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Signal integration on plant promoters
A case study in maize

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Gene promoters perceive numerous signals and integrate this information into a single response, the transcriptional activity of a gene. It was speculated that covalent modification of histones on the promoters might have an important function in storage and integration of signals. Using the genes for the core proteins of C4 metabolism in maize as a model, we associated the perception of specific signals with the establishment of individual histone modifications. Core elements of the histone code defined in these studies are conserved on all C4 genes and on other maize genes that respond to similar stimuli. Moreover, the code is used in independent C4 lineages. However, our data also advise caution because interpretation of histone modifications might differ dependent on the promoter position of the modification. The model provided here constitutes a starting point for genome-wide decoding of stimulus-modification pairs in epigenetic gene regulation.

In behavioral sciences, information integration theory describes the valuation and integration of information derived from different sources or signals into a response.[1] On a molecular level, information integration is a major function of gene promoters that translate numerous developmental or environmental inputs into a single output, the transcriptional rate. Eukaryotic genes are packed into chromatin. Research from the last two decades established chromatin as a key player in gene regulation.[2] The basic repeat unit of chromatin is the nucleosome that is made up of each two copies of the histone proteins H2A, H2B, H3 and H4 plus approximately 147 bp of DNA wound around this body.[3] Multiple amino acid residues on histone proteins can be modified in numerous ways with acetylation and methylation of the N-terminal domains of histones H3 and H4 being the best studied modifications.[4,5] Two different models for the function of histone modifications in gene regulation were proposed in the past (**Fig. 1A**): First, some histone modifications can lead to neutralization of positively charged histone tails, thereby weakening the interaction with the negatively charged DNA. This might allow better access for RNA polymerases and other transcription factors (charge neutralization model).[6,7] This model would imply that information integration takes place before chromatin is modified and that chromatin modifications would just be used to control the response function of the integrator. Alternatively, specific histone modifications are themselves recognized by transcription factors (histone code model).[8,9] In this model, histone modifications would be controlled by defined signals and used to store and integrate information on promoters. Thus, histone modification would act on the level of the integration function.

The C4 carbon concentrating mechanism in maize is an excellent system to study signal integration on chromatin, because the corresponding genes are highly transcribed and regulated in a similar manner by many different developmental and external signals. Major developmental stimuli include organ specificity (i.e., genes are only transcribed in leaves, but not in roots) and tissue specificity (i.e., within a leaf, most C4 genes are either transcribed in mesophyll cells or bundle sheath cells, but not in both tissues). Most important responses to external signals are a strong induction by light and a downregulation at low nitrogen availability or high leaf sugar levels.[10,11] In addition to the high degree of regulation of promoter activity, initial studies revealed that epigenetic factors are involved in the regulation of C4 genes.[12,13] It was shown that de-methylation of four specific cytosines in the upstream promoter region of the C4 gene encoding phosphoenolpyruvate carboxylase (C4-*Pepc*) occurred in a light- and tissue-specific manner. Using the same promoter as a model, we identified histone modifications associated with gene regulation (**Fig. 1B**). Among others, these analyses revealed that core promoter histone modifications were controlled by specific stimuli, e.g., acetylation of lysine 9 on histone H3 (H3K9ac) and H4K5ac were exclusively controlled by light,[14] whereas trimethylation of H3K4 (H3K4me3) potentiated the gene for activation

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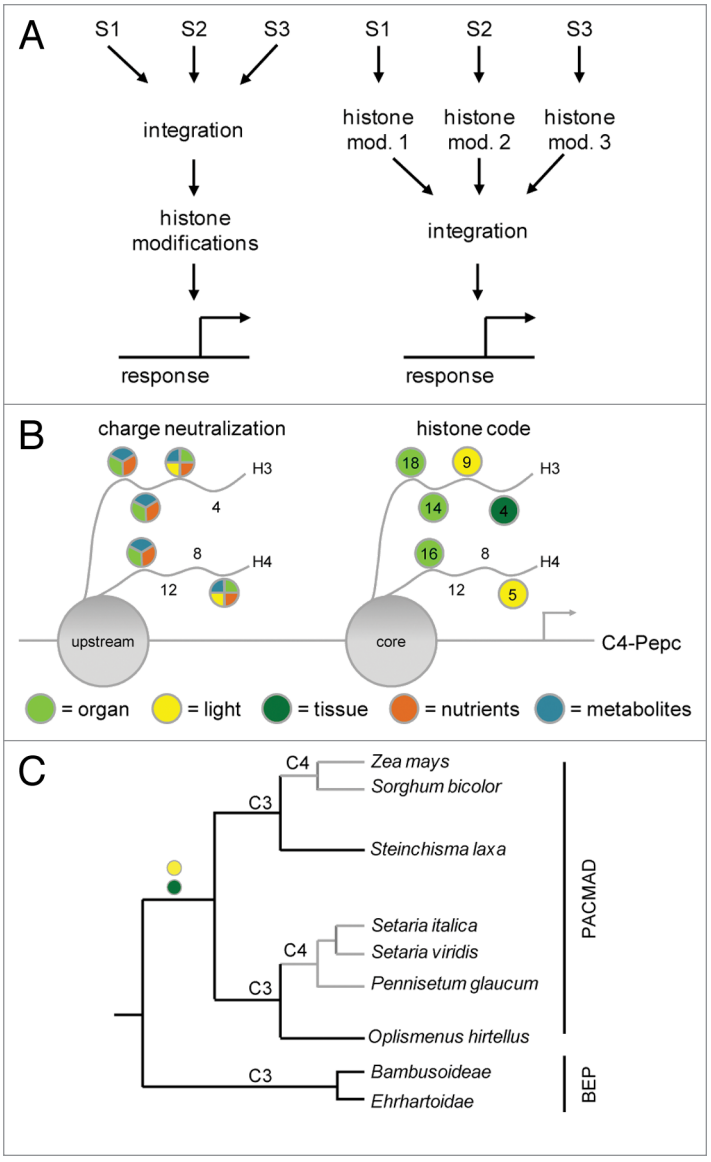

Figure 1. Histone modification models and phylogeny of the Poaceae. (**A**) Two different models for the function of histone modifications in signal integration. Charge neutralization model (left), histone code model (right). (**B**) Schematic model of the function of histone modifications in C4-*Pepc* gene regulation. Numbers represent lysine residues on the N-terminal tails of histones H3 and H4. The color represents as listed in the figure. Core promoter modifications follow the histone code model, upstream promoter modifications follow the charge neutralization model. (**C**) The cladogram illustrates the phylogenetic relationship of representative C4 and C3 species among the Poaceae. The PACMAD clade contains both C4 (gray branches) and C3 species (black branches), whereas the BEP clade contains only C3 species. The most recent possible phylogenetic origins of light-induced histone acetylation (yellow dot) and tissue-specific histone methylation (green dot) are indicated.

in mesophyll cells.[15] Such stimulus-modification pairs were compatible with a histone code model and argued for a function of histone modifications in signal integration. However, in more upstream promoter regions, histone modifications responded to all tested stimuli, including nitrogen availability and metabolite repression, in a similar and dose-dependent manner suggesting that histone modifications were only used to control the response function of the promoter as predicted in the charge neutralization model.[14]

We now analyzed whether other C4 genes in maize use the same histone code.[16] Key features of the code such as light-dependent histone acetylation and tissue-specific histone methylation were highly reproducible on all genes encoding enzymes of the C4 core cycle.[16] Here, we add data for the core promoters of four additional maize genes (**Fig. 2**). *Glk*1 encodes a kinase with possible function in mesophyll chloroplast development,[17] whereas *Cp*24, *Cp*26 and *Cp*29 encode elements of the light harvesting complex II.[18] All these genes are preferentially transcribed in mesophyll cells of illuminated maize leaves (data not shown and ref. 19). Accordingly, we found high H3K9ac only in illuminated leaves, but not in leaves from plants exposed to prolonged darkness. H3K4me3 levels were increased in mesophyll compared with bundle sheath cells, even when leaves were never illuminated. These additional results substantiate our hypothesis that a universal code is used for the control of promoters by light and tissue-specific signals in maize.

The question remains to which extent this histone code has been established during evolution of C4 metabolism or whether a previously existing code has been recruited into C4. Due to its recent evolutionary origin (approximately 25 million years), the C4 syndrome is an outstanding example for parallel evolution with more than 60 independent origins in different plant lineages.[20] Recent analyses of DNA sequence elements responsible for C4-specific gene expression indicated that these elements were active in different C4 lineages[21] and already found in the C3 orthologs of some of these genes.[22,23] We therefore compared chromatin patterns on C4-*Pepc* and a second C4 gene, C4-malic enzyme (C4-*Me*) in maize, sorghum and *Setaria italica*.[16] Whereas maize and sorghum share the same C4 origin, C4 photosynthesis in *S. italica* evolved independently (**Fig. 1C**, altered after refs. 24 and 25). All three species belong to the PACMAD clade of the *Poaceae* family that contains both C3 and C4 plants whereas the sister BEP clade exclusively contains C3 plants.26 The comparative chromatin analyses again revealed light induction of H3K9ac, but tissue-specific control of H3K4me3 in all three species.16 Thus, the two core features of the maize C4 histone code were retrievable in independent C4 lineages. These results indicate that elements of the histone code had been recruited into C4 from a preexisting mechanism. The most recent possible phylogenetic origin of this mechanism was after separation of the PACMAD and BEP clades (yellow and green dots in **Fig. 1C**). Further comparative analyses will show whether the origin can be dated back to even earlier time points.

In conclusion, data from previous work and the chromatin analyses on additional genes added here point to an important role of histone modifications in the integration function of plant

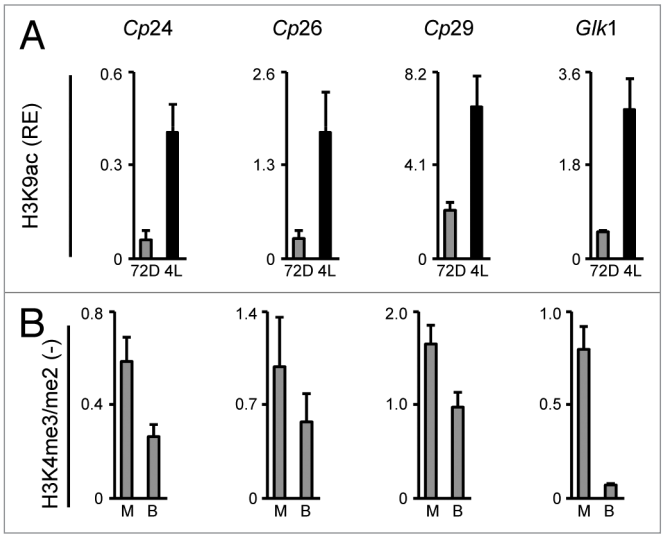

Figure 2. Light regulation of histone acetylation and cell-type specific histone methylation on four maize genes. (**A**) Light-dependent acetylation of histone H3 lysine 9 (H3K9ac) in leaves from plants that were exposed to 72 h darkness (72D, gray columns) and from plants that were illuminated for 4 h (4L, black columns). Values are presented as the relative enrichment (RE) of modifications per nucleosome over modifications per nucleosome found on the *Actin*1 promoter. (**B**) Ratio of the histone H3 lysine 4 trimethylation (H3K4me3) and histone H3 lysine 4 dimethylation (H3K4me2) in mesophyll (M) or bundle sheath (B) cells isolated form etiolated leaves. *Cp*24, *Cp*26 and *Cp*29 encode components of light harvesting complex II, *Glk*1 a kinase involved in mesophyll chloroplast development. All data points are based on at least four independent experiments. Vertical lines indicate standard errors.

promoters. The histone code used to display the perception of specific stimuli seems to be highly conserved. Dependent on the modification and the position on the promoter, histone modifications might in addition help to implement the response function of promoters.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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