Chromatin and Epigenetics

Wrapping DNA around protein beads, to form so-called nucleosomes, is an ingenious invention of nature allowing the condensation of an enormous amount of genomic information into a tiny package. The assembly of DNA with histone proteins, the chromatin, increases genome stability, but it also hinders the access of enzymes needed to transcribe the encoded information. Bliss or burden, the chromatin provides an extra layer of control over gene expression. This control is exerted through a plethora of chemical modifications of the histones and the DNA, so-called epigenetic marks, which alter chromatin structure and provide specific recognition sites for regulatory factors. Evidence has grown exponentially over the past decade of the importance of chromatin-based processes for plant development, physiology, and adaptation to environmental stimuli, catapulting chromatin research to the forefront of plant science research. The increasing availability of affordable high-throughput sequencing services, antibodies for modified DNA or histone residues, and genome sequences have enabled laboratories worldwide to introduce chromatin-related questions into their research.

As epigenetics research goes mainstream, publications on this topic are beginning to appear in journals with remits well beyond that of genetics and cell biology. The importance of chromatin-based regulation is slowly emerging, not only for plant development but also for growth, physiology, metabolism, and stress responses. As a result, *Plant Physiology* is seeing an increasing number of submissions in this area. Therefore, it is timely to share this advance with our readership in the form of a Focus Issue. Publications in the field of Chromatin and Epigenetics not only offer an enormous amount of exciting fundamental science but are attractive also to readers with more applied interests. The emerging relationship between epigenetics and phenotypic variation is animating crop breeders, while each discovery of yet another component of the chromatin machinery opens new avenues in biotechnology and synthetic biology. This Focus Issue on Chromatin and Epigenetics showcases the breadth of research undertaken in this area of plant science. As exemplified in the study by Qian et al. (2015), gene families with functions in chromatin structure and remodeling have considerably expanded from fungi to plants. We are only just starting to appreciate the complexity of interactions between the myriad proteins associated with chromatin at any given time during developmental transitions and responses to the environment. How plants employ these proteins to generate a regulatory system that allows dynamic and plastic processes to occur within the context of a highly compact, valuable structure is a big question that can only be tackled in a concerted effort. Our selection of Update reviews and original articles is no more than a snapshot of the current activity in this field. Nevertheless, we hope that the following menu of papers caters to a wide range of interests in our readers.

OUR UPDATES

The Update by Donà and Mittelsten Scheid (2015) on chromatin and DNA-damage repair (DDR) provides an excellent start into the topic, as it exemplifies the problem of how essential repair machinery gets to a site that is buried under a compact structure, with minimal disturbance. A nucleosome can be shifted, evicted, or exchanged, its composition can be altered, and its subunits can be covalently modified. As the authors walk us through these possibilities, they cover a lot of ground that is useful for understanding not just DDR but many other chromatin-related topics. They also point out that understanding the chromatin environment of DDR will be helpful for improving gene-editing technology.

Paramutation describes the ability of one (epi)allele at a specific locus to transmit its activity state heritably to the second allele. In maize (*Zea mays*), paramutation can generate striking patterns of kernel pigmentation, one of the earliest epigenetic phenomena to be systematically investigated (Brink, 1956). Yet, 60 years on, the molecular mechanisms that generate and maintain paramutations are still under debate. In their Update, Giacopelli and Hollick (2015) discuss different ways to explain paramutation mechanisms. They provide strong evidence for a model based on RNA-directed DNA methylation, in which the production of 24-nucleotide RNAs from a paramutagenic allele influences 5-methyl cytosine patterns of a paramutable allele in trans, but they also consider histone modifications as a potential mechanism to account for transgenerational inheritance of paramutagenic states. Another well-described phenomenon that still awaits clear and comprehensive molecular explanation is hybrid vigor. Based on the identification of epigenetic changes in hybrid nuclei through genome-wide methylome profiling, Greaves et al. (2015) argue that DNA methylation associated with 24-nucleotide small interfering RNAs could exhibit transallelic effects in hybrids of Arabidopsis (*Arabidopsis thaliana*) and other species. However, the link to transcriptional changes and the hybrid phenotype remains to be confirmed. New hybrid-like pure breeding lines are expected to shed light on this question and could have valuable applications in crop systems.

DNA methylation not only silences transposable elements and other heterochromatic regions but also occurs in transcribed genes. However, genes responding to environmental or developmental signals generally do not show body methylation. In their Update, To et al.

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(2015) discuss recent evidence for mechanisms underpinning the intronic heterochromatin and gene body methylation of housekeeping genes.

How epigenetic markers can integrate a variety of developmental and environmental cues is the topic of the remaining two Updates. Floral transition as an environmentally controlled developmental phase change is the model par excellence for this research. Hepworth and Dean (2015) bring us up to date with lessons learned from *FLOWERING LOCUS C* (*FLC*). *FLC* must be the single most-studied gene that is epigenetically regulated, and the diversity and complexity of the regulatory pathways culminating in its control are astounding. Yet, the authors show that the combination of detailed molecular information with mathematical modeling has led to a relatively simple picture in which a few core chromatin complexes antagonize each other to enable fine-tuning of the flowering developmental program to multiple inputs from the environment. Another central regulator in flowering is WUSCHEL, a transcription factor that integrates signals from multiple regulatory pathways to ensure the temporally precise maintenance and determinacy of the floral meristem. Cao et al. (2015a) summarize the current state of knowledge on the regulation of WUSCHEL, which includes a range of chromatin-based processes and raises many new questions. The authors conclude that, in order to fully understand the molecular mechanisms that control the timing of the floral meristem, a combination of tissue- or cell type-specific analyses with genome-wide and computational approaches will be necessary in the future.

FLOWERING CONTROL IN MAIZE, ARABIDOPSIS, AND RICE

Considering the importance of epigenetic regulation for flowering, it is not surprising that several of the original studies in this Focus Issue home in on this process. Mascheretti et al. (2015) provide evidence that distinct mechanisms regulate florigens in response to autonomous and photoperiod pathways. They compared histone modification profiles over florigen loci in nonflowering, *indeterminate1* (*id1*) mutants of modern day-neutral temperate maize with those of a photoperiod-responsive maize ancestor grown under floral inhibitory photoperiods. Their results showed that *ID1* function promotes histone H3 hyperacetylation, whereas short-day induction is associated with increased histone H3 dimethylation and trimethylation at Lys-4. The importance of Polycomb group proteins in the transition of Arabidopsis from juvenile to adult phase is stressed by the study of Pico et al. (2015). They found that mutants impaired in different components of the Polycomb group machinery displayed either delayed or accelerated flowering due to misexpression of microRNAs miR156 or miR172, respectively, in juvenile plants. Finally, Cao et al. (2015b) report that histone H2B monoubiquitination, mediated by two E3 ligases in concert with histone H3 Lys-4 dimethylation, is required for anther development in rice (*Oryza sativa*). Analysis of the anther transcriptome revealed that transcript levels of several key tapetum degradation-related genes were decreased in E3-ligase defective rice mutants defective for the E3 ligases.

A HEN-AND-EGG PROBLEM

The relationship between chromatin modifications and gene expression is a hen-and-egg problem. It is further complicated by the fact that multiple chromatin modifications can occur in the same locus and that the same modification can have different roles at different sites of a locus. We are far from understanding the causal relationship between transcriptional activity and chromatin marking. Despite being purely correlative at this stage, the comparative profiling of concurrent genome-wide chromatin and transcriptome changes in different tissues and developmental stages or under different environmental conditions nevertheless provides useful information, as it identifies potentially important loci and target sites for the direct interplay of transcriptional and epigenetic regulation. Several such studies are included in this Focus Issue.

Brusslan et al. (2015) monitored genome-wide histone H3 Lys-4 trimethylation (H3K4me3), histone H3 Lys-9 acetylation, and transcript levels in mature Arabidopsis leaves during developmental senescence. There was no tight correlation between transcript changes and epigenetic marks, but 22% of genes up-regulated over the time course also showed an increase in H3K4me3. Interestingly, it was the breadth of the H3K4me3 islands that increased over time, indicating spreading of the mark out of the gene body as transcriptional activity increased. Monitoring the same two marks during the development of maize leaves, Perduns et al. (2015) found that changes in transcript levels and histone marks were better correlated than absolute values. They also identified secondary histone H3 Lys-9 acetylation peaks farther upstream in the promoter regions that were strongly up-regulated in the leaf blade compared with the leaf base and occurred preferentially in genes with functions in C4 photosynthesis. Both studies highlight the importance of temporal and spatial resolution when comparing histone marks with gene expression.

The relationship between the variation of context-specific DNA methylation and genetic variation as well as differential gene expression was investigated by Li et al. (2015b) in five maize inbred lines. Several thousand regions differed in context-specific methylation between any two lines and, although less common than in genetically varying regions, differences also occurred without local sequence variation. Differential methylation was correlated with on/off differences of gene expression in a context-specific manner, but no correlations were found with quantitative differences in transcript levels of expressed genes. For rice, Xing et al. (2015b) report genome-wide DNA methylation patterns during seed development, showing greater embryonic hypermethylation near short transposable elements and non-transposable element genes than those in the endosperm. In particular, genes with low expression levels are enriched in hypermethylated genes, and early endosperm developing stages had reduced levels of methylation. From a different perspective, Kim et al. (2015) examined the possible relationship between DNA methylation and duplicated genes from polyploid (whole-genome duplication [WGD]) events in soybean (*Glycine max*) and common bean (*Phaseolus vulgaris*). Soybean had a relatively recent WGD, and both soybean and common bean share an older WGD. The authors found that CG methylation in gene bodies was enriched in WGD-duplicate genes in soybean and in the shared single-copy genes in both species and that these types of genes tended to have higher expression levels.

NEW TARGET PROCESSES

Moving underground, the importance of chromatin-based gene regulation for root development is starting to emerge. As reported here by Li et al. (2015a), Arabidopsis mutants defective in HISTONE DEACTYLASE6 (HDA6) produce ectopic root hair cells in nonhair positions of the root epidermis. The authors found that HDA6 regulated acetylation and transcript levels of two transcription factors with central roles in root cell patterning. Tissue differentiation in response to pests and pathogens is another area where epigenetic processes could play a regulatory role. In soybean roots, the formation of a multinucleated feeding site (syncytium) during susceptible interaction with cyst nematodes involves massive gene expression changes. A genome-wide methylome analysis by Rambani et al. (2015) identified several hundred syncytium-specific differentially methylated genes, which will aid further dissection of the regulatory pathways underpinning plant responses to root pests. Finally, a relatively unexplored field for epigenetic research is plant mineral nutrition. In this Focus Issue, Xing et al. (2015a) show that mutation of the histone acetyltransferase General Control Nonrepressed5 (GCN5) leads to impaired iron homeostasis in Arabidopsis. The phenotype was linked to GCN5-mediated histone H3 Lys-9/Lys-14 acetylation of the citrate efflux protein-encoding gene *Ferric Reductase Defective3* (*FRD3*) and could be partially rescued by *FDR3* overexpression or citrate application.

WRITERS AND READERS

At the mechanistic level, two studies in this Focus Issue shed new light on the establishment and recognition of chromatin modifications. In a forward-genetic screen with a highly tissue-specific GFP-reporter line of Arabidopsis, Hristova et al. (2015) identified a unique allele of HDA6, *hda6-8*, which was impaired in histone deacetylation and transgene silencing in young leaves. Surprisingly, *hda6-8* did not show the strong effect of previously described *hda6* mutant alleles on DNA methylation or the release of transposable elements. The study proved that HDA6 has clearly separable activities in euchromatin and heterochromatin and identified a role of a conserved E-R motif in HDA6 for the repression of de novo CG methylation at certain target genes. HDAs and histone demethylases erase the marks established by acetyltransferases and methyltransferases, and the net activity of these enzymes determines the epigenetic code. Decoding activities include histone-binding proteins that specifically bind to certain modifications. As reported by Jin et al. (2015), knockdown of the histone-binding protein Morf-Related Gene702 (MRG702) in rice mimicked brassinosteroid-deficient and late-flowering phenotypes of loss-of-function mutants of the histone H3 Lys-36 methyltransferase Set-Domain Group725. The authors further show that MRG702 binds directly to chromatin at target gene loci in a histone H3 Lys-36 dimethylation/trimethylation-dependent manner, thereby proving that MRG702 functions as a reader of this mark.

COUNTING BEADS

Nucleosomes have been compared with beads on a string. The digestion of chromatin with micrococcal nuclease, which cuts preferentially nucleosome-free linker DNA, allows the isolation of those parts of the string that are directly associated with beads. Furthermore, combining micrococcal nuclease with deep sequencing can generate high-resolution maps of nucleosome positions along the DNA. Such studies in yeast and animal species (including humans) have shown that nucleosome positioning is highly dynamic and has crucial relevance for the expression, regulation, and evolution of eukaryotic genes. This Focus Issue includes, to our knowledge, the first comprehensive analysis of genome-wide nucleosome dynamics in plants. Using leaf tissue of rice and both leaf and flower tissues of Arabidopsis, Zhang et al. (2015) have developed high-volume nucleosome occupancy and positioning data sets enabling the comparison of nucleosome occupancy in specific genomic loci between species and between tissues. The analysis revealed that plants and animals share the fundamental characteristics associated with nucleosome dynamics and provided for differential nucleosome occupancy in tissue-specific genes. The data sets represent a useful new resource for plant genomic and epigenomic research.

WHAT'S IN IT FOR AGRICULTURE?

Understanding chromatin and epigenetics is essential for improving agriculturally important traits in crops. The ability of epigenetic regulators to determine the set point of basal expression levels and of transcriptional sensitivity for environmental cues provides new opportunities to alter plant behavior through the epigenetic manipulation of signaling pathways. In the context of breeding, the discovery of permanently or tissue-specifically silenced methylated genes (Li et al., 2015b) is significant, as this cryptic information could provide a reservoir for new trait variation if reactivated. Moreover, epigenetic marks themselves can be selected for through the process of epigenetic breeding, which involves successive selection over several generations of the best-performing individuals in isogenic populations. Verkest et al. (2015) have used this procedure to select for improved drought tolerance in canola (*Brassica napus*). In an attempt to identify the molecular causes of trait improvement, they compared transcriptome and genome-wide H3K4me3 patterns in stable epilines with enhanced drought-tolerant and/or energy-use efficiency. The analysis showed that the drought-tolerant epilines displayed differential expression and histone marking of drought-responsive genes. The authors conclude that the canola epigenome can be shaped by selection to increase yield and stress tolerance. Hence, these findings support the further development of strategies to incorporate epigenetics into breeding.

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