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Nuclease sensitivity of a maize HRGP gene in chromatin and in naked DNA

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The sensitivity to different nucleases of a gene coding for a cell wall hydroxyproline-rich glycoprotein in *Zea mays* has been studied. Hypersensitive sites are found in the proximal 5′ flanking region of the gene. The main hypersensitive site around position −200, corresponds to a region that also shows a peculiar hypersensitivity to S1 degradation in naked negatively supercoiled DNA. The region where most of the changes in chromatin structure occurs is concentrated in approximately one nucleosome indicating that sequences for transcriptional control may be tightly clustered in plants.

Key words: chromatin; HRGP; maize; nuclease; S1-nuclease

Introduction

Cell wall protein genes show complex patterns of expression in plants. In dicotyledonous species, the best known of these genes, those coding for extensins (hydroxyproline-rich glycoproteins present in cell walls) constitute small multigene families whose members exhibit distinct patterns of mRNA accumulation. Extensin genes appear to be developmentally regulated [1] and they are induced by wounding or by pathogen infection [2].

In monocotyledonous species, genes sharing some functional or structural homology to extensin appear to have a simpler organization than in dicots. cDNA clones coding for a proline-rich protein from maize have been obtained [3]. Accumulation of the corresponding mRNA is observed mainly in organs enriched in dividing cells, as a response to wounding of young organs [4] and in sites of early vascular differentiation [5]. These clones detect a small group of genes (one or two) in the genomes of four different maize inbred lines. These genes are located at a single genetic locus on chromosome 2 as judged by RFLP analysis [5].

While the use of DNase hypersensitivity has been very useful in animal systems [6], very few observations have been reported in plants [7]. The reasons for this situation may be several. On the one hand, the large genome size of the most common crops and the inability to prepare nuclei with intact components from different tissues make this approach difficult for technical reasons. On the other hand, the lack of suitable probes with clear regulatory patterns and belonging to either single genes or small multigene families presents another difficulty. In any case, the phenomenon of nuclease sensitivity of active genes and nuclease hypersensitivity in their vicinity is present in plant systems as has been shown in a limited number of cases [7]. In the present article we report the identification of general DNase sensitivity in the region of a gene coding for a cell wall hydroxyproline-rich glycoprotein from maize and the detection of hypersensitive sites in its vicinity that correlate in one case with a S1 hypersensitive region in negatively supercoiled DNA.

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Materials and Methods

Plant material and isolation of nuclei

Seeds of maize W64A pure inbred line were germinated by imbibition in water at 25°C in the dark. Six-day-old plumules were obtained by blade cutting. Nuclei were isolated by a modification of the method described for plant nuclei isolation [8]. Three grams of hand-dissected plumules were suspended in isolation buffer and were disrupted with a VirTis homogenizer. The nuclei were isolated by successive centrifugations. Intactness of nuclei was controlled by light microscopy after staining with carmine acetic acid and by micrococcal nuclease digestion to give the typical nucleosomal ladder.

Nuclease digestion and DNA purification

Nuclei suspensions were adjusted to 0.5–1.0 μg DNA/μl as determined spectrophotometrically after lysis with 1% SDS. DNase I (Amersham) digestion was performed at 4°C for 5 min at different nuclease concentrations. A control reaction mixture containing no exogenous DNase I was included. Naked DNA was digested as a control. Genomic DNA was isolated by direct lysis of cells and purified on a CsCl gradient. DNA aliquots (0.1 μg/μl) were digested under the same conditions with increasing amounts of DNase I. Endogenous nuclease digestion of nuclei was performed at 37°C in digestion buffer. Digestions were terminated after different digestion times by dilution into 2.5 mM EDTA (pH 8.0), 0.2 M NaCl and 0.5% SDS.

Restriction enzyme digestion and Southern hybridization

Samples were digested with appropriate restriction endonuclease (10 units/μg) to completion as indicated by the supplier. Approximately 10 μg of each DNA sample was separated on 0.8–1.0% agarose gels and transferred to nylon membranes (Zeta probe, Bio-Rad). Hybridization was carried out in 180 mM NaCl, 10 mM sodium phosphate, 10 mM EDTA, 1% SDS, 0.5% defatted milk, 0.5 mg/ml salmon sperm DNA at 68°C. Last washes were done in 0.1 × SSC, 0.1% SDS at 65°C. The DNA probes were labelled with ^{32}P to a specific activity of 0.5–2 × 10^9 cpm/μg by random priming (Boehringer).

S1-sensitive sites

Negatively supercoiled plasmids were digested at 15°C for 30 min with S1 nuclease (0.02 units/μg DNA) in 50 mM sodium acetate (pH 4.5), 50 mM NaCl, 3 mM $ZnCl_2$. The reactions were stopped with phenol/chloroform extraction. The nicked band was isolated and digested with *Hin*dIII. After complete digestion the samples were end-labelled with [^{32}P]ATP and DNA polymerase (Klenow) or polynucleotide kinase. After digestion with *Bam*HI the positions of S1 cleavage were analyzed on urea-acrylamide gels.

Results

Chromatin sensitivity and hypersensitivity of the maize HRGP gene

The gene coding for a hydroxyproline-rich glycoprotein (HRGP) from maize is highly expressed in plumules [3]. An endogenous nucleolytic activity present in this organ was used in order to test whether the HRGP gene is found in a less condensed chromatin structure compared with a gene not expressed in it. The HRGP gene shows a higher sensitivity to endogenous nucleolytic degradation than the glutelin-2 a typical endosperm specific gene [9]. This result is shown in Fig. 1. The higher sensitivity of the HRGP gene can also be seen in the figure where the result of the densitometric analysis of the blots expressed as the intensity of the band in relation to zero time digestion has been plotted as a function of the time of incubation.

The hypersensitive zones in the regions flanking the maize HRGP gene may be located by using the indirect end-labelling technique. It is possible to use the endogenous nuclease activities as probes for the chromatin structure in the vicinity of active genes [10,11]. This is the experiment shown in Fig. 2A. After incubation at 37°C of plumule nuclei allowing the endogenous nuclease to act on chromatin, the DNA was further digested with Sac I in order to use a SnaB I-Sac I probe (Fig. 2C) for indirect end-labelling. Sac I digestion produces a main band at 10 kb, that corresponds with the

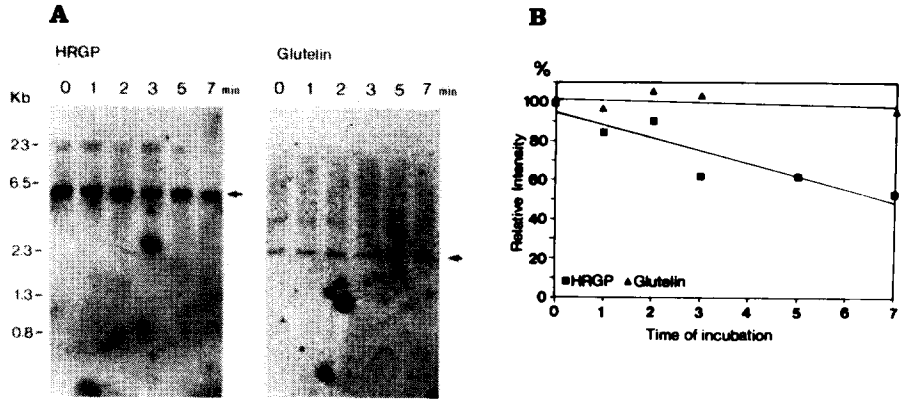

Fig. 1. Sensitivity of maize plumule chromatin to an endogenous nucleolytic activity. (A) Nuclei were digested with endogenous nuclease for 0–7 min at 37°C. The DNA was extracted, digested with *Hin*dIII, blotted and hybridized with HRGP and glutelin-2 cDNA probes. (B) Graphic representation of the relative sensitivity of the HRGP to the glutelin-2 DNA sequences plotted as the percentage of densitometric intensity relative to zero time.

band found in the Southern genomic analysis [5] and other bands that hybridize less intensely. Digestion with the endogenous nuclease produces three bands whose intensities correlate with the decrease in intensity of the 10 kb band. The three bands produced correspond to zones centered around positions −290 (band I) (that, as digestion proceeds, becomes increasingly broad to define a

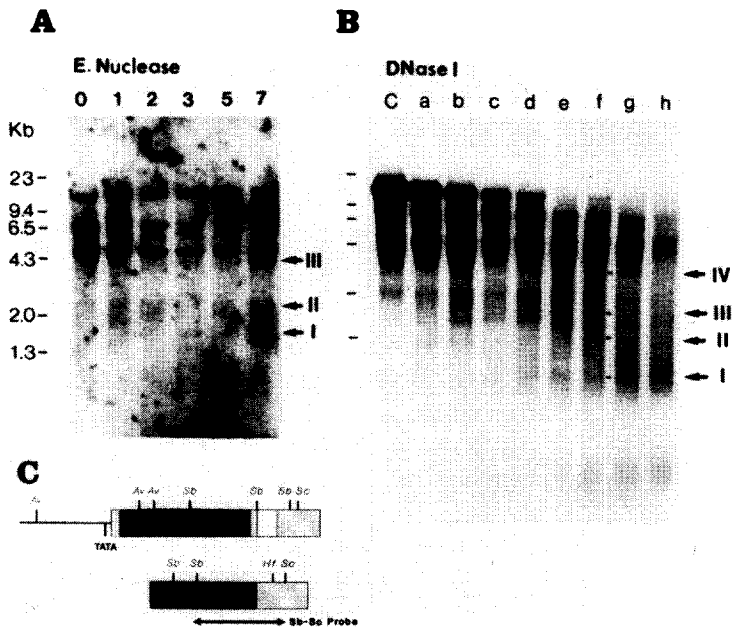

Fig. 2. Endogenous nuclease and DNase I hypersensitive sites of the maize HRGP genes in plumule. (A) Nuclei were digested with the endogenous nuclease for 0–7 min. (B) Nuclei were digested with DNase I. (a)–(h) 0.3, 0.6, 0.9, 1.2, 1.8 and 2.1 units/mg DNA. Bands I–IV, subfragments generated by the cleavage of DNase I at the hypersensitive sites. Other subfragments were also present in the control of naked DNA digested with DNase I. (C) Scheme of the HRGP gene and cDNA structures showing the fragment SnaBI-SacI used as a probe.

band between −220 and −370), −930 (band II) and −2680 (band III) from the ATG.

The endogenous nucleolytic activity in plumule decreases drastically with temperature, so that no activity is detected when incubation is carried out at 4°C. Under these conditions, it is feasible to determine DNase I hypersensitive sites (Fig. 2B). Four DNase I hypersensitive sites are obtained located at positions −330 (band III) and −1380 (band IV) and two less prominent ones within the transcribed region at the initial ATG (position 0) (band II) and +400 (band I) from the ATG. Controls with naked DNA (not shown) indicated that none of these points were hypersensitive in the absence of the chromatin structure. When the different nucleotide sequences showing hypersensitivity were compared (not shown) no significant homology was found that could explain the results observed here.

Sensitivity of S1 nuclease to specific sequences of supercoiled plasmids

The presence of nuclease hypersensitive sites in chromatin frequently correlates with the presence of nuclease hypersensitive sites in negatively supercoiled naked DNA, suggesting that the DNA sequences located at these sites are structurally versatile, being able to adopt altered non-B DNA conformations [12]. To investigate whether this is also the case for the HRGP 5′ region, the Alu I–Alu I fragment which spans from position −55 to −465 was cloned into the pDPL6 plasmid [13] and its sensitivity to degradation by S1 nuclease in the negatively supercoiled plasmid determined (Fig. 3). A strong S1 hypersensitive site is found located around position −190 in the top strand. The bottom strand shows two hypersensitive sites which are also centered around position −190. The precise nucleotides cut by the nuclease are indicated in Fig. 3.

Discussion

The global sensitivity to endonucleases of the sequence coding for a maize hydroxyproline-rich glycoprotein (HRGP) has been studied using a plumule endogenous nucleolytic activity. The degree of condensation of the HRGP chromatin appears to depend on the state of expression as, when a gene not expressed in plumule is analyzed, it shows a significantly lower sensitivity to the nucleases. These results are consistent with the

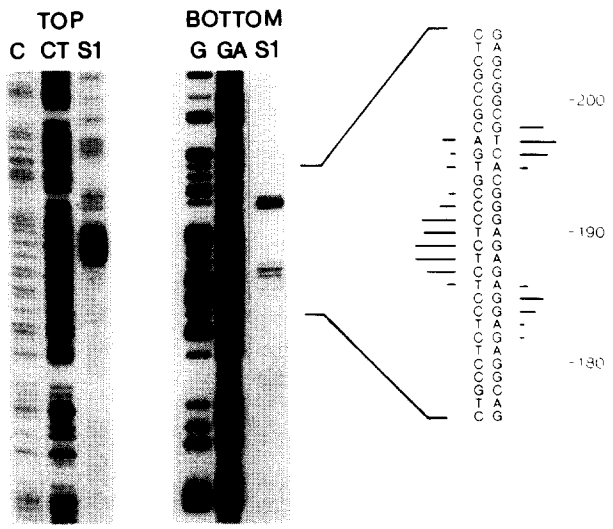

Fig. 3. High resolution mapping of S1 single strand nicks in the promoter region of the HRGP gene. Lanes C, CT, G, GA, contain the Maxam and Gilbert sequencing ladders. Lanes S1 contains S1 digests of supercoiled plasmid in the top strand and the bottom strand. The sequence shows the most prominent S1 nicks mapped in the region.

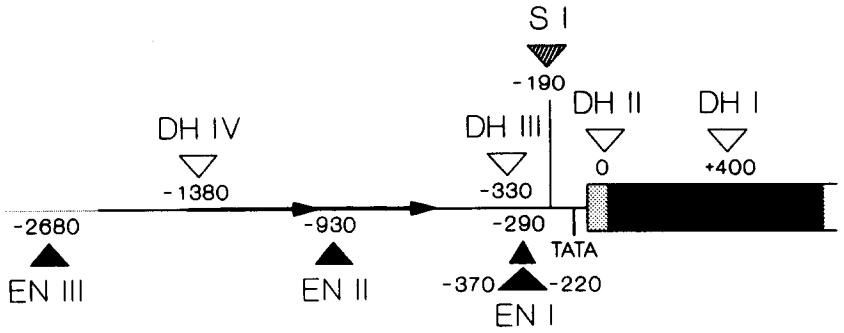

Fig. 4. Summary of nuclease hypersensitive sites at the 5′ end of the HRGP gene. Positions of DNase I hypersensitive (DH-), endogenous nuclease (EN-) and S1 sensitive sites are shown as arrow heads relative to the map of the HRGP genomic clone. The positions are referred to the translation start point.

proposal that the structural changes observed upon transcriptional activation are the consequence of a more labile higher order chromatin structure [14].

Three different nuclease assays have been used in the present work to probe the structure of DNA in the regions surrounding the maize HRGP gene. The effects of endogenous nuclease at 37°C and DNase I at 4°C were studied in nuclei and those of S1 in negatively supercoiled DNA in vitro. A summary of the results is presented in Fig. 4. The sequence of the 5′ region of the maize HRGP gene displays a number of well-defined zones [5]. A single consensus TATA-box is found at −112 from the ATG. A zone with mostly unique sequences containing short repeats is found from −112 to −555. At this point two repeated sequences 425 and 445 bp long are present. In these repeats two hypersensitive sites are found at positions −930 and −1380. They are located near homologous sequences within the two repeats. However the region showing most of the hypersensitive sequences is the one nearest to the TATA-box. Interestingly, the same DNA region which is hypersensitive to nucleases in chromatin also shows a peculiar sensitivity to S1 nuclease in negatively supercoiled naked DNA indicating that it contains elements capable of adopting non-B DNA conformations. This S1-hypersensitive site maps at a polypyrimidine-polypurine stretch 190 bp upstream from the ATG.

The main region hypersensitive to nucleases expands approximately through 200 bp, a DNA length equivalent to a nucleosome. In this sense it may be proposed that in this zone lie the main sequences responsible for transcriptional control and that one nucleosome is either removed or structurally modified when the gene is transcriptionally active. This is consistent with recent results [15] showing that multiple *cis*-elements, each one able to confer different type of transcriptional regulation in plants, are very close in a region near the TATA-box. Sequences having particular structures, such as the polypyrimidine-polypurine stretch could be relevant in the induction of the structural modifications of specific nucleosomes.

Acknowledgments

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