Mol Gen Genet (1988) 214:325–327

MGG

DNA methylation of the maize transposable element *Ac* interferes with its transcription

Reinhard Kunze [1], Peter Starlinger [1], and Drew Schwartz [2]

[1] Institut für Genetik, Universität zu Köln, Weyertal 121, D-5000 Köln 41, Federal Republic of Germany
[2] Department of Biology, Indiana University, Bloomington, IN 47405, USA

Summary. We find a correlation between genetic activity, DNA methylation and transcription of the maize *Ac* element: in the active phase the *Ac* DNA is unmethylated and normally transcribed, in the inactive phase the DNA is highly methylated and no *Ac* transcript is detectable. After reversion to *Ac* activity the DNA is partly demethylated and the element is transcribed again. The distribution of CpG and GpC dinucleotides in the transcribed part of the *Ac* sequence is reminiscent of a CpG island and a neighboring inter-island region described in mammalian genes. A different distribution is observed in the termini of *Ac* where the dinucleotide GpC is strongly under-represented.

Key words: *Zea mays* – Inactivation of transposition – Modification – Expression – Regulation

Introduction

The *Ac* element is the autonomous member of the *Ac/Ds*-transposable element family in maize; it is auto-transposable and can mobilize *Ds* elements *in trans* (McClintock 1951). McClintock observed that the *Ac* element in the *wx-m7::Ac* allele can undergo changes in phase from active to inactive and back to an active state (McClintock 1964, 1965).

Recently, it was shown that the cycling of the *Ac* element in the *wx-m7::Ac* allele between active and inactive phases can be correlated with DNA modification (Chomet et al. 1987). Schwartz and Dennis (1986) described the occurrence of a cycling *Ac* in the *wx-m9::Ac* allele, resembling the behavior of the *wx-m7::Ac* allele. The inactivated *Ac* element was designated *wx-m9::Ds-cy* because it behaves like a *Ds* element: it can transpose in the presence of an additional active *Ac*, but has lost its transposase activity (Schwartz and Dennis 1986).

By Southern analysis of genomic DNA it was demonstrated that in the normal, active phase only the cluster of 3 *Hpa*II sites close to one end of the element is methylated, whereas at least some of the other 10 *Hpa*II restriction sites in *Ac* (which are all located near the other end) are cleavable by the enzyme. After the switch to the inactive state in the *wx-m9::Ds-cy* allele, all 13 CCGG sites within *Ac* are methylated, but the *Hpa*II sites in the maize DNA bordering the element remain cleavable. Upon reversion from the inactive to the active state ($wx\text{-}m9::Ac_{rev}$) partial demethylation of the *Hpa*II restriction sites within *Ac* occurs. However, due to the incomplete and variable demethylation of the *Hpa*II sites in the first generation revertants, they clearly differ from the progenitor in their methylation pattern (Schwartz and Dennis 1986). Apparently, the demethylation process can be progressive: in the third backcrossed generation after the reversion to the active state of the element the *Ac* DNA can be completely demethylated (data not shown).

McClintock observed reversible changes in phase also in the *Spm* controlling element (McClintock 1959), and there are indications that the molecular mechanism responsible is DNA methylation (Masson et al. 1987). A correlation between activity and DNA modification was also described for Robertson's Mutator (Bennetzen 1985, 1987; Chandler and Walbot 1986).

Results and discussion

For this analysis we studied the transcription of the transiently inactivated *Ac* derivative in the *wx-m9::Ds-cy* allele and a revertant to full *Ac* activity, $wx\text{-}m9::Ac_{rev}$.

Poly(A)$^+$ RNA was isolated from seedlings and from leaves of single young plants of the described strains and additionally from seedlings containing an active *Ac* in the *wx-m7::Ac* allele and an *Ac*-free control strain. The RNA was size-fractionated on a Northern gel, blotted onto nitrocellulose and hybridized with a single-stranded DNA probe homologous to part of *Ac*. The result of the hybridization is shown in Fig. 1. The RNA from the *wx-m7::Ac* control strains contain the 3.5 kb *Ac* transcript (lanes 1, 2). It was shown earlier that in plant material from the *wx-m9::Ac* allele, which carries the *Ac* element in a different position in the *Wx* locus, a transcript of the same size hybridizes with the *Ac* probe (Kunze et al. 1987). This transcript appears also in tobacco plants stably transformed with *Ac* (lane 9). In poly(A)$^+$ RNA from maize seedlings (lane 7) and a single plant (lane 5) both carrying the inactivated *Ac* element (*wx-m9::Ds-cy*) the *Ac* transcript is not detectable. The other transcripts which hybridize to the *Ac* probe are not *Ac* specific, because they are lacking in some strains with *Ac* activity and two of them are also found in the *Ac* free control strain (lane 6). In a young plant grown from a kernel showing phenotypic reversion to *Ac* activity a small amount of the *Ac* mRNA is synthesized (lane 4). The DNA of this plant is still partially methylated. The plants (lane 3)

Offprint requests to: P. Starlinger

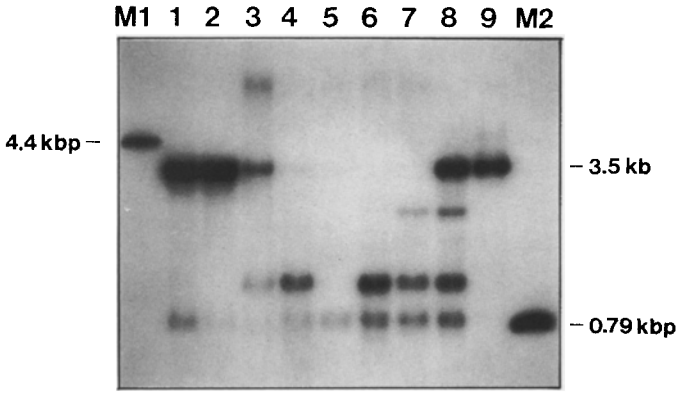

Fig. 1. Transcription analysis of *Ac*, an inactivated derivative and revertants of it to *Ac* activity. Poly(A)$^+$ RNA was isolated, electrophoresed, transferred to nitrocellulose and hybridized with a single-stranded DNA probe containing a 324 nucleotide *Bgl*II-*Acc*I *Ac* fragment as described (Kunze et al. 1987). Denatured plasmid fragments containing *Ac* sequences were used as size markers: Lane M1 contains 6 pg of a 4.4 kb DNA fragment; lane M2 contains 2 pg of a 0.79 kb DNA fragment. Lanes 1–8 contain 3 μg poly(A)$^+$ RNA each from different maize materials: lane 1: *wx-m7::Ac*, seedling roots; lane 2: *wx-m7::Ac*, whole seedlings; lane 3: *wx-m9::Ac*$_{rev}$ (3rd backcross generation), young plant; lane 4: *wx-m9::Ac*$_{rev}$ (1st generation), young plant; lane 5: *wx-m9::Ds-cy*, young plant; lane 6: *Ac*-free *wx*, young plant; lane 7: *wx-m9::Ds-cy*, seedlings; lane 8: *wx-m9::Ac*$_{rev}$ (3rd backcross generation), seedlings. Lane 9 contains 2 μg poly(A)$^+$ RNA from transgenic tobacco plants carrying *Ac*

and seedlings (lane 8) of the third backcross generation after reversion, the DNA of which was completely demethylated, contain normal amounts of the 3.5 kb *Ac* transcript. However, we have not yet confirmed the inverse correlation between the degree of methylation and the amount of *Ac* RNA synthesized in other plants.

These results corroborate our earlier conclusion that only the 3.5 kb *Ac* transcript, and not the other mRNAs homologous to *Ac*, correlates with *Ac* activity. This activity seems to be correlated with the degree of DNA methylation. In the case of *Ac*, methylation of the element could prevent the expression of the transposase gene. Indeed, genetic analysis showed that the inactivated *Ac* cannot mobilize *Ds* elements, which means that no functional transposase protein is synthesized in that state (Schwartz and Dennis 1986). This conclusion is supported by the observation that the only specific *Ac* transcript, the 3.5 kb mRNA, disappears upon inactivation.

Alternatively, methylation of the target sites for the transposase could interfere with transposase binding or function. Such a mechanism was described for the bacterial insertion sequence IS*10* and the composite transposon Tn*10* (Morisato and Kleckner 1987; Roberts et al. 1985). However, assuming that methylation of the *Ac* transposase target sites would be sufficient to prevent transposition, one would expect that the modified element would behave like a stable insertion mutation. This is not the case: the inactivated *Ac* element behaves like a *Ds* element, as it can be transposed if an active *Ac* element provides the transposase *in trans*.

In several cases, it was reported that methylation of one or a few sequences in the promoters of viral or mammalian genes can reduce or even prevent gene expression (Doerfler 1983; Doerfler et al. 1985). Our data show that the *Ac* promoter can also be regulated by methylation.

The distribution of CpG dinucleotides and CpNpG trinucleotides in the *Ac* element is conspicuously non-random. The overall GC content in the first 240 bases of the *Ac* element (Fig. 2, segment A), including the putative promoter region, is 108 G+C (45%). However, it contains 26 CpG (23 CpNpG) and only 1 GpC (2 GpNpC). One of these CpG is part of a *Hpa*II recognition site which was shown to be uncleavable in the inactivated state of *Ac* (Fig. 2). Up to now we do not know which distinct sequences in the *Ac* terminus exhibit promoter activity, but the results from experiments of G. Coupland et al. (manuscript in preparation; Coupland et al. 1988) indicate the possibility that the transposase target region and the *Ac* regulatory region could overlap, and that there is some positive interaction or negative interference between DNA binding proteins involved in transposition and transcription.

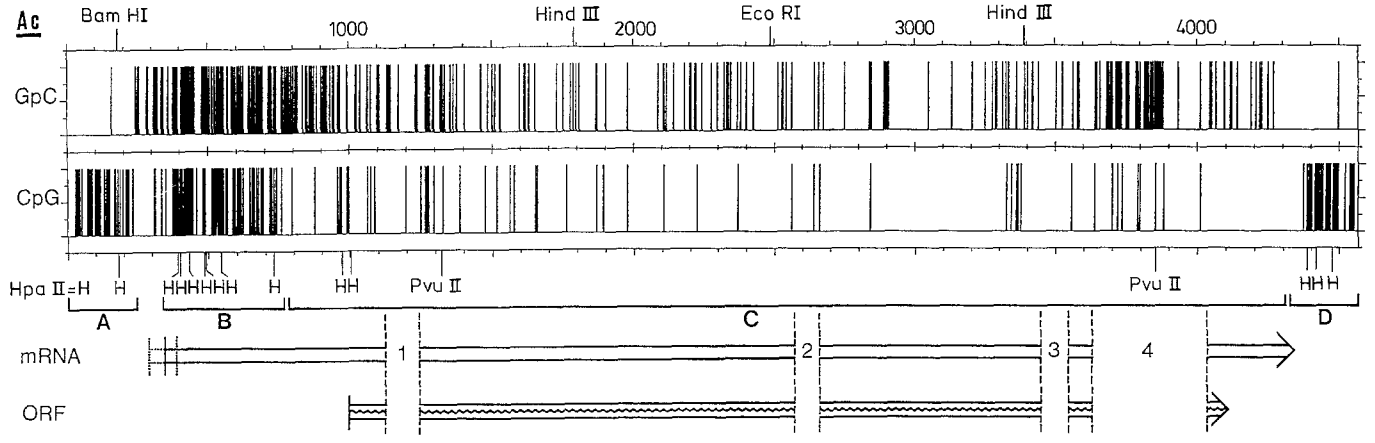

Fig. 2. Distribution of CpG and GpC dinucleotides along the *Ac* element. On top of the figure the position of the *Bam*HI, *Hind*III and *Eco*RI sites on the 4565 bp long *Ac* element is shown. CpG and GpC: each *vertical line* indicates the occurrence of the dinucleotide in the *Ac* sequence. The methylation-sensitive *Hpa*II and *Pvu*II sites are marked on the bottom line. The *Ac* transcript is shown as an *open arrow* (mRNA). The multiple transcription start points lie between the two *dotted vertical lines*; the *solid vertical line* marks the major transcription start site. The long open reading frame encoded by the *Ac* mRNA is diagrammed as the *arrow with the wavy line* (ORF). The introns (1–4) are indicated

A bias in CpG distribution very similar to that of the 5′-end is observed at the 3′-end of *Ac* (Müller-Neumann 1985): the terminal 240 nucleotides of the element (Fig. 2, segment D) contain an overall of 95 G+C (40%), 24 CpG (21 CpNpG) and 1 GpC (6 GpNpC). In this terminal *Ac* segment, the three *Hpa*II sites are located which are methylated even in the active state of *Ac*. The similarity in base and sequence composition between the two *Ac* termini is striking and may reflect partially identical sequence requirements for transposition (Müller-Neumann et al. 1984).

Except for the 240 terminal bases of both *Ac* ends, the *Ac* sequence is remarkably reminiscent of a CpG island (or HTF island) identified in vertebrate DNA and a neighboring inter-island region (Bird 1986; Lavia et al. 1987). Approximately the first 650 bases of the *Ac* transcript constitute a long untranslated leader sequence. The G+C content in this leader is 68%, compared to only 38% in the following open reading frame. Eight of the 13 *Hpa*II sites of *Ac* (marked H in Fig. 2) are located in the leader sequence. However, in contrast to the terminal 240 bp, there is no bias in the distribution of CpG/GpC dinucleotides observed in the first two-thirds of the leader: its first 430 nucleotides (Fig. 2, segment B) contain 62 CpG and 60 GpC; this segment resembles a CpG island.

Further downstream, the distribution of these dinucleotides shifts drastically. In the remaining 3.1 kb of the 3.5 kb long transcribed region, the methylation-prone CpG sequence is under-represented (51 CpG vs 181 GpC), thereby resembling the inter-island regions in mammalian genes.

Conclusion

The data presented for the *Ac* element of *Zea mays* provide evidence that the transcription of its transposase gene can be regulated via DNA methylation. Obviously, the DNA methylation of inactivated *Ac* is not restricted to the region upstream of the transcription start, but extends several hundred nucleotides into the untranslated leader sequence. We cannot distinguish experimentally between premature termination of transcription and an inhibition of transcription initiation. Termination of transcription could yield an unpolyadenylated transcript which would be excluded from the poly(A)$^+$ RNA fraction and, therefore, would not be detectable in our experiments.

References

Bennetzen JL (1985) The regulation of Mutator function and Mu1 transposition. In: Freeling M (ed) Plant Genetics, UCLA Symp Mol Cell Biol, vol 35. Liss, Inc, New York, pp 343–353

Bennetzen JL (1987) Covalent DNA modification and the regulation of Mutator element transposition in maize. Mol Gen Genet 208:45–51

Bird AP (1986) CpG-rich islands and the function of DNA methylation. Nature 321:209–213

Chandler VL, Walbot (1986) DNA modification of a maize transposable element correlates with loss of activity. Proc Natl Acad Sci USA 83:1767–1771

Chomet PS, Wessler S, Dellaporta SL (1987) Inactivation of the maize transposable element *Activator* (*Ac*) is associated with its DNA methylation. EMBO J 6:295–302

Coupland G, Plum C, Baker B, Schell J, Starlinger P (1988) Analysis of *Ac* sequences required for transposition. Maize Genet Coop News Lett 62:46–47

Doerfler W (1983) DNA methylation and gene activity. Annu Rev Biochem 52:93–124

Doerfler W, Langner KD, Knebel D, Weyer U, Dobrzanski P, Knust-Kron B (1985) Site-specific promoter methylations and gene inactivation. In: Razin A, Cantoni G (eds) Biochemistry and Biology of DNA Methylation. Liss, Inc, New York, pp 133–155

Kunze R, Stochaj U, Laufs J, Starlinger P (1987) Transcription of transposable element *Activator* (*Ac*) of *Zea mays* L. EMBO J 6:1555–1563

Lavia P, MacLeod D, Bird A (1987) Coincident start sites for divergent transcripts at a randomly selected CpG-rich island of mouse. EMBO J 6:2773–2779

Masson P, Surosky R, Kingsbury JA, Fedoroff NV (1987) Genetic and molecular analysis of the *Spm*-dependent *a-m2* alleles of the maize *a* Locus. Genetics 177:117–137

McClintock B (1951) Chromosome organization and gene expression. Cold Spring Harbor Symp Quant Biol 16:13–47

McClintock B (1959) Genetic and cytological studies of maize. Carnegie Inst Wash Year Book 58:452–456

McClintock B (1964) Aspects of gene regulation in maize. Carnegie Inst Wash Year Book 63:592–602

McClintock B (1965) Components of action of the regulators *Spm* and *Ac*. Carnegie Inst Wash Year Book 64:527–536

Morisato D, Kleckner N (1987) Tn10 Transposition and Circle Formation in vitro. Cell 51:101–111

Müller-Neumann M (1985) Molekulare Isolierung und Charakterisierung des transponierbaren Elementes *Activator* am *Waxy*-Locus von *Zea mays* L. PhD thesis, Köln, FRG

Müller-Neumann M, Yoder JI, Starlinger P (1984) The DNA sequence of the transposable element *Ac* of *Zea mays* L. Mol Gen Genet 198:19–24

Roberts D, Hoopes BC, McClure WR, Kleckner N (1985) IS10 transposition is regulated by DNA adenine methylation. Cell 43:117–130

Schwartz D, Dennis E (1986) Transposase activity of the *Ac* controlling element in maize is regulated by its degree of methylation. Mol Gen Genet 205:476–482

Communicated by H. Saedler

Received May 4, 1988