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Detection and abundance of mRNA and protein encoded by transposable element *Activator (Ac)* in maize

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Summary. The 3.5 kb long mRNA of the maize transposable element *Ac* contains an open reading frame (ORFa) which encodes a polypeptide of 807 amino acids, the putative transposase of *Ac*. The *Ac* mRNA is a rare transcript: we now estimate the fraction of *Ac* mRNA in *wx-m7*::*Ac* seedlings to be $2–13 \times 10^{-5}$ of the polyA RNA. Assuming that maize cells contain similar amounts of polyA RNA as another monocot (0.16 pg/cell), this is equivalent to 1.5–10 transcripts in each cell. A protein with an apparent molecular weight of 112 kDa is detected, by five antisera directed against different segments of ORFa, exclusively in nuclear extracts from *Ac*-containing maize. This protein is most likely the full-length *Ac* ORFa protein. We estimate its concentration to be in the range of 3×10^{-7} of the nuclear proteins, or about 1000 molecules per triploid endosperm cell containing one *Ac* element.

Key words: Transposase – DNA-binding protein – Zea mays – mobile DNA

Introduction

The transposable element *Ac* of *Zea mays* L. was discovered and characterized genetically by Barbara McClintock (1947, 1951, 1965). Its molecular structure was determined by cloning and DNA sequencing (Fedoroff 1983; Behrens et al. 1984; Pohlman et al. 1984; Müller-Neumann et al. 1984). Only one *Ac*-specific transcript, 3.5 kb in length, was detected. The structure of this RNA was analysed by Northern blotting, S1 protection experiments and cDNA cloning (Kunze et al. 1987). A ca. 650 nucleotide untranslated leader sequence precedes the first methionine codon on the *Ac* mRNA, which initiates an open reading frame (ORFa) encoding a polypeptide of 807 amino acids. The *Ac* ORFa protein, the putative transposase, is a DNA-binding protein that recognizes repetitive sequence motifs near the termini of *Ac* (Kunze and Starlinger 1989).

In cases where genes expressing a particular phenotype are inactivated by the insertion of an *Ac* or *Ds* element, excision events can be observed as revertant sectors on a mutant background and as germinal revertants. Transposition of *Ac* and of the *Ac*-dependent *Ds* elements is usually a rare event (McClintock 1949): typically, *Ac* or *Ds* elements excise in only a small fraction of cells, although reversion frequencies can vary considerably between different mutants (for review see Döring 1989). In some cases the majority of sectors in a tissue, for instance in the aleurone layer of the endosperm, have similar sizes. This observation could indicate that the timing of transposition reactions during development may be regulated by the host (McClintock 1948; Levy and Walbot 1990).

As well as the phenomenon of regulated timing which is also observed with other transposable elements, another *Ac*-specific regulatory phenomenon is the 'dosage effect' (McClintock 1948, 1951). With an increasing number of *Ac* elements, excisions occur at progressively later times during development, and the excision frequency apparently drops. The molecular mechanism(s) underlying these phenomena is not known.

We report here on experiments that were designed to obtain an estimate of the amount of mRNA and protein encoded by *Ac*. This estimate is important for the identification of the rate-limiting factor(s) for the transposition reaction. Based on a comparison of the intensities of *Ac* mRNA bands with bands containing known amounts of DNA on Northern blots, Kunze et al. (1987) estimated the *Ac* transcript concentration in different tissues to be $1–4 \times 10^{-7}$ of the polyA RNA. We show here that the *Ac* transcript concentration was underestimated by this method. By determining the frequency of cDNA clones in a library, Finnegan et al. (1988) estimate that the *Ac* mRNA constitutes $5–10 \times 10^{-6}$ of polyA RNA. We re-evaluated the concentration of the *Ac* transcript by using RNA concentration markers and by S1 mapping, and show that DNA is

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unreliable as a concentration standard on Northern blots.

In order to identify and estimate the amount of *Ac* translation product in maize nuclei, we have performed immunoblotting experiments. A protein with an apparent molecular weight of 112 kDa is recognized by antisera directed against different segments of the large open reading frame (ORFa) of *Ac* in nuclear protein extracts from maize plants containing an *Ac* element, but is absent from *Ac*-free plants. An estimate of the amount of *Ac* protein per maize cell was achieved by calibration with the *Ac* protein synthesized in *Escherichia coli*.

Materials and methods

Plant material. PolyA RNA was isolated from ca. 5-day-old maize seedlings homozygous for *wx-m7::Ac* or *wx-m9::Ds-cy* (Schwartz and Dennis 1986). Nuclei were prepared from maize endosperm harvested 20 days after pollination, containing either one, two or three *wx-m7::Ac* alleles.

RNA isolation, Northern hybridization, and preparation of single-stranded DNA probes. Standard nucleic acid manipulations were performed as described by Kunze et al. (1987).

S1 protection assay. The S1 protection experiments were carried out essentially as described by Burke (1984). Five micrograms of polyA RNA were hybridized to ^{32}P-labelled single-stranded DNA probes for 5 h at 50° C in 80% formamide, 0.4 M NaCl, 40 mM Pipes, 1 mM EDTA, pH 6.5. S1 nuclease was added at a concentration of 65 units per µg RNA in 200 µl S1 buffer consisting of 0.4 M NaCl, 30 mM sodium acetate, 4.5 mM zinc acetate, pH 4.5, and incubated for 10 min at 37° C. The reaction was stopped by precipitation with 2-propanol and analysed on 4% sequencing gels.

In vitro transcription. Plasmid pSpAc-a contains the central *Hin*dIII fragment of *Ac* integrated between the T7 and SP6 RNA polymerase promoters of the pGEM™-2 vector (Promega Biotec). Two micrograms of this plasmid were linearized with *Pvu*I or *Hin*cII and transcribed with 24 units of T7 RNA polymerase in 80 µl transcription buffer containing 40 mM TRIS-HCl, 6 mM $MgCl_2$, 10 mM NaCl, 10 mM dithiothreitol (DTT), 50 ng/ml bovine serum albumin (BSA), 40 units RNasin (Boehringer Mannheim), and 0.6 mM each of ATP, GTP, CTP, and TTP, ph 7.9, for 60 min at 37° C. Halfway through of incubation period another 12 units of T7 RNA polymerase were added. After DNaseI digestion the in vitro transcription product was purified by phenol/chloroform extraction and precipitated twice with ethanol. The transcribed RNA migrated as a single band on an ethidium bromide-stained denaturing agarose gel. The yield of RNA was determined photometrically.

Plasmid pGem4-Sh contains a 2.8 kb *Shrunken* cDNA insertion (Werr et al. 1985), including a polyA tract, in the pGem™-4z vector (Promega Biotec). After linearization with *Nde*I the plasmid was transcribed in vitro as described for pSpAc-a and yielded a 3 kb RNA, which was purified by oligo(dT)-cellulose chromatography.

Preparation of nuclei. Maize endosperm nuclei were prepared according to Rowland and Strommer (1985) with the following modifications: 2.4 M sucrose was used for the gradient instead of Percoll. Honda buffer (Luthe and Quatrano 1980) contained 0.1% rather than 0.5% Triton X-100. After purification by sucrose gradient the nuclei were washed three times with modified Honda buffer without 2-mercaptoethanol. An aliquot was removed for DNA determination (Burton 1956), and the number of genome equivalents in the preparation was calculated based on the weight of the maize genome (Bennett 1985). Proteins were extracted by shaking the nuclei in 2 × PEB (20 mM TRIS-HCl, pH 7.5, 600 mM NaCl, 20 mM $MgCl_2$, 0.6 mM phenyl methyl sulfonyl fluoride, 20 µg/ml each of antipain, chymostatin, leupeptin, pepstatin) for 4 h at 4° C. Insoluble components were removed by centrifugation. The protein content of the supernatant was determined by the Micro BCA Assay (Pierce Chemical Co.; Wiechelman et al. 1988), and the nuclear extract was stored at −70° C. As internal control for the DNA and protein measurements defined amounts of DNA and BSA, respectively, were added to the samples.

Western blotting. Protein electrophoresis was carried out as described by Lugtenberg et al. (1975) and proteins were blotted by the semi-dry procedure of Kyhse-Andersen (1984) onto nitrocellulose. Blocking was performed in phosphate-buffered saline (PBS) with 10% fat-free milk powder. Incubation with the primary antiserum was in PBS with 10% milk powder for 4 h at room temperature. Subsequently, the filters were washed four times for 15 min each in ANT buffer (Swerdlow et al. 1986) and for 10 min in PBS (137 mM NaCl, 13 mM KCl, 81 mM $Na_2HPO_4 \cdot 2H_2O$, 15 mM KH_2PO_4) with 10% milk powder. For autoradiographic detection of Western blots the filters were incubated for 2 h at room temperature with (^{125}I)-labelled goat anti-rabbit IgG (0.7 µCi/ml; 200 µl/cm^2). Finally, the filters were washed four times for 5 min each in ANT buffer. The dried filters were exposed at −70° C with two intensifier screens.

Generation of antisera. Segments encoding amino acids 1–97, 189–807, and 663–807 of the 807-amino acids-long open reading frame of *Ac* (ORFa) have been cloned under the transcriptional control of a T7 promoter (Rosenberg et al. 1987) and give rise to unfused polypeptides. Segments encoding amino acids 559–663 and 663–744 have been cloned as C-terminal fusions downstream of the 102 N-terminal amino acids of the MS2 polymerase (Strebel et al. 1986). Details of the cloning and expression procedures used are available from the authors upon request. Inclusion bodies were prepared by centrifugation of a French Press lysate of induced *E. coli* cells. Sedimented proteins were size-fractionated by denaturing SDS-polyacrylamide gel electrophoresis (PAGE).

The recombinant protein band was excised and electro-eluted into a high-salt barrier. The eluted protein was dialysed against PBS and used to immunize rabbits (Gilles et al. 1980).

Estimation of the protein concentration of Ac *ORFa in maize.* The concentration of *Ac* ORFa protein synthesized in *E. coli* was estimated by comparing the band intensity of the recombinant protein with that of a defined amount of β-galactosidase after Coomassie staining (Bickle and Traut 1971) or silver staining (Heukeshoven and Dernick 1985) of the gels. The concentration of *Ac* ORFa protein in maize nuclear extracts was estimated by comparing its signal intensity with that of defined amounts of the recombinant *Ac* ORFa protein mixed with appropriate amounts of maize nuclear extracts from Ac^- plants on Western blots. The corresponding number of *Ac* ORFa protein molecules was calculated using the theoretical molecular weight of 92 kDa.

Results

Selection of appropriate conditions for determination of mRNA concentration

We wished to compare the hybridization signals obtained from *Ac* DNA and *Ac* RNA after Northern blotting. In plasmid pSpAc-a the 1.5 kb *Hin*dIII fragment from the *Ac* cDNA was cloned downstream of the T7 promoter. Digestion of the plasmid with *Pvu*I and *Xba*I gives rise to a DNA fragment that contains the complete *Ac* cDNA segment and has an overall length of 3.15 kb. Linearization of pSpAc-a with *Pvu*I and in vitro transcription with T7 RNA polymerase yielded a 3.05 kb RNA.

Unexpectedly, about two orders of magnitude more RNA than denatured DNA have to be applied to Northern gels to generate hybridization signals of similar intensities (Fig. 1; compare lanes 3 and 4 with lanes 6 and 7, respectively). A parallel experiment was done with a 3 kb sucrose synthase RNA transcribed in vitro and corresponding DNA fragments. Here, too, about 100 times more RNA than DNA was required to generate equal band intensities (data not shown). Therefore, this phenomenon is not peculiar to *Ac* RNA.

The transfer efficiencies of DNA and RNA during capillary transfer to the nitrocellulose filter are identical (>90%), as tested with radiolabelled nucleic acids. During preincubation and overnight hybridization, 70%–80% of the bound RNA, but only 5%–10% of the DNA was lost from the filter (data not shown). This preferential loss of RNA can partially account for the difference in hybridization yield, but does not, however, explain the 100-fold difference obtained.

Abundance of the Ac *transcript*

Northern blot experiments were performed with defined amounts of polyA RNA from *Ac*-containing maize material, using denatured DNA fragments, or in vitro transcribed RNA as standards. The 3.5 kb *Ac* transcript contained in 3 μg of polyA RNA from *wx-m7*::*Ac* containing maize seedlings generated a band intensity similar to that obtained with 0.4–0.8 pg of the 3.15 kb denatured dsDNA fragment from the plasmid pSpAc-a. This result is in agreement with earlier experiments (Kunze et al. 1987). To obtain the same band intensity with in vitro transcribed *Ac* RNA, 200–400 pg of the 3.05 kb RNA from pSpAc-a were required (Fig. 1). This experiment was repeated severalfold, using various polyA RNA preparations from *wx-m7*::*Ac* seedlings and three different single-stranded DNA probes. Depending on the mRNA preparation and the probe, we calculated abundances of the 3.5 kb *Ac* mRNA in the polyA RNA fraction of $2–13 \times 10^{-5}$.

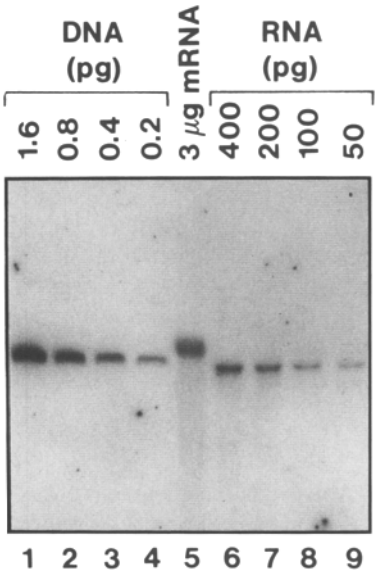

Fig. 1. Quantification of *Ac* mRNA by Northern blot analysis. PolyA RNA from maize seedlings was run on a denaturing agarose gel together with different amounts of in vitro synthesized RNA and DNA fragments, respectively. The nucleic acids were blotted onto nitrocellulose and hybridized to a single-stranded DNA probe spanning most of the central *Hin*dIII fragment of *Ac* (see Fig. 2a). Lanes 1–4, decreasing amounts of a 3.1 kb *Pvu*I-*Xba*I DNA fragment from pSpAc-a; lane 5, 3 μg *wx-m7*::*Ac* polyA RNA; lanes 6–9, decreasing amounts of in vitro synthesized *Ac* RNA

In addition, we wanted to determine the *Ac* mRNA concentration by a method that avoids any potential problems attributable to the filter-binding procedure. An S1 protection assay was designed that allows one to distinguish the protected bands derived from the *Ac* mRNA and in vitro transcribed *Ac* RNA (Fig. 2a). The expected digestion products of the 3.5 kb *Ac* mRNA and the in vitro transcribed *Ac* RNA are 753 and 669 nucleotides long, respectively. In addition, a 453 nucleotides protected band is generated by both RNAs.

Defined amounts of in vitro synthesized *Ac* RNA were mixed with maize polyA RNA from *Ac*-free, *wx-m7*::*Ac*, or *wx-m9*::*Ds-cy* seedlings, respectively, hybridized to the nearly homogeneously labelled single-stranded DNA probe, incubated with S1 nuclease, and

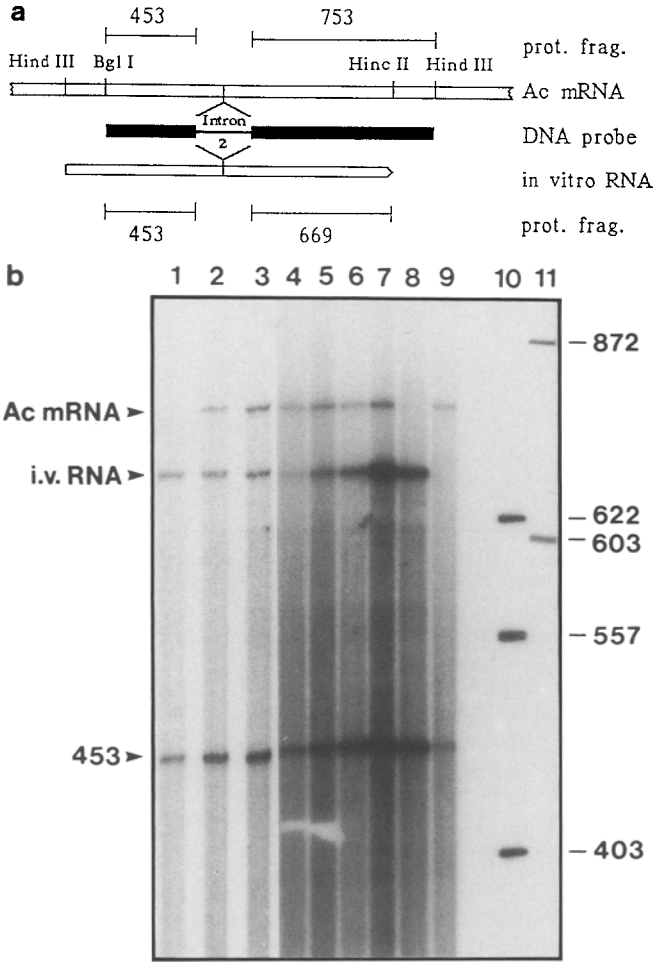

Fig. 2a and b. Quantification of the *Ac* mRNA by S1 protection analysis. **a** Protected fragments after nuclease S1 digestion. The *upper open bar* shows a part of the *Ac* mRNA. The *lower open bar* depicts the complete in vitro synthesized *Ac* RNA. The *solid bar* in between is the single-stranded DNA probe. The thin top and bottom *lines* show the length of the double-stranded fragments protected against S1 digestion. Both RNAs protect a 453 bp fragment. In addition, the *Ac* mRNA protects a fragment of 753 nucleotides, whereas the in vitro synthesized RNA protects a fragment 669 bp in length. **b** S1 protection experiment. Lane 1, 100 pg in vitro synthesized RNA was mixed with 5 µg *wx-m9::Ds-cy* polyA RNA; lanes 2 and 3, 100 pg in in vitro transcribed RNA was mixed with 5 µg of two different *wx-m7::Ac* polyA RNA preparations; lanes 4–7, 5 µg *wx-m7::Ac* polyA RNA was mixed with 25, 50, 100, and 200 pg in vitro synthesized RNA, respectively; lane 8, 100 pg in vitro synthesized RNA; lane 9. 5 µg *wx-m7::Ac* polyA RNA; lanes 10 and 11, DNA size markers. i.v. RNA, in vitro synthesized *Ac* RNA

electrophoresed on a sequencing gel (Fig. 2b). No band of 753 nucleotides in length is protected in polyA RNA from the *Ac*-free control (not shown) or from *wx-m9::Ds-cy* plants (lane 1) containing a transiently inactivated *Ac* that is not transcribed (Kunze et al. 1988). When 50–100 pg in vitro transcribed RNA was mixed with 5 µg *wx-m7::Ac* polyA RNA, the intensities of the 753 and 669 nucleotide protected bands were comparable (lanes 2, 3, and 5). Using other polyA RNA preparations from *Ac*-containing material, equal signal intensities were generated by 50–200 pg in vitro transcribed

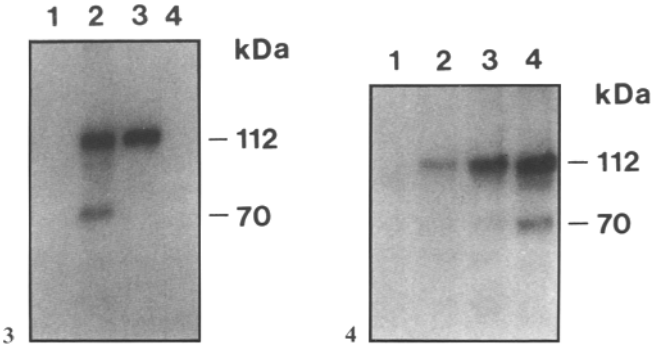

Fig. 3. Detection of *Ac* protein in nuclear extracts from maize endosperm by Western blotting. Six hundred micrograms of protein extract from *Ac*-free endosperm (lanes 1 and 4) or *Ac/Ac/Ac* endosperm (lanes 2 and 3) were size fractionated, blotted onto nitrocellulose and incubated with antisera directed against amino acids 559–663 (lanes 1 and 2) or 1–97 (lanes 3 and 4) of *Ac* ORFa protein

Fig. 4. Western blot with 600 µg nuclear protein from maize endosperm containing: 0 (lane 1), 1 (lane 2), 2 (lane 3), or 3 *Ac* elements (lane 4) per triploid endosperm genome

RNA (data not shown). From these results it was calculated that the 3.5 kb *Ac* mRNA constitutes a fraction of $1\text{–}4 \times 10^{-5}$ of the polyA RNA in *wx-m7::Ac* seedlings, which is in agreement with the results obtained with RNA concentration standards on Northern blots.

Detection of Ac *encoded protein in maize*

In order to obtain antisera with specificities directed against different parts of ORFa, fragments of the *Ac* cDNA were expressed in *E. coli* either unfused or as C-terminal fusions to the MS2 polymerase, and used to immunize rabbits. Nuclear protein extracts were prepared from *wx-m7::Ac* and *Ac*-free kernels harvested 20 days after pollination, electrophoresed and blotted to cellulose nitrate filters.

Figure 3 shows Western blots probed with antiserum raised against the 97 N-terminal amino acids (lanes 3 and 4) and against amino acids 559–663 (lanes 1 and 2) of the 807 amino acids long *Ac* ORFa protein, respectively. The major protein band in extracts from *Ac*-containing maize (lanes 2 and 3) detected by all five antisera obtained has an apparent molecular weight of about 112 kDa. No such protein band was detected in extracts from *Ac*-free plants (lanes 1 and 4).

A smaller amount of a second protein with an apparent molecular weight of 70 kDa was detected by four of the five antisera in extracts from *Ac*-containing plants. As the appearance of this protein depends on the extraction conditions, we do not yet know if this protein is a preparation artefact. Other signals are produced by individual antisera. These are present in *Ac*-free as well as in *Ac*-containing extracts and, therefore, are presumably unrelated to *Ac* activity.

Ac ORFa protein was expressed in insect cells infected with a recombinant baculovirus (Hauser et al. 1988), and in *E. coli*. When these proteins were run in

lanes adjacent to the nuclear proteins of maize, the ORFa protein band from the insect cell extracts was indistinguishable in electrophoretic mobility from the maize *Ac* protein (data not shown). The ORFa protein synthesized in *E. coli* appears to migrate slightly faster (Fig. 5). We conclude that the 112 kDa *Ac* protein in maize is probably the full-length 807 amino acid translation product of ORFa.

The Ac *ORFa protein concentration increases with the* Ac *dosage*

As was shown earlier, the concentration of the *Ac* mRNA increases with the copy number of *Ac* elements in the genome (Kunze et al. 1987). To determine the correlation between the *Ac* copy number and the relative concentration of the *Ac* protein, Western blots were performed with equal amounts of nuclear proteins from 20-days-old *wx-m7*::*Ac* endosperm containing 0, 1, 2, or 3 doses of *Ac*. The *Ac* protein concentration increases with the copy number of *Ac* (Fig. 4).

Estimation of Ac *protein concentration in maize nuclei*

Due to the low amount of *Ac* protein present in maize tissue, no direct quantitative determination is possible. Therefore, we used an indirect approach. *Ac* ORFa protein was expressed in *E. coli* and inclusion bodies were purified. The inclusion bodies consist mainly of complete and fragmented ORFa protein. Since the full-length ORFa protein is widely separated from other proteins on the gel, the amount of protein contained in this band could be determined by comparison with the intensity of a β-galactosidase band in a parallel lane after staining of the gel. The results obtained after Coomassie staining and silver staining were in agreement (data not shown).

Subsequently, maize nuclear extracts from *wx-m7*::*Ac* endosperm containing one *Ac* copy per cell equivalent were size-fractionated in parallel with a dilution series of the bacterial protein, and the *Ac* ORFa protein was visualized by Western blotting (Fig. 5). The estimated absolute amount of *Ac* protein in 600 µg nuclear protein is ca. 0.17 ng, representing about 3×10^{-7} of the total protein. Although the nuclear proteins constituted only a fraction of all cellular proteins, this is one to two orders of magnitude less than the concentration of *Ac* mRNA in total polyA RNA.

For the conversion of this concentration into the number of *Ac* protein molecules per haploid genome, we used the predicted molecular weight of the *Ac* ORFa protein (92 kDa) instead of its apparent molecular weight of 112 kDa. To determine the number of nuclei used to prepare the protein extract the DNA content of an aliquot was measured by a Burton reaction (Burton 1956). The number of cells was calculated on the assumption that the weight of the haploid maize genome is 2.8×10^{-12} g (Bennett 1985). This led to an estimated yield of 0.8 ng nuclear protein per cell, or 2.4×10^{-4} pg *Ac* protein per haploid genome. As final results of three

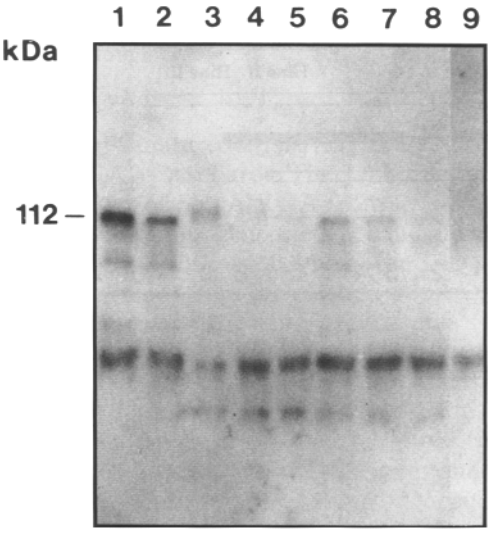

Fig. 5. Quantitation of *Ac* protein by Western blotting: 600 µg (lane 3), 300 µg (lane 4), or 150 µg (lane 5) nuclear proteins from maize endosperm containing 1 *Ac* element in the genome, 600 µg nuclear protein from *Ac*-free endosperm mixed with 0 ng (lane 9), 1 ng (lane 1), 0.5 ng (lane 2), 0.25 ng (lane 6), 0.12 ng (lane 7), and 60 pg (lane 8) *Escherichia coli*-derived *Ac* ORFa protein were blotted and probed with an antiserum directed against *Ac* ORFa amino acids 663–774

independent experiments we obtained values of 900, 1100, and 1800 *Ac* ORFa protein molecules per haploid genome, respectively. If the smaller 70 kDa protein seen on the Western blots is a degradation product of the full-length protein, the true values will be slightly higher. However, because the 70 kDa protein is much less abundant than the 112 kDa protein, the error should be small.

Discussion

By using DNA fragments as concentration standards on Northern gels, the abundance of the 3.5 kb *Ac* mRNA had been estimated in previous experiments to be between 1 and 3×10^{-7} of the polyA RNA (Kunze et al. 1987). A re-evaluation of the amount of *Ac* mRNA per cell with RNA concentration standards showed that this use of DNA standards leads to a more than 100-fold underestimation of the *Ac* transcript concentration; in *wx-m7*::*Ac* seedling material we determined the *Ac* mRNA fraction to be between 2 and 13×10^{-5} of the polyA RNA. Very similar values were obtained with S1 experiments, whereas determination of the frequency of *Ac* clones in a cDNA library (Finnegan et al. 1988) results in about 10-fold lower values.

The amount of mRNA per cell in maize is unknown, but the value has been determined for other plants: the monocot *Tradescantia palludosa* contains ca. 0.16 pg messenger RNA per cell in shoot cells (Willing and Mascarenhas 1984), *Petroselinum sativum* roots contain 0.08 pg mRNA/cell (Kiper et al. 1979), and in tobacco leaves 0.38 pg mRNA were found per cell (Goldberg et al. 1978). Assuming that maize contains a similar

amount of polyA RNA per cell as *Tradescantia*, the *Ac* mRNA concentrations are equivalent to an average frequency of 1.5 to 10 transcript molecules per cell. The *actual* distribution of *Ac* mRNA in the plant tissue is unknown, however.

McClintock observed that the dosage of *Ac* elements alters the reversion pattern of *Ds* mutations. With increasing dosage of *Ac*, excisions of the *Ds* element occur later in the development of the endosperm (McClintock 1948, 1951). This observation suggests that *each* cell of the endosperm reacts to an increase in number of *Ac* elements in the genome with a reduced probability of an excision event at a certain developmental state. Such a reaction requires expression of *Ac* in every cell. If the number of mRNA molecules per cell were below 1.0, an increase in *Ac* dose should increase the number of cells with one mRNA molecule, but not the number of molecules per cell. It would be difficult to envisage a decrease in excision events. With more than one molecule per cell and thus an increase in the number of mRNA molecules per cell with increasing *Ac* doses a negative regulation of excision could be more easily envisaged. Our data are compatible with this interpretation of the negative dosage effect, if the 1.5 to 10 *Ac* mRNA molecules per cell are evenly distributed in the tissue.

Schwartz (1989) reported recently that in the absence of *Ac* activity the *Bam*HI site of the *Ds9* element is modified in every cell, as assessed by Southern blotting. In the presence of an active *Ac* element, however, the *Bam*HI site is completely cleavable. This finding also can only be explained if the *Ac* element is expressed in every cell or at least in one of its progenitors.

Despite the putative expression in each cell, transposition of *Ac* and *Ds* elements is usually a rare event; it occurs only in a fraction of the cells in a tissue. In different unstable mutants, however, this proportion can vary considerably (reviewed by Döring 1989). Levy and Walbot (1990) recently measured the excision frequency of the *Ds2* element from the *bz2*::*Ds2* allele in endosperm tissue, and found that at any stage during development, transpositions occur in between 0.2% and 1% of all the cells. If the *Ac* mRNA is evenly distributed among the cells, transcription cannot be the rate-limiting step for the transposition event. This conclusion is strengthened by the observation that the transcription rate of *Ac* increases with the number of *Ac* elements in the genome (Kunze et al. 1987).

The Western blotting experiments with maize nuclear extracts and the long open reading frame of *Ac* (ORFa) overexpressed in insect cells or *E. coli* make it likely that the 112 kDa protein detected in *Ac*-containing maize is the full-length *Ac* ORFa protein. The calculated molecular weight of the *Ac* ORFa protein is only 92 kDa. The *Ac* ORFa protein is phosphorylated in insect cells (Hauser et al. 1988); however, preliminary experiments indicate that dephosphorylation does not abolish the gel migration anomaly (H. Fußwinkel, unpublished). Because the *E. coli*-derived *Ac* ORFa protein migrates at nearly the same position in denaturing gels, we consider the possibility that protein modifications like glycosylation are responsible for the migration anomaly to be unlikely. We speculate rather that a structural peculiarity of the *Ac* protein causes this behaviour. This idea is supported by earlier observations that the in vitro translation product comprising the first 230 amino acids of the *Ac* protein migrates with an apparent molecular weight of 39 kDa instead of the calculated 25 kDa (Kunze et al. 1987). Apparent molecular weights higher than those predicted have been described for several proteins, including DNA-binding proteins (Freytag et al. 1979; Benedum et al. 1986; Kleinschmidt et al. 1986; Benson and Pirotta 1987; Dingwall et al. 1987; Franssen et al. 1987; Ollo and Maniatis 1987).

The *Ac* protein was estimated to constitute a proportion of about 3×10^{-7} of the nuclear proteins. The relative concentration of *Ac* mRNA in the total polyA RNA is, however, approximately 100 times higher. This might be an indication of instability of the *Ac* protein, and/or of a low translation efficiency of the *Ac* messenger. Support of this hypothesis is provided by the calculated ratio of less than 1000 protein molecules translated from each messenger RNA, which is less than the average translation rate of eukaryotic messengers (Darnell et al. 1986). In view of the sequence environment of the first methionine codon on the *Ac* mRNA (CAG*ATG*A), which deviates from the eukaryotic (Kozak 1986) and plant (Lütcke et al. 1987) consensus sequences, this would not be surprising.

With an increasing number of elements in the genome both the transcript and protein concentrations are raised. Therefore, the negative dosage effect of *Ac* must be expressed at the post-translational level. A very simple explanation could be that a single transposase molecule has to interact with both ends of the transposable element to initiate transposition. In that case, at higher protein concentrations separate transposase molecules might bind to the ends, thereby preventing transposition. This model is not satisfactory, however, because it cannot explain the very different variegation patterns observed with distinct mutable alleles. Possibly, the relation between (a) cellular factor(s) that might be involved in the transposition reaction and the *Ac* ORFa protein modulates the transposition frequency and pattern.

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