Characterisation of transcriptional and chromatin events in relation to floral transition and identification of nuclear organisation determinants

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Summary and concluding remarks

Transcriptional gene activity is dynamic and modulated through the integration of three main layers of regulation. The first layer is based on the information stored in the linear genome and the epigenome, which consists of a large repertoire of histone post-translational modifications and requires specific nucleosome distribution and compaction. The second layer involves the formation of chromatin loops at a locus scale or larger. Lastly, the third layer relies on the spatial position and organization of the locus inside the nucleus (Chapter 1).

Polycomb Repressive Complexes (PRCs) control transcriptional gene repression, and play a key role in these three layers of regulation (Chapter 2). PRC proteins mediate both biochemical modifications of histone tails, changing the epigenetic topography of the genome, and biophysical modifications impacting on chromatin compaction. They can also affect the three-dimensional (3D) conformation of chromatin. PRCs are involved in the regulation of a variety of biological processes, such as developmental transitions, cell-fate decisions, but also stress-response processes. Developmental phase-transitions are characterised by large transcriptional reprogramming and can thus be used as model system to investigate the three layers of gene regulation. PRCs have a key role in flowering time by controlling the sequence and timing of the developmental switch throughout the regulation of flowering-gene expression. We thus choose to study the floral transition to address questions regarding these different layers of gene regulation with a specific focus on LHP1, a PRC1 subunit.

Flowering time is fine-tuned by various environmental and endogenous cues to ensure the success of offspring production. Photoperiod is one of the most important factors regulating the initiation of flowering in angiosperms. Leaves play a crucial role in light perception and in the production of the florigen signal that converges to the SAM. Despite the important role of leaves in flowering, limited data are available for their gene network during the developmental transition.

In Chapter 3, we present an inductive system to characterise the morphological and molecular events in the leaves that are associated with the floral transition. We use a short-day (SD) to long-day (LD) shift that allows to induce the flowering time. We defined the floral transition window using the expression of key flowering genes. *CO* and *FT,* key genes expressed in the leaves with the light stimulus and involved in the activation of downstream flowering genes, showed a peak of expression 3 days after the transfer to LD. *AP1,* a floral meristem marker, which marks the completion of the transition was detected after 5 days. While establishing the temporal window we noticed that the longer the plants grow in SD, the quicker they bolt after the transfer in LD, suggesting that the flower initiation is related to the size or age of the plants and that it might depend on their capability to produce certain metabolites. During the SD-LD switch we observed a high endocycle activity, without leaf growth in the 3rd and 4th leaves. Therefore, this pair of mature leaves was chosen to further investigate the early transcriptional events associated with floral transition, independent of leaf developmental and growth processes.

In Chapter 4, we use a genome-wide RNA-seq approach to unravel the composition and the dynamics of the gene network in mature leaves during the floral transition and to highlight the main molecular processes. We observed large transcriptional reprogramming with major changes in photosynthetic activity, lipid localisation, protein metabolism and DNA replication, altogether highlighting the complexity of this developmental switch. We detected deregulation of several chromatin-related genes, reflecting changes in chromatin composition and reorganization that underly the floral switch. From the analysis of endocycles associated genes, we showed the redirection of leaf cells towards endoreduplication. This phenomenon can be seen as a strategy to quickly increase leaf metabolism to provide energy required for the developmental switch, suggesting a role of these genes in the regulation of the floral transition. Furthermore, a comparative analysis with transcriptome data in shoot and root tissues during a similar floral transition was performed to enlarge the overview of the flowering gene networks. We highlight common pathways to the different organs with the perspective to have an integrated view of the floral transition process at the whole plant level. The analysis provides a list of interesting new candidates involved in the floral transition in mature leaves. Further

characterisation at the molecular level and by reverse genetic approaches will clarify their role in flowering time. Moreover, the experimental set-up allowed to identified differentially expressed long non-coding RNA (lncRNAs), other important players in flowering. In the near future, we aim to explore their mechanism of action and bring new insight on their roles during the floral transition.

In Chapter 5, we focused on the PRC1 subunit LIKE HETEROCHROMATIN PROTEIN 1 (LHP1). To investigate the effect of LHP1 dosage on chromatin and on key flowering-genes, we developed transgenic lines to conditionally alter LHP1 expression in a specific temporal window. This allowed to study the relation between the induced misregulation of LHP1 and development events, while avoiding the potential compensatory effects which are inherent to constitutive knockdown and over-expression lines. We observed that short-term modulation of LHP1 dosage alters its binding pattern on target gene regions, with broader enrichments all along the analysed loci. We showed that induced high levels of LHP1 increase the deposition of H3K27me3, giving insight into the interaction between LHP1 and PRC2. The modulated LHP1 level also had an impact on H3K4me3, suggesting a new role for LHP1 in the formation of bivalent chromatin regions. Despite these chromatin modifications, only transient transcriptional changes of LHP1 target-genes were detected after one day of LHP1 dosage alteration. Moreover, the effect of LHP1 modulation varies between the gene targets. Therefore, the regulation of the transcriptional state by LHP1 seems to be more complex than a simple on or off mechanism. Our study suggests a dosage-dependent effect of LHP1 on its chromatin distribution and on the deposition of the histone marks, highlighting the role of LHP1 in chromatin dynamics.

In Chapter 6, we aimed to identify molecular determinants involved in the 3D nuclear architecture. Using innovative 3D spatial statistical tools we analysed the nuclear morphology, heterochromatin features and spatial organization of chromocenters in the nuclear domain of mutants affected in genes encoding nuclear-envelope proteins. The quantitative analysis revealed a role for CRWN and KAKU proteins in the 3D position of chromocenters and the nuclear shape.

Moreover, we observed that the remodelling of the 3D nuclear architecture was dependent on the specific protein localization.

Overall, this thesis has linked the flowering-gene network with molecular processes that accompany the floral transition events in mature leaves perceiving a photoperiodic inductive signal. Our study provides new insights into the role of LHP1 and the genetic determinants involved in the 3D nuclear- and heterochromatin-architecture. Moreover, the deep characterisation of the floral transition window and the development of the LHP1 transgenic lines can be exploited to further understanding the relation between PRCs and the three layers of gene regulation. Chromatin dynamics could be investigated using ChIP-seq experiments to determine the genome-wide distribution and dynamics of LHP1 binding during the transition. Furthermore, the analysis of the nuclei of the transgenic lines could enable to decipher the role of LHP1 in nuclear organization.