Project for Master Thesis *2018/2019*

Molecular evolution of an antagonism between MYB transcription factors

Supervisor: Célia V. Romão, cmromao@itqb.unl.pt,
Co-supervisor: Prof. Maria Manuela Ribeiro Costa manuela.costa@bio.uminho.pt
ITQB NOVA, Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa
Department of Biology, University of Minho

The origin of morphological novelties is a long-standing challenge in evolutionary biology. Its understanding demands elucidation of developmental and genetic mechanisms that produce such new structures. Dorsoventral asymmetry of flowers is thought to have evolved many times independently in different plant lineages from radially symmetric ancestors and it provides a good system to study the molecular interactions responsible for the establishment of a novel morphology.
Antirrhinum majus flowers are asymmetric along their dorsoventral axis, having distinct dorsal, lateral and ventral organ petals and stamens. In this model species, dorsoventral asymmetry of the flower and its component organs requires the combined activity of four transcription factors: *CYCLOIDEA* (*CYC*), *DICHOTOMA* (*DICH*), *RADIALIS* (*RAD*), and *DIVARICATA* (*DIV*) [1-5]. *CYC*, *DICH* and *RAD* are expressed dorsally in the flower primordia and promote dorsal petal and stamen identity. Genetic and molecular studies have revealed that *RAD* acts downstream of *CYC* and *DICH* and is thought to antagonise the activity of DIV post-transcriptionally, both cell and non-cell autonomously [1, 2, 3, 6, 7]. *DIV* is expressed in the whole floral primordium, even though it only has a phenotypic effect in more ventral regions of the flower. In *div* mutants, ventral identify of the flower is lost [2, 4]. Plants carrying strong *rad* mutant alleles have almost fully ventralised flowers [8]. Therefore, when dorsal identity is mutated, ventral identity expands to more dorsal parts of the flower. Because RAD and DIV encode related MYB-like transcription factors, one possibility would be that DIV might act as a transcriptional activator to promote ventral identity, while RAD might inhibit DIV through competition for a common protein or DNA targets. Recently in our lab, two new Antirrhinum MYB-like transcription factors were identified in a yeast two-hybrid screening as being interactors for both RAD and DIV (DIV and RAD interacting proteins; DRIF1 and DRIF2) [6].

The goal of this present proposal is by using a multidisciplinary approach to evaluate how the interactions between the proteins establish the antagonistic module of transcriptional control of petal dorsoventral identity, which has evolved to be co-opted in different biological processes in different species. In conclusion the results within this project will contribute to further enhance our understanding on the establishment of asymmetric gene expression along the dorsoventral axis of the Antirrhinum flower meristem.

Work Plan

During this project the following experiments will be performed:
(1) The amino acids responsible for the interactions between the DRIF-RAD and DRIF-DIV will be studied. Site-directed mutagenesis will be performed and the strength of interaction analysed in a Quantitative Yeast Two-Hybrid Interaction Assay. The proteins will also be expressed in *E. coli*, and purified by affinity and size-exclusion chromatography. After, the proteins will be submitted to several biochemical and biophysical studies, such as SDS-PAGE, Western-Blotting, thermal-shift assays.
(2) Protein-protein interactions studies will be performed by Isothermal titration calorimetry (ITC) and Surface Plasmon Resonance (SPR)
(3) Structural studies will be performed for the individual proteins using X-ray crystallography and for the protein-protein complexes using Small Angle X-ray scattering (SAXS).
This work is part of an on-going collaboration between the University of Minho and the Instituto Tecnologia Química e Biológica (ITQB NOVA, Lisboa) and a period of work in both labs might be required.

References:

1. Luo D. et al 1996 Nature 383, 794
2. Almeida J. et al 1997 Development 124, 1387
3. Luo D. et al 1999 Cell 99, 1
4. Galego L. & Almeida J. 2002 Genes Dev. 16, 880
5. Corley S.B. et al 2005 PNAS 102, 5068
6. Costa M.M.R. et al 2005 Development 132, 5093
7. Baxter C.E.L. et al 2007 Plant J. 52, 105
8. Raimundo J. et al 2013. Plant J. 75, 527