Native RNA targets of Arabidopsis RNA-binding proteins

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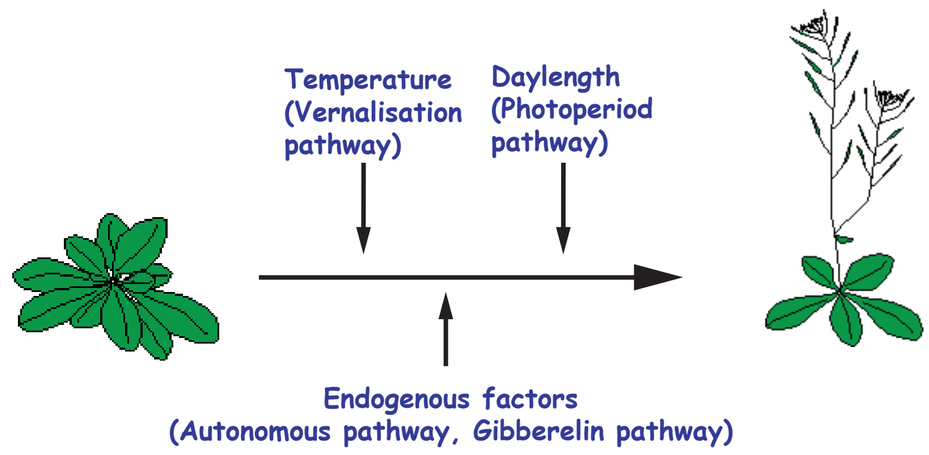
INTRODUCTION

Like all living organisms, plants must respond and adapt appropriately to environmental changes in order to survive. To take up this challenge, they have evolved complex and exquisitely regulated molecular networks to tightly control expression of their genes. Since we are interested in understanding the regulation of gene expression and its downstream physiological outputs, plants represent ideal model organisms to study.

In *Arabidopsis thaliana*, flowering is a unique event in the life of an individual. To maximise the chance of descent survival, decision of flowering time must integrate information from the environment (upper row), as well as endogenous factors (lower row).

Schematic flowering transition in Arabidopsis



Genetics allowed the isolation of many genes involved in controlling flowering time. Mutations in these genes affect the expression of only a few key regulators of flowering time, indicating a complex and intricate network of gene expression regulation.

Genes encoding RNA-binding proteins feature prominently in flowering time control. However the mechanisms by which these proteins may regulate gene expression are poorly documented.

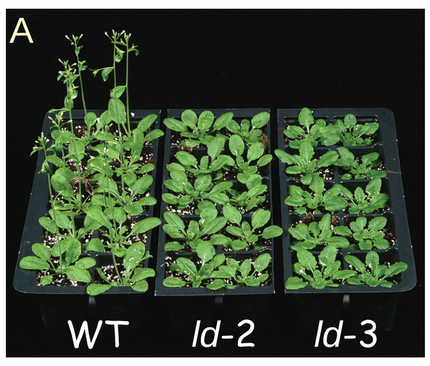
By studying FPA, a poorly characterised RRM-containing protein, we aim to deepen our knowledge of the roles of RNA-binding proteins in regulating gene expression *in vivo*.

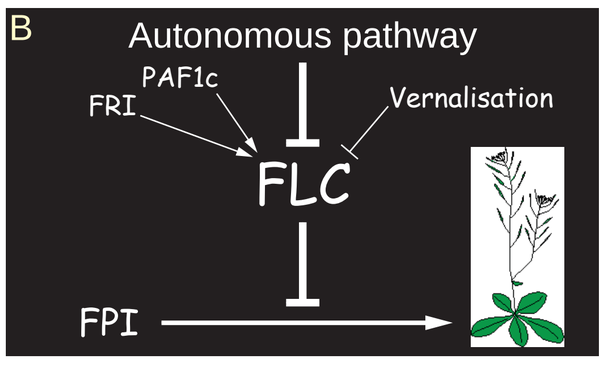
FPA, A RRM-CONTAINING PROTEIN INVOLVED IN DOWN-REGULATION OF GENE EXPRESSION

FPA (At2g43410) promotes flowering through the autonomous pathway. Like the other mutations in this pathway (*ld*, *fca*, *fy*, *flk*, *fve*, *fld*, *ref6*), *fpa* mutants flower later than wt in both long and short days (A).

As genetically defined, the major function of the autonomous pathway is the down-regulation of *FLC* expression (At5g10140). *FLC* is a strong repressor of flowering because it down-regulates the expression of the Floral Pathway Integrators (FPI), namely *FT*, *SOC1* and *LEAFY* (B).

FPA comprises 3 RNA-recognition motifs (RRM) located in its N-terminus, suggesting a function in RNA interaction and/or processing. However the direct RNA targets of FPA, as well as the mechanism used to down-regulate *FLC*, are unknown.





CHARACTERIZATION OF FPA FUNCTION AND DIRECT RNA TARGETS

We aim to characterize the direct RNA targets of FPA and to understand its role in gene expression regulation by combining the advantages of Arabidopsis genetics with biochemistry.

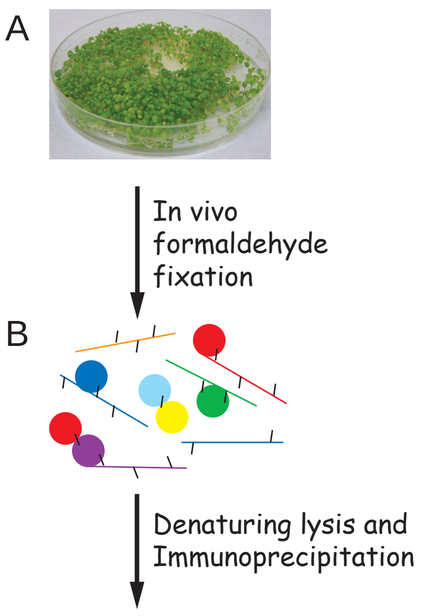
We are characterizing a T-DNA insertion mutant, *fpa*-7. To gain insights into the mechanism of FPA-regulated gene expression, we will compare the gene expression profiles of *fpa*-7 and Col0 (WT) by microarray analysis.

To characterize the direct RNA targets of FPA *in vivo*, we are adapting to Arabidopsis two powerful techniques (see below): RNA Immunoprecipitation (RIP) and Cross-Linking and Immuno-Precipitation (CLIP).

Since RIP is a modification of Chromatin Immunoprecipitation (ChIP), we have already set up ChIP for our laboratory.

Finally, to bring cell biology insights to our understanding of FPA function, we have produced vectors encoding GFP-tagged FPA to determine its *in vivo* localization.

RNA IMMUNOPRECIPITATION



RNA Immunoprecipitation (RIP) is a powerful technique allowing the *in vivo* characterization of the physiological RNA targets of a given RNA-binding protein.

Like ChIP, RIP is based on *in vivo* formaldehyde fixation of plant tissues or young seedlings (A).

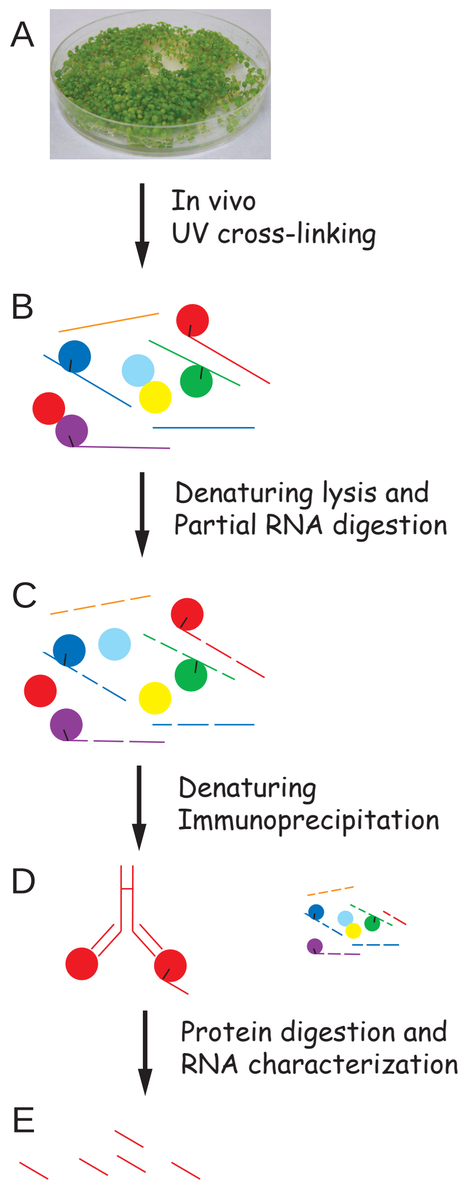
Formaldehyde creates intermolecular and reversible bridges (B, dark lines) between biological components such as RNA (lines) and proteins (spheres). Therefore it fixes the physiological *in vivo* interactions between RNA- binding proteins and their RNA targets. Moreover it prevents the re-arrangement of RNA-protein complexes that occurs during lysate preparation (Mili S. and Steitz JA, 2004).

RIP requires specific and high-affinity antibodies because RNA-protein complexes containing a given RNA-binding protein are recovered by immunoprecipitation under denaturing conditions (C).

We raised antibodies against FPA and are currently assessing their specificity and affinity. In parallel, we are using commercially available anti-GFP antibodies to set up RIP by using seedlings over-expressing U2B"-GFP, a well-characterized RNA-binding protein interacting specifically with U2 snRNA.

Finally, the specific RNA targets of a given RNA protein are recovered (D) and will be characterized either at a genomic scale by microarray or by target-specific RT-PCR.

CROSS-LINKING AND IMMUNO-PRECIPITATION



Cross-Linking and Immuno-Precipitation (CLIP) is a powerful technique allowing the *in vivo* characterization of the physiological binding site(s) of a given RNA-binding protein.

CLIP is based on *in vivo* cross-linking of plant tissues or young seedlings by UV irradiation (A). Unlike RIP, CLIP specifically characterizes direct protein-RNA interactions because UV irradiation only creates molecular bridges (B, dark lines) between proteins (spheres) and nucleic acids (RNA in our example, lines) that are in direct contacts.

In addition, CLIP allows the characterization of the binding site of a given RNA-binding protein because it contains an additional step in which the RNAs are partially degraded with RNases (C). This step leads to the formation of RNA-protein complexes containing only small RNA tags of 70 nucleotides comprising the binding site of the protein.

Like RIP, CLIP requires specific and high-affinity antibodies to recover RNA-protein complexes containing a given RNA-binding protein by denaturing immunoprecipitation (C). The characterization of FPA antibodies and the use of seedlings expressing U2B"-GFP, as described for RIP, will be equally useful for CLIP.

Finally, the specific RNA targets of a given RNA protein are recovered (D) and will be characterized by large scale cloning.

CONCLUSIONS AND PERSPECTIVES

The characterization of FPA *in vivo* RNA targets by RIP and CLIP, together with assays aiming to reveal the function of FPA, will shed new light on the direct role of this RNA-binding protein in gene expression regulation.

Moreover RIP and CLIP will add invaluable tools towards the characterization of the role and function of uncharacterized RNA-binding proteins in *Arabidopsis thaliana*.

ACKNOWLEDGMENTS

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