Novel Insights Into Gene Silencing Mechanisms in Zea Mays and Arabidopsis Thaliana

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Summary

In eukaryotic cells genomic DNA is compartmentalized in the cell nucleus. DNA, together with DNA-bound proteins and RNA forms chromatin, which is not randomly distributed in the nucleus, but folded in a hierarchical manner. In the nucleus, DNA is transcribed into RNA, which is subsequently transported out of the nucleus to the cytoplasm and translated into proteins. Transcription can be positively or negatively influenced by the binding of regulatory proteins to certain DNA sequences. The transcription level of DNA sequences is additionally influenced by chemical modifications of DNA and histone proteins, as well as by interactions with particular DNA sequences.

Multi-cellular organisms consist of many different cell types. In each cell type a specific set of genes is actively transcribed, while other genes are silenced. Thereby, only proteins that are needed for the cell-type-specific functionality are expressed. Besides genes, the genome also contains a large proportion of other sequences, of which some have gene-regulatory potential. This thesis addresses two different silencing mechanisms, targeting either non-coding regulatory sequences or genes during plant development.

A silencing mechanism specific to plants is RNA-directed DNA methylation (RdDM). This mechanism uses RNA molecules that are 24 nucleotides short and also called short interfering RNAs or siRNAs. These siRNAs mediate the addition of DNA methylation to DNA sequences that are complementary to the sequence of the siRNAs. As a consequence, these DNA sequences are transcriptionally repressed. Transcriptional repressive marks that are found at RdDM-targeted loci are, among others, a specific type of DNA methylation and a histone mark named H3K9me2. In maize, RdDM is known to be involved in the transfer of silencing information between alleles of particular genes. During this process, which is called paramutation, a plant inherits a silenced, paramutagenic allele of a specific gene from one parent and an active, paramutable allele of the same gene from the other parent, and this active allele gets silenced. Moreover, when the originally silenced allele is crossed out, the originally active allele will remain silenced, and will even obtain the ability to silence other, active alleles. When components of the RdDM pathway are mutated, paramutation is impaired and paramutagenic alleles can lose their repression. Here, we focused on two different paramutagenic loci, *B'*, encoding a factor in plant pigmentation, and *vNYR*, a transgenic locus encoding a fluorescent marker protein. We studied the level of DNA methylation and histone marks at the *B'* and *vNYR* loci, and how the levels of these marks changed in RdDM mutants. Although both the *B'* and *vNYR* locus are paramutagenic and require RdDM components for their repression, their DNA methylation level and chromatin marks were different. While *B'* was enriched for the histone mark H3K9me2 and had low levels of a specific type of DNA methylation, *vNYR*

was devoid of H3K9me2 and had high levels of this particular type of DNA methylation. Within the whole range of genome-wide loci that are targeted by RdDM, an inverse correlation between the two repressive marks DNA methylation and H3K9me2 was observed. We propose that *vNYR* and *B'* are two distinct, rather extreme cases of RdDM-loci. A future perspective is to determine whether there is a correlation between the repressive marks they carry and their behavior in paramutation. It would also be interesting to examine if different types of RdDM loci serve distinct functions in general.

Paramutation causes a permanent, heritable silencing of a formally active gene. While it may be beneficial to permanently silence particular sequences, other sequences have to be silenced or re-activated throughout development in a dynamic way. The repression of developmentally regulated genes is accomplished by polycomb proteins, via a process that is widely conserved among multicellular organisms, including plants and animals. An example of a gene that is developmentally silenced by polycomb proteins is the *Flowering Locus C* (*FLC*) of the model plant Arabidopsis. *FLC* encodes a flowering repressor that ensures that the plant does not flower too early after germination. However, to allow flowering at a later time point, *FLC* has to be silenced for the rest of the plant life cycle. Silencing of *FLC* is induced by vernalization, which is a prolonged cold treatment of the plant. We studied the chromosomal interaction pattern of the *FLC* gene and tested whether certain interactions change after polycomb silencing of this gene. The results indicated that, once the *FLC* gene itself was repressed by polycomb, it interacted preferably with the nearest polycomb targets rather than with non-polycomb targets. However, sequences that were not a polycomb target are not completely avoided. This indicates that particular interaction partners might be preferred, but are not exclusive. Furthermore, we noticed genome-wide changes in chromosomal interactions during the cold treatment. These changes correlated with a larger size of nuclei during the cold than before and after the cold treatment. The larger size of nuclei, together with the global changes in chromosomal interactions, suggest that during the cold treatment the chromatin is packed in the nucleus in a less compact manner. To get a better understanding of the correlation between chromatin reorganization and the size of nuclei further research is needed.