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.
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.

We now analyzed whether other C4 genes in maize use the same histone code. 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. Here, we add data for the core promoters of four additional maize genes (Fig. 2). Glk1 encodes a kinase with possible function in mesophyll chloroplast development, whereas Cp24, Cp26 and Cp29 encode elements of the light harvesting complex II. 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. Recent analyses of DNA sequence elements responsible for C4-specific gene expression indicated that these elements were active in different C4 lineages and already found in the C3 orthologs of some of these genes.

We therefore compared chromatin patterns on C4-Pepc and a second C4 gene, C4-malic enzyme (C4-Me) in maize, sorghum and Setaria italica. 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 species whereas the sister BEP clade exclusively contains C3 plants. The comparative chromatin analyses again revealed light induction of H3K9ac, but tissue-specific control of H3K4me3 in all three species. 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
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.

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Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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