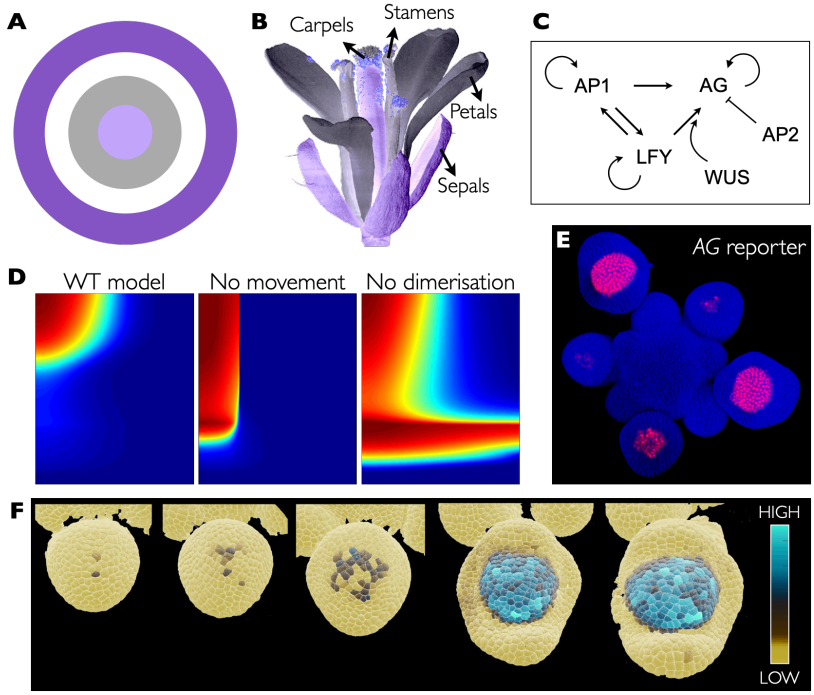
The so-called ABC model of flower development was postulated over thirty years ago. This model describes the establishment of four distinct whorls of floral organ identities (sepals, petals, stamens and carpels) via the combinatorial activities of three classes of genes. A lot is known about the key regulatory interactions that generate the robust expression patterns of these gene classes.

Our focus is on the C class gene *AGAMOUS* (*AG*), which is necessary for proper third and fourth identities. It is known to be expressed in the central dome of the flower at stage 3 of flower development (just as sepals begin to arise), about 72 hours after the flower bud first forms on the flanks of the shoot apical meristem. However, between stages 1 and 3, the flower undergoes an eight-fold change in volume, alongside several rounds of cell division. Thus the gene network that regulates *AG* expression is precisely established in time and space even as the flower grows rapidly.

We have used mathematical modelling to carry out a theoretical exploration of the key parameters underlying the spatial and temporal dynamics of *AG* expression in the flower. This has allowed us to postulate that AG protein multimersation and cell-cell movement, as well as *AG* autoregulation, are critical characteristics.

To test these hypotheses experimentally, we developed a fluorescent AG translational reporter using an 8.5-kb genomic fragment containing all the known regulatory sequences of *AG*, which is able to rescue the *ag* mutant. We then quantified expression at cell-resolution using custom-made software. This analysis has revealed that *AG* expression initiates stochastically at a much earlier developmental stage than previously thought, but then undergoes stereotypical changes until the known robust pattern emerges.

In the next phase of the project, we will explore the effects of mutating the binding sites of four known regulatory proteins. Lines carrying these constructs have already been generated. The visiting student will first carry out both static and time course experiments on these lines in WT and *ag* mutant backgrounds (to examine titration effects). These data will be quantified in 3D or 4D using the MorphoGraphX software and analysed in the context of the WT reporter. Next, the student will image and analyse two other established floral reporters to determine whether their expression patterns might explain the stochastic origins of *AG* expression. Lastly, if time permits, the student may also examine whether epigenetic mechanisms impact stochasticity by studying *AG* expression in mutants in key epigenetic pathways. The student's training will also extend to helping prepare figures and text for a manuscript on the project.



(A-B) Overlapping whorled expression of three gene classes leads to four organ identities. (C) Gene network regulating floral development. (D) Simulations of the floral model for the WT flower (left) as well as without movement (middle) or dimerisation (right). (E) Shoot apical meristem showing expression of an *AGAMOUS* translational reporter. (F) Cell-resolution quantifications showing AG protein levels over time.