ChromAct Report Summary

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Final Report Summary - CHROMACT (Chromatin dynamics for gene activation in the developing flower)

In the ChromAct project, we analysed the influence of chromatin structure on gene expression (or transcription), which corresponds to how the packaging of DNA around nucleosomes regulates the reading through the genome. Chromatin conformation is employed as a regulatory means to hinder or grant accessibility of genes for the transcriptional machinery in all eukaryotes. This is mediated through chromatin remodellers that alter the positioning of histones (the components of nucleosomes), and through chromatin modifiers that set histone marks (the post-translational modifications on histone tails). We focussed our work on the transcriptional activator ULTRAPETALA1 (ULT1), postulated to be involved in several steps of chromatin-mediated activation of genes controlling flower formation. ULT1 had been shown to genetically antagonise the Polycomb Group (PcG) protein CURLY LEAF (CLF), a factor responsible for deposition of repressive histone marks [1]. At single gene level, ULT1 was shown to be necessary to maintain wild type levels of both repressive H3K27me3 (Lysine 27 of histone 3 tri-methylation) and activation-associated H3K4me3 marks. ULT1 was proposed to be involved in the removal of repressive and deposition of active marks. Additionally, there are hints that ULT1 interacts with the transcriptional machinery during initiation of transcription [2-5].

The ChromAct project aimed at analysing the genome-wide role of ULT1 during transcriptional initiation, its interplay with other factors and the temporal evolution of gene activation events during early flower development. The project requires quantitative analysis of histone mark abundance by chromatin immuno-precipitation followed by next generation sequencing (ChIP-seq). We developed a protocol to generate high quality ChIP samples, as well as an analysis and visualisation pipeline for the output dataset.

To investigate the genome-wide role of ULT1 in histone-mark-mediated gene regulation, we analysed the distribution of H3K27me3 and H3K4me3 in ULT1 loss-of-function (lof) and gain-of-function (gof) mutants, at an early stage of plant development (in seedlings). We found that more than 900 genes show both a reduction in gof lines and an elevation in lof lines for the H3K27me3 repressive mark. The genes include know regulators of flowering time and flower development. We found more than 30 % overlap between ULT1-regulated and CLF-regulated genes [6], however in opposite direction as expected for antagonistic factors.

Thus, we could further consolidate the role of ULT1 as a trithorax group (trxG) factor antagonising PcG function for gene activation at a genome-wide level.

We further analysed a putative interaction of ULT1 with the H3K27me3 demethylase RELATIVE OF EARLY FLOWERING 6 (REF6). Mutations in REF6 suppress the CLF lof phenotype in a similar way as ULT1 lof does and REF6 gof leads to phenotypes resembling CLF lof and ULT1 gof [7]. However, we found weak to no genetic interaction in combinations of lof and gof lines for both genes. Furthermore, we analysed genome-wide influence of REF6 lof on H3K27me3 and H3K4me3 at the same stage and in the same conditions employed for ULT1 and found only a minute overlap between genes changing in both directions. Thus, we postulate that ULT1 and REF6 function rather independently in the activation of target genes.

Currently, we are performing ChIP-seq experiments to identify direct target genes of ULT1 and to assess correlation with ULT1 effects on marks (submission for publication planned for August 2015). Additionally, we studied genetic interaction of ULT1 and the floral organ identity gene regulator LEAFY (LFY) and found that ULT1 and LFY act separately in regulating identity and determinacy at the floral meristem [8]. To investigate the mode of ULT1 targeting to chromatin, we analysed the DNA binding
motif of a transcription factor, interactor of ULT1, and will correlate the results with the ChiP-seq-resolved ULT1 direct target sites.

To study early events of flower formation with a temporal resolution, we employ a floral induction system to induce synchronised flower development from inflorescence meristem-like tissue [9]. We generated a developmental time series including vegetative leaf tissue, young developing flowers at zero (t0) and two days after induction (t2) and fully expanded inflorescences. Although we found very similar numbers of H3K27me3 and H3K4me3 target genes, many genes display quantitative changes in those marks, especially between different tissue types (e.g. >60% of target genes change quantitatively from leaf to t0). To correlate changes in histone marks with changes in transcriptional activation, we analysed expression in the same tissues by RNA-seq. For this purpose, the researcher was trained by local specialists and acquired the necessary knowledge to perform the analysis of RNA-seq data. This analysis revealed that the quantitative changes in histone marks are widely correlated with changes in expression (in the expected direction, e.g. elevated expression for decreased H3K27me3 and elevated H3K4me3 levels). The study (submission of publication planned for July 2105) constitutes the first part of a collaborative effort between three European laboratories to unravel dynamics of the chromatin landscape during flower development in Arabidopsis (Wellmer group, Dublin; Kaufmann group, Potsdam; Carles group, Grenoble). Our pioneering dataset revealed the strong genome-wide dynamics of histone modification dynamics during flower formation in Arabidopsis. It furthermore constitutes the basis for future research aimed at unravelling the orchestration of histone mark changes and transcription factor binding during flower development, in correlation with transcription. Thus, we generated a valuable data source for research in molecular regulation of flower development, and that also sheds light on plant transcriptional regulation in general.

Taken together, our work gave insights on the impact of ULT1 on gene regulation and on the extend and nature of chromatin dynamics during flower development. ULT1 is conserved in crop species such as tomato, maize and wheat, which are widely cultivated in Europe and of economic relevance. Therefore, it is very likely that transcriptional activation functions in a similar way in these plant. Thus, our results can provide the basic knowledge to study timing and mechanisms of gene activation in crops, especially since important traits for yield, as flowering time, flower and fruit development are regulated by PcG repression and trxG activation, and more particularly by ULT1 [3; 10].

References

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