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Article Addendum

Chromatin architecture: a new dimension in the dynamic control of gene expression

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Abstract

As the most recent evidence of eukaryotic cell complexity, genome architecture has astounded the scientific community and prompted a variety of technical and cognitive challenges. Several technologies have emerged and evidenced the integration of chromatin packaging and topology, epigenetic processes, and transcription for the pertinent regulation of gene expression. In the present addendum we present and discuss some of our recent research, directed towards the holistic comprehension of the processes by which plants respond to environmental and developmental stimuli. We propose that the study of genome topology and genomic interactions is essential for the understanding of the molecular mechanisms behind a phenotype. Even though our knowledge and understanding of genome architecture and hierarchy has improved substantially in the last few years -in Arabidopsis and other eukaryotes -, there is still a long

way ahead in this relatively new field of study. For this, it is necessary to take advantage of the high resolution of the emerging available techniques, and perform integrative approaches with which it will be possible to depict the role of chromatin architecture in the regulation of transcription and ultimately, physiological processes.

The physiological processes behind the plant responses to environmental and developmental stimuli have been exhaustively characterized. Thanks to this, nowadays there is a wide understanding in stress biology, hormonal signaling, and their ultimate impact on gene expression. Even though epigenetics and epigenomics have risen as an intriguing and exciting research field, giving insights into the molecular mechanisms behind gene expression, there are still plenty of interesting questions to be answered. Epigenomic and 3D-chromatin conformation approaches have emerged as tools to address these questions from an integrative perspective; tools to find the missing links between physiological, cellular and molecular processes that, despite being coordinated and corregulated, have been habitually independently addressed by the scientific community.

Some studies have characterized specific cases that illustrate the importance of the physical configuration of the chromatin fiber on the regulation of plant development, and responses to environmental stimuli[1]. It is generally accepted that high levels of DNA compaction are correlated with low gene expression and vice versa, this phenomenon being mainly attributed to the covalent modifications of histone tails and DNA. However, it is becoming clearer that the panorama in the eukaryotic nucleus is significantly more complex, and that the interactions between distal and proximal genomic regions have a great impact over transcription.

A Hi-C study depicted the general 3D-genome organization in Arabidopsis, finding a non-random distribution of diverse genomic regions. Furthermore, such conformation was proven to be different to the one described in animal nuclei -and characterized by a higher frequency of intrachromosomal

interactions enriched with H3K27me3, H3.1 and H3.3 marks-[2]. There does not seem to be abundant interactions between highly transcribed genes in Arabidopsis, being long-range interactions mainly between heterochromatic regions of plant chromosomes[3]. Nevertheless, short-range interactions occur between regulatory elements and their gene-encoding targets in the same chromosome, in order to establish a dynamic transcriptional network based on physical proximity[4]. Gene loops, a type of short-range interaction, have been proven to have an important role in the transcriptional regulation and memory in animals, yeast and plants[5,6,7]. These consist in dynamic three-dimensional local interactions, which structure is a determinant factor for the regulation of transcription[6].

In one of our studies we described an example of these latter interactions, where BAF60, a subunit of the SWI/SNF chromatin remodeling complexes, regulates cell division and differentiation during development in *Arabidopsis* plantlets. The mechanism by which this occurs is through the targeting of *IPT3* and *IPT7,* genes involved in cytokinin synthesis. BAF60 binds to these loci and to the cell cycle negative regulator *KRP7*, preventing the formation of gene loops in each locus, and repressing their expression. Such repression leads to a decrease in cytokinin levels and has a positive effect in the progression of the cell cycle and root growth[8].

Interestingly, BAF60 also prevents the formation of a gene loop in *FLC* (FLOWERING LOCUS C), a very well described flowering repressor in *Arabidopsis* [9,10]. Under long day conditions BAF60 binds *FLC* and promotes the addition of H3K27me3, which, together with the loop repression, leads to the downregulation of this gene. In the absence of FLC the molecular events related

to flowering are positively regulated and the development of sexual organs occurs [11,1].

In a more recent study we described the role of long nonconding intergenic RNAs (lncRNAs) on genome topology and gene expression[12]. With this study we were able to evidence the plasticity of chromatin conformation and gene expression, as a result of the integration of hormone signaling, DNA methylation, noncoding transcription, and histone modifications. Thus, we elucidated some of the mechanisms by which the expression of the *PINOID (PID)* gene, an important regulator of the spatial localization of auxin transporters, is regulated. Robust evidences have shown that *PINOID* expression relies on *APOLO*, a lncRNA capable of triggering several downstream molecular pathways involved in the determination of chromatin topology[12]. A Chromosome Conformation Capture (3C) assay showed that the promoter region of *PID* interacts with the *APOLO* locus through the formation of an LHP1-mediated -and auxin sensitive- loop. Auxin signaling induces the activity of several DNA demethylases, leading to a reduction of DNA methylation along *APOLO*. Such changes result in the opening of the loop encompassing the *PID* promoter region, thereby exposing the locus to the transcriptional machinery. PolII divergent transcription starts at both, *PID* and *APOLO* loci, a phenomenon coupled with the increase in the H3K9 acetylation at the 5' region of *PID*.

Interestingly, it is the accumulation of *APOLO* transcripts what leads to the recruitment of LHP1, a PRC (Polycomb Repressive Complex) protein that participates in the subsequent reestablishment of the loop. In parallel, POLIV/V transcription triggers the RNA dependent DNA Methylation (RdDM) pathway and the recruitment of PRC1 and PRC2 is in charge of the deposition of H3K27

trimethylation of *APOLO.* Recently, we demonstrated that LHP1 controls the spreading of H3K27me3 towards the 3´of its genomic targets, suggesting that this protein may be contributing to loop formation[13]. One of the most notorious and exciting characteristics of LHP1 is its potential to co-regulate the expression of distant genes through the establishment of LHP1-dependent physical interactions. Even though LHP1 can control genome topology and hence expression patterns of several genes[13], there is still a need for identifying the precise mechanisms by which this occurs.

Hence, we propose the existence of at least several hundreds of this type of loops and genomic interactions in the eukaryotic cells, which may be influencing the transcription of diverse genomic regions[14,15]. The discovery of genome "interactomes" and their role in the tuning of on gene expression have become the foundations of genome topology research. These basic structures have encouraged the scientific community to elucidate their complexity and hierarchical organization. Our understanding of the complexity and nature of genome topology and conformation has continuously increased in the last years, as the technical robustness and resolution of the available and new techniques improve[16]. However, there are still many questions to be addressed, and their answer will probably need from an integrative molecular biology. With these approaches we will be able to discover some of the missing shackles in the well defined, and intensely studied, relation between genotype and phenotype, in plants and other eukaryotes.

1. Rodriguez-Granados, N. Y., Ramirez-Prado, J. S., Veluchamy, A., Latrasse, D., Raynaud, C., Crespi, M., … Benhamed, M. (2016). Put your 3D glasses on: plant chromatin is on show. *Journal of Experimental Botany*, erw168.
2. Wang, C., Liu, C., Roqueiro, D., Grimm, D., Schwab, R., Becker, C., … Weigel, D. (2015). Genome-wide analysis of local chromatin packing in\n Arabidopsis thaliana. *Genome Research*, *25*(2), 246–256. http://doi.org/10.1101/gr.170332.113
3. Liu, C., Wang, C., Wang, G., Becker, C., & Weigel, D. (2016). Genome-wide analysis of chromatin packing in Arabidopsis thaliana at single- gene resolution. *Genome Research*, 1–30.
4. Louwers, M., Bader, R., Haring, M., van Driel, R., de Laat, W., & Stam, M. (2009). Tissue- and expression level-specific chromatin looping at maize b1 epialleles. *The Plant Cell*, *21*(3), 832–42. http://doi.org/10.1105/tpc.108.064329
5. Singh, B. N., Laine, J., Krishnamurthy, S., & Hampsey, M. (2009). A physiological role for gene loops in yeast, 2604–2609. http://doi.org/10.1101/gad.1823609.Genes
6. Hampsey, M., Singh, B. N., Athar, A., Laine, J., & Krishnamurthy, S. (2011). Control of eukaryotic gene expression: gene loops and transcriptional memory, *4*(164), 118–125. http://doi.org/10.1126/scisignal.2001449.Engineering
7. Oti, M., Falck, J., Huynen, M. A., & Zhou, H. (2016). CTCF-mediated chromatin loops enclose inducible gene regulatory domains. *BMC Genomics*, *17*(1), 252. http://doi.org/10.1186/s12864-016-2516-6
8. Jegu, T., Domenichini, S., Blein, T., Ariel, F., Christ, A., Kim, S. K., …

Benhamed, M. (2015). A SWI/SNF chromatin remodelling protein controls cytokinin production through the regulation of chromatin architecture. *PLoS ONE*, *10*(10), 1–18. http://doi.org/10.1371/journal.pone.0138276

9. Michaels, S. D., & Amasino, R. M. (2001). Loss of FLOWERING LOCUS C activity eliminates the late-flowering phenotype of FRIGIDA and autonomous pathway mutations but not responsiveness to vernalization. *The Plant Cell*, *13*(4), 935–41. http://doi.org/10.1105/tpc.13.4.935 http://doi.org/10.1093/jxb/erw168
10. Searle, I., He, Y., Turck, F., Vincent, C., Fornara, F., Kr??ber, S., … Coupland, G. (2006). The transcription factor FLC confers a flowering response to vernalization by repressing meristem competence and systemic signaling in Arabidopsis. *Genes and Development*, *20*(7), 898–912. http://doi.org/10.1101/gad.373506
11. Jégu, T., Latrasse, D., Delarue, M., Hirt, H., Domenichini, S., Ariel, F., … Benhamed, M. (2014). The BAF60 subunit of the SWI/SNF chromatin-remodeling complex directly controls the formation of a gene loop at FLOWERING LOCUS C in Arabidopsis. *The Plant Cell*, *26*(2), 538–51.

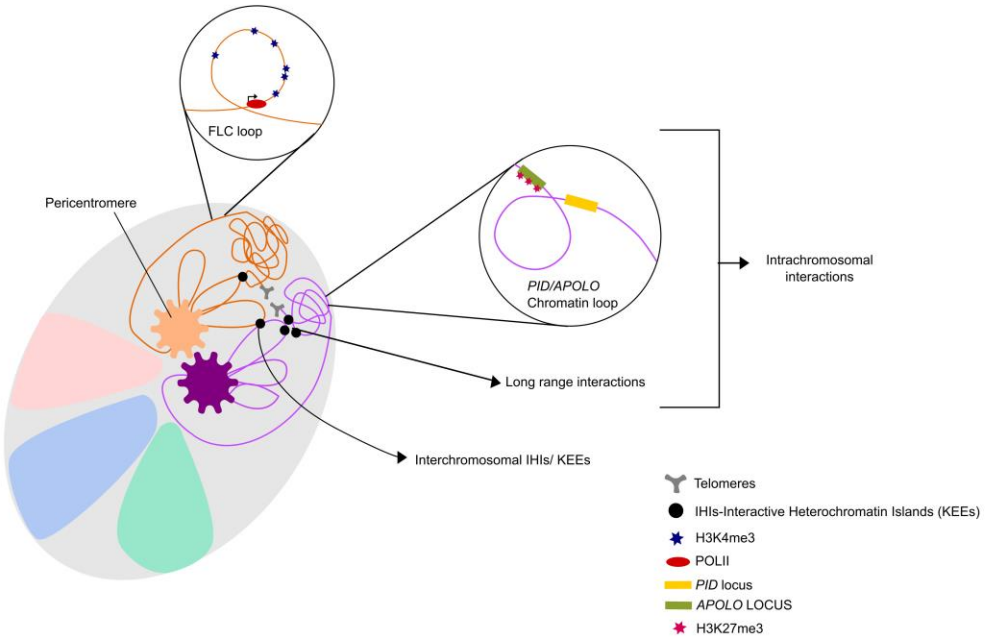

Fig1. Schematic representation of chromatin organization in *Arabidopsis thaliana*. Chromosomes territories are represented with different colors, being two of chromosomes showed in detailed with the most relevant interactions, observed in previous 3C-derived analyses. Pericentromeric regions are recognized for establishing strong intra- and inter-chromosomal interactions between them. Interactive Heterochromatic Islands (IHIs) can be found in different regions along the five chromosomes. These islands are mainly located in proximal and distal regions of the chromosome arms and converge in the three dimensional space, where they form a *KNOT*, or a superstructure that consists of inter and intra-chromosomal interactions of both long and short-range. Regulatory elements and genes belonging to the same chromosome are commonly connected by the formation of loops or higher order chromatin structures. For instance, *FLC* expression is activated by the formation of a gene loop between its 3' and 5' flanking regions and H3K4 trimethylation along the locus. Moreover, chromatin loops encompassing different genes have also been involved in gene expression fine-tuning; the regulation of the *PID* gene by the formation of a loop between *PID* and *APOLO* loci is a clear example of these structures. Ploidy level of the cell and genomic position of the interactive regions shown above are not considered.