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Green chromatin dynamics in Zurich
Meeting summary based on the European Workshop on Plant Chromatin 2009 in Zurich, Switzerland

Claudia Köhler,∗ Valerie Gaudin2 and Lars Hennig3

1Department of Biology and Zurich-Basel Plant Science Center; Swiss Federal Institute of Technology; ETH Centre; Zurich, Switzerland; 2Laboratoire de Biologie Cellulaire; UR501 Institut J.P. Bourgin; Institut National de la Recherche Agronomique; INRA; 3Versailles, France

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In September 2009, the 1st European Workshop on Plant Chromatin took place in Zurich, Switzerland. The workshop covered a variety of chromatin related topics, including the mechanisms of Polycomb group protein function, long-range interactions of regulatory elements, genome-wide reconfiguration of epigenetic marks during gametogenesis and protection of chromosomal ends by epigenetic marks. Some of the highlights of this meeting are summarized in this report.

Introduction

At the recent European Workshop on Plant Chromatin in Zurich, Switzerland, the fundamental importance of chromatin states for plant development was apparent. Many reports focused on the mechanisms of Polycomb group (PcG) protein action, from recruitment to target genes over deposition of histone modifications to the interpretation of such modifications as gene silencing signals. It became also evident that epigenetic control of gene activity does not act in isolation and that for a comprehensive understanding of the interaction of different chromatin marks cell type-specific epigenetic profiles are needed. What is more, we need to expand our view to three dimensions to understand the impact of long-range gene interactions within the nucleus.

This report conveys several of the main topics presented during this meeting and discusses recent progress and insights into this exciting research field. We apologize to those colleagues whose contributions were not mentioned due to space constraints.

Polycomb Group Recruitment and Action

How PcG proteins are recruited to their target loci is still an open question in the field, and B. Rutjens (Norwich, UK) reported on COOLAIR antisense transcripts derived from the FLC locus that might be required for the initial repression of FLC transcription. COOLAIR is spliced and polyadenylated and rapidly increases during cold treatment, coinciding with FLC silencing (Fig. 1). The COOLAIR promoter is cold-inducible and can drive silencing of a transgene reporter, suggesting that cold-induced COOLAIR transcription, or the transcript itself initiate silencing. As with many new results, these findings raise more questions than they give answers; for instance, it will be important to find out whether the presence of COOLAIR is indeed required for stable FLC silencing, given that vernalization-induced silencing of FLC transgenes lacking the COOLAIR promoter has been reported.1 If confirmed, these findings would have striking parallels to the non-coding HOTAIR RNA mediated silencing at the human HOX loci, with HOTAIR being required for Polycomb Repressive Complex 2 (PRC2) occupancy and histone H3 lysine 27 trimethylolation (H3K27me3).3

F. De Lucia (Norwich, UK) described a vernalization-induced PHD-PRC2 complex that in addition to the core PRC2 components contains the PHD domain proteins VRN5, VIN3 and VEL1, which mediate high density H3K27me3 required for stable FLC silencing.3 Thus, Arabidopsis PHD-PRC2 is likely to act similarly to Pcl-PRC2 of Drosophila1 and PHF1-PRC2 of mammals.3

Another open question in the field is whether plant PcG proteins have specific target genes during different phases of development and in defined tissues. Results presented by D. Schubert (Düsseldorf, Germany) revealed that the H3K27me3 profile in the shoot apical meristem differed substantially from the profile in leaves, demonstrating that PcG proteins have indeed tissuespecific targets genes. Understanding how these tissue specific differences are established is one of the burning questions that need to be addressed.

Whereas intensive research is currently focusing on mechanisms leading to PcG mediated gene silencing, little is known so far about the regulation of PcG genes themselves. C. Köhler (Zurich, Switzerland) reported that the chromatin-remodeling factor PICKLE acts rather unanticipated as a transcriptional activator of PcG target genes and is also directly activating expression of PcG genes SWINGER and EMF2 in primary roots.4 Therefore, expression of embryonic traits in pickle mutant roots and dedifferentiation of mutants lacking PcG protein activity5 have a common mechanistic base.

*Correspondence to: Claudia Köhler; Email: koehlerc@ethz.ch
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An Emerging Plant PRC1

Probably the most intriguing question connected to PcG proteins concerns the mechanism leading to gene silencing. L. Hennig (Zürich, Switzerland) reported that LHP1 binding to H3K27me3 is essential for silencing of certain PcG target genes. W.-H. Shen (Strasbourg, France) described genetic and physical interactions of LHP1 with two RING domain proteins (AtRING1a/b), constituting part of a potential plant PRC1. Knockout mutants in the AtRING1a/b genes form ectopic meristems and show homeotic conversions, implicating that PcG proteins are required to confine shoot apical meristem activity. Interestingly, mutations in AtRING1a/b enhance the lhp1 phenotype, suggesting that LHP1 might not be the only protein recognizing H3K27me3, but that there are other pathways acting in parallel to LHP1.

M. Calonje (Heidelberg, Germany) presented exciting new data on the identification of novel PcG proteins from Arabidopsis that together with LHP1 and AtRING1a/b might form a plant PRC1 complex. The identified proteins were previously predicted as plant homologs of the fly PRC1 subunit BMI1. The Calonje laboratory analyzed mutants in the BMI1 homologs and found that they closely resemble mutants lacking PRC2 activity, strongly suggesting that the identified BMI1 homologs act in the same genetic pathway as PcG proteins.

V. Gaudin (Versailles, France) addressed the question what determines specificity of LHP1 binding to H3K27me3 in vivo, given that LHP1 does not discriminate between H3K27me3 and H3K9me2 in vitro. She presented a novel LHP1 interacting RNA-binding protein that might participate in the mechanism determining specificity. Thus, it is possible that recruitment of plant PRC1 might require RNAs as well, similar to the Xist noncoding RNA dependent recruitment of PRC1 in mammals.

Propagation of Epigenetic States through Replication

Epigenetic states need to be stably transmitted through mitosis; however, the underlying molecular mechanism is far from understood. M. Piñeiro (Madrid, Spain) described the identification of early in short days (esd7) that is allelic to the ado4 mutant, which has recently been shown to be defective in the catalytic subunit of DNA polymerase epsilon. Esd7 and ado4 are early flowering, likely caused by reduced H3K27me3 levels, concomitantly with increased active epigenetic marks at the F7 locus, leading to increased F7 expression. He reported that ESD7/ADO4 interacts with LHP1 in vitro, suggesting a connection of DNA replication and maintenance of PcG mediated gene repression. A similar connection was recently suggested based on the finding that weak mutants in the catalytic subunit of DNA polymerase alpha cause similar phenotypic defects as PcG mutants and importantly, LHP1 and DNA polymerase alpha interact as well in vitro. Together, these findings support previous studies revealing that animal PRC1 remains bound to replicated templates and suggest that propagation of PcG mediated gene repression through cell division requires the association of plant PRC1 with the replication machinery.

Chromatin Modifications Regulate Mitosis and Meiosis

Chromatid separation during mitosis and meiosis needs to be tightly regulated to prevent unbalanced chromatid segregation and its fatal consequences. One central player in this process is the Aurora protein kinase, and D. Demidov (Gatersleben, Germany) discussed the functional role of plant Aurora protein kinases for chromatid segregation during mitosis. Aurora kinases phosphorylate histone H3 at serine 10 (H3S10), and although its functional role seems to be conserved. In mammals, H3S10 phosphorylation originates at the pericentromere, spreads throughout the chromosome during the G2-M transition and is likely to be required for chromosome condensation. In contrast, in plants, H3S10 phosphorylation originates at the pericentromere, spreads throughout the chromosome during the G2-M transition and is likely to be required for chromosome condensation. In contrast, in plants, H3S10 phosphorylation is most prominent at the pericentromeric regions, suggesting a functional role for chromatid cohesion in metaphase chromosomes during mitosis and meiosis. D. Demidov presented evidence that Aurora kinases in plants are required for H3S10 phosphorylation and that inhibiting Aurora function by drug treatment leads to lagging chromatids during mitosis, suggesting that the function of Aurora kinases in monitoring the complete attachment of kinetochores to the spindle and activating the spindle checkpoint has been evolutionarily conserved.

Resetting the Epigenome during Gametogenesis

Unlike in animals where a germline is set aside early during embryogenesis, plant gametes are generated from somatic cells in the adult organism. In plants, the cells formed by meiosis go through several rounds of mitosis before fertilization re-establishes the diploid state. The female haploid spore undergoes...
three nuclear divisions without cytokinesis forming a syncytium before cellularization establishes the mature embryo sac containing two female gametes, the egg and central cell, as well as two accessory cell types (the synergids and antipodals). The omnipotent state of the zygote, formed upon fertilization of the egg cell, likely requires the resetting of epigenetic marks of the differentiated founder cell. Because, accessory cells do not share the gametic cell fate, resetting possibly occurs, at least partly, post-meiotically. Indeed, C. Baroux (Zurich, Switzerland) presented evidence that gametic nuclei show visible differences in chromatin organization compared to accessory cell nuclei. These differences, which include distinct patterns of heterochromatin distribution, chromatin compaction, as well as LHP1 deposition, seem to be established before cellularization of the embryo sac. These findings point towards a general post-meiotic change of the epigenetic status of plant female gametes that might be required for the switch from sporophytic to gametophytic development (Fig. 2).

The two female gametic cells have different developmental fates, and M. Pillot (Monpellier, France) presented data showing that these different fates are reflected by different epigenetic states. Whereas the central cell nucleus is almost devoid of heterochromatic H3K9me2, the egg cell nucleus contains high levels of H3K9me2. This epigenetic asymmetry of the two gametes is inherited by their descendants upon fertilization and is reflected by a transcriptional asymmetry, with the zygote being transcriptionally quiescent and the endosperm being transcriptionally active during the first divisions. Recently published studies revealed an asymmetric DNA methylation distribution in embryo and endosperm at later stages of seed development; whereas the endosperm is globally hypomethylated, the egg cell contains high levels of DNA methylation in the descendent embryo and endosperm, respectively. Given that H3K9me2 and DNA methylation are self-enforcing each other, both findings are likely to be mechanistically connected.

### Chromatin Structure in Three Dimensions

Long-range interactions of genes with distantly located regulatory elements and the spatial clustering of genes emerges as an important new dimension that needs to be considered when attempting to understand genome regulation. The ability to explore this level of regulation largely depends on chromatin conformation cartography (3C) and related methods that are used to detect the physical interaction of distantly located genomic loci in cis and in trans. M. Stam (Amsterdam, Netherlands) presented evidence based on 3C experiments for long-range chromosomal interactions that were responsible for the differential expression of two epialleles of the b1 gene in maize. High expression levels of b1 require the interaction of a hepta-repeat sequence, which is located 100 kp upstream of b1, with the transcription start site of b1. The physical interaction of the repeats with the transcription start site occurs in a tissue- and expression level-specific manner, thus, long-range interactions in the nucleus are dynamic and rely on mechanisms that still need to be elucidated.

Long range gene interactions monitored by the lac operator/lac repressor::GFP system were reported by B. Borisova (Gatersleben, Germany). Based on previous observations that lac operator transgene loci frequently associate with each other and with heterochromatic chromocenters, Borisova and colleagues investigated the underlying molecular mechanism for this interaction. They found that the depletion of H3K9me2 reduced pairing, but to a much lesser extent than DNA hypomethylation in the mutant ddm1. This suggests that DNA methylation might act largely independently of H3K9me2 as a major mark for DNA pairing and that H3K9me2 has an additional minor role in pairing.

### The End of Chromosomes

The end of linear eukaryotic chromosomes needs to be marked to distinguish them from DNA double strand breaks and to protect genome integrity. This is achieved by telomeres, nucleoprotein structures that not only protect the end of chromosomes from the DNA damage response pathway but also allow the complete replication of chromosome ends preventing a continuous shortening of chromosomes with each replication cycle. Telomeres in many organisms including plants are heterochromatic structures comprised of tandem repeat sequences that regulate the length of the telomere primarily by controlling telomerase activity and telomere accessibility. Telomeres are enriched for heterochromatin associated epigenetic marks, and F. Fajkus (Brno, Czech Republic) presented results showing that DNA hypomethylation does not affect telomere length in Arabidopsis, in contrast to the strongly increased telomere length in hypomethylated mammalian cells driven by increased homologous recombination. Surprisingly, mutations in the chromatin assembly factor 1 (CAF-1) leading to increased homologous recombination in Arabidopsis cause a decrease in telomere length, strengthening previous observations that equivalent recombination processes have different outcomes in different organisms.

![Figure 2. Scheme of the Arabidopsis female gametophyte and images of nuclei from gametophytic cells. Nuclei from accessory cells (synergids and antipodals) and gametic cells (egg and central cell) have distinct size and heterochromatin content. Scale bar: 2 μm. Courtesy of C. Baroux (Zurich, Switzerland).](image-url)
Emerging Directions

Although there are many similarities in the regulation of chromatin structure and function among eukaryotes, there are as well many lineage-specific differences. Plant and animal development differs in many ways, and it will be one of the challenges for the coming years to discover how these differences are reflected by differences in the regulation of chromatin structure and function. Thus, identifying plant specific mechanisms of chromatin regulation and understanding why these differences might have evolved will advance our understanding of the underlying principles of developmental plasticity of plants. Chromatin research (like many other research directions) heavily depends on the development of new techniques that will give new types of data, such as information about defined chromatin states of a specific cell at a specific time in development or about position of any gene of interest within the nucleus at any given time point. Thus, promotion of technical developments that will allow us to pursue these questions will be a worthy investment in the near future.

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