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INVESTIGATING THE IMPACT OF REDUCED TETRAMER FORMATION AND DNA BINDING COOPERATIVITY ON MADS COMPLEX TRANSCRIPTIONAL ACTIVITY

<u>Véronique Hugouvieux</u>, Xuelei Lai, Arnaud Stigliani, Jérémy Lucas, Agnès Jourdain, François Parcy, and Chloé Zubieta

Laboratoire de Physiologie Cellulaire and Végétale, CNRS, CEA, INRA, Université Grenoble-Alpes, IRIG, UMR 5168, Grenoble 38000, France.

Differentiation of the floral organs depends on the formation of specific transcription factor (TF) complexes belonging to the MADS transcription factors family. Among them, SEPALLATA3 (SEP3) is described as a key factor during floral organ development in Arabidopsis thaliana. This MADS transcription factor is involved in the development of all reproductive organs: sepals, petals, stamens and carpels. In the quartet model, SEP3 interacts specifically with other MADS TFs, including APETALA1, APETALA3, PISTILLA and AGAMOUS (AG) to form functional and specific tetramers that drive specific target gene expression and developmental fate through cooperative DNA binding. As an example, the SEP3/AG hetero-tetramer is described for its importance in carpel development. Using a naturally occurring SEPALLATA3 splice variant (SEP3Δ161-174 also called SEP3Atet) that lacks 14 amino acids in the tetramerisation interface, we probed the requirements of MADS tetramers and cooperative binding for flower development in the sep123 mutant background. Indeed, SEP3/Atet shows a significant impairment in the ability to form SEP3/AG hetero-tetramers in vitro, but still can form hetero-dimers. Our in vivo and in vitro experimental data pointed to the requirement of SEP3/AG tetramer formation and cooperative DNA binding for floral meristem determinacy through the regulation of CRABSCLAW (CRC), a transcription factor described to be required for nectary formation and carpel development. To further investigate the impact of reduced SEP3/AG DNA binding cooperativity on MADS complex transcriptional activity, we have compared RNA-seq experiments between plants expressing SEP3 or SEP3Atet. Interestingly, RNA-seq data highlight very similar patterns of gene expression between plant expressing SEP3 or SEP3∆tet, indicating that regulation of only few genes, including CRC, are affected by reducing SEP3/AG cooperative binding. These data will be discussed together with our recent DAP-seq analysis comparing SEP3/AG dimer and tetramer binding to DNA.

E-mail address of the presenting Author: veronique.hugouvieux@cea.fr