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Article Addendum

Next-generation sequencing reveals complex relationships between the epigenome and transcriptome in maize

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Epigenetic modifications and small RNAs play an important role in gene regulation. Here, we discuss results of our Solexa/Illumina 1G sequencing-based survey of DNA methylation, activating and repressive histone modifications, small RNAs and mRNA in the maize genome. We analyze tissue-specific epigenetic patterns, discuss antagonistic relationships between repressive epigenetic marks and highlight synergistic relationships between activating histone modifications. We discuss our observation that small RNAs show a tissue-specific distribution in maize. Whereas 24-nucleotide long small interfering RNAs (siRNAs) accumulated preferentially in shoots, 21-nucleotide long micro RNAs (miRNAs) were the most abundant group in roots, which follows the transcript level of *mop1*. Furthermore, we discuss the possibility that a novel class of 22-nucleotide siRNAs might originate from long double-stranded RNAs in an RNA-dependent RNA polymerase (RdRP)-independent manner. This supports the intriguing possibility that maize possesses at least two distinct pathways to generate siRNAs, one of which relies on RdRP and a second one that might be RdRP-independent.

Epigenetic Modifications Regulate Genome Activity

Histone proteins are targeted by a number of epigenetic modifications, whose identification and functional characterization have become the focus of major research efforts in recent years.[1,2] Instead of being static entities mainly involved in physically condensing the DNA double-helix, it is now clear that histones play a major role in actively regulating gene expression genome-wide. In fact, it has been proposed that combinations of histone modifications form a histone code, thereby extending the genetic code embedded in the DNA sequence.[3] Acetylation of histone residues generally leads to gene activation, whereas methylation can have both activating and repressive effects.[4-8] The regulatory effects of histone modifications are complemented by epigenetic modifications of the DNA itself: cytosine methylation is highly prevalent in repetitive sequences and transposable elements (TEs), but is also found in promoters and coding regions of genes.[9-16] Additionally, small RNAs, such as small interfering RNAs (siRNAs) have been shown to be associated with DNA methylation and to be involved in the guidance of epigenetic modifications to specific genomic regions.[16,17]

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Next-Generation Sequencing and Epigenomics in Maize

In recent work we have employed Illumina/Solexa 1G next-generation sequencing to analyze the relationships between exemplary histone modifications (H3K4me3, H3K9ac, H3K27me3, H3K36me3), DNA methylation, small RNAs and mRNA in the genome of maize inbred line B73.[18] Confirming previous findings for other species, we found that H3K4me3, H3K9ac and H3K36me3 were mainly associated with transcriptionally active regions of the genome, whereas H3K27me3 and DNA methylation were most abundant in transcriptionally inactive genomic segments. Intriguingly, we observed that H3K9ac and H3K27me3 were twice as common in shoots compared to roots of 14-day-old seedlings, which might be indicative of tissue-specific epigenetic patterns in the maize genome. We found that the length of modified regions strongly depended on the nature of the modification. Specifically, DNA methylation affected regions that were on average only 200 bp long, but it was the most common modification surveyed. In contrast, H3K36me3 covered the least regions in the maize genome, but the individual regions it covered were by far the longest (on average 1,600 bp). Differences could also be detected when the nature of the underlying sequences was considered. For example, H3K27me3 and DNA methylation were considerably less common in genes than in TEs or repetitive sequences. In contrast, TEs were eight times more likely to be modified by DNA methylation than at histone H3. Interestingly, the two repressive modifications surveyed (H3K27me3 and DNA methylation) were mutually exclusive but most activating modifications co-occurred genome-wide. This potentially suggests

different regulatory mechanisms between repressive and activating epigenetic modifications.

While the overall epigenetic landscape in maize is comparable to findings for Arabidopsis and rice, we found that genic DNA methylation in maize is similar to that of rice but differs substantially from Arabidopsis. While in maize and rice DNA methylation peaks around the ATG, it is most abundant in transcribed regions in Arabidopsis. It would be interesting to determine whether this is a general feature of grasses or monocots and whether similar fundamental differences exist among the epigenomes of plants. Even more significant epigenetic differences can be observed for H3K27me3 patterns when plants and animals are compared. In general, H3K27me3 is considered as a mark of transcriptional quiescence in plants, but it has recently been shown that it can also be associated with transcriptional activity in mouse.[19] Furthermore, H3K27me3 covers much shorter regions in plants than it does in animals, where it is known to span multiple genes and to form large domains. This suggests a tantalizing scenario in which some histone modifications have evolved very different spreading mechanisms and functions in plants and animals, whereas the characteristics of others are virtually conserved across phyla.

Small RNAs Leave Their Mark in Maize

Small RNAs significantly contribute to the complexity of the maize epigenome and add to its dynamics. We found that small RNAs preferentially accumulated at TEs that were not DNA methylated but were lacking at TEs that were highly DNA methylated. The biological relevance of this antagonism is not clear at this point. However, it has recently been shown that siRNAs direct DNA methylation to specific regions of the genome and might also be involved in the demethylation of DNA.[20,21] Thus, it is possible that the strong accumulation or virtual lack of small RNAs we observed for TEs in maize might be two endpoints of a dynamic process in which small RNAs specifically methylate and demethylate the DNA of TEs. Furthermore, we found that maize shows a tissue-specific distribution of small RNAs. Whereas 24-nt long siRNAs were the most common size class in shoots, 21-nt long micro RNAs (miRNAs) were the most abundant group in roots. A recent study showed that while most plant small RNAs are 21 or 24 nt long, maize is an exception even among other monocots in that it possesses an additional group of 22-nt siRNAs.[22] We were interested in determining the source of this unusual 22-nt siRNA size class and found that these siRNAs might originate from long double-stranded RNAs (dsRNAs) that are over 1 kb in length. Previous studies in mouse have shown that siRNAs can in fact be generated from long dsRNAs without involvement of RNA-dependent RNA polymerase (RdRP),[23,24] an enzyme which is involved in the canonical biogenesis of siRNAs. This brings up the intriguing possibility that maize might possess at least two distinct pathways to generate siRNAs, one of which relies on RdRP and a second one that seems to be RdRP-independent. Interestingly, we found that a decrease of 24-nt siRNAs relative to 21-nt miRNAs was not only tissue-specific, but was also clearly associated with the transcript level of *mop1*, an *RdRP* gene in maize, which supports previous locus-specific results.[25]

Maize has been an important model system in plant genetics for decades, and it is of primary importance as crop, feeding millions of people worldwide. Understanding the complex interplay between its epigenome and transcriptome will not only benefit basic research but will also be of great value to enhance crop productivity in molecular breeding programs. We hope that our survey of the maize genome will be a first step into that direction.

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