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Binding of a nuclear factor to a consensus sequence in the 5′ flanking region of zein genes from maize

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The genomic organization of the zein structural genes and of regulatory loci influencing their expression suggests that control of zein gene expression will involve interactions between *cis* elements in the flanking DNA sequences and products from *trans*-acting genes. The interaction between fragments from the 5′ flanking region of a zein gene and specific, double-stranded oligonucleotides with crude nuclear extracts from maize endosperm have been studied by nitrocellulose filter binding, gel retention and DNase I footprinting assays. Specific binding of a nuclear factor was observed and the exact position of the protein binding site was determined. The 22-nt binding site included 14 bp of a 15-bp sequence conserved in all zein genes.

Key words: zein/DNA-protein binding/nuclear factor/DNase I footprinting/nuclear extract

Introduction

The study of gene expression has recently been advanced in animal systems by the identification of DNA–protein interactions between nuclear factors and specific *cis*-acting DNA sequences. These *cis* sequences, for example TATA and CAAT boxes and enhancers (Breathnach and Chambon, 1981; Dynan and Tjian, 1985, for reviews) are thought to regulate the transcription of RNA polymerase II-transcribed genes by interaction with *trans*-acting factors (Khoury and Gruss, 1983; Dynan and Tjian, 1985; Sassone-Corsi and Borelli, 1986; Voss *et al.*, 1986, for reviews). The binding of nuclear protein factors can turn transcription on or off, can influence the level of transcription or can be responsible for tissue-specific transcription. These effects have been extensively reviewed (Khoury and Gruss, 1983; Dynan and Tjian, 1985; Sassone-Corsi and Borelli, 1986; Voss *et al.*, 1986). Plant gene systems exhibit similar features in the structure of their 5′ regions. Many plant genes contain TATA and CAAT boxes as found in animal systems (Messing *et al.*, 1983). Further upstream sequences have also been described which may play a role in gene regulation. Such sequences are the AGGA box (Heidecker and Messing, 1986) which in some genes replaces the CAAT box, enhancer-like sequences which contain sequences homologous to animal enhancer sequences, and other sequences which are conserved in all members of specific gene families. Examples of this latter group are the legumin box (Bäumlein *et al.*, 1986), found in the legumin storage protein genes of broadbean, pea and soybean and in the globulin storage protein genes of oats and rice, the −300 box found in some cereal (barley, maize and wheat) storage protein genes (Forde *et al.*, 1985), and sequences necessary for light-induced transcription during chloroplast maturation (Morelli *et al.*, 1985; Timko *et al.*, 1985; Fluhr *et al.*, 1986).

In maize the major storage proteins fall into two size classes, 19 and 21 kd, coded by ~100 genes located on chromosomes 4, 7 and 10 (Soave *et al.*, 1981, 1982; Valentini *et al.*, 1979; Viotti *et al.*, 1982). The expression of these genes is developmentally regulated in a tissue (endosperm)-specific and highly coordinated manner. Genetic mutants exist which reduce the synthesis of both classes (*floury*-2, Di Fonzo *et al.*, 1977) or preferentially the 21- or 19-kd class [*opaque*-2 and *opaque*-7, respectively (Soave *et al.*, 1976;Di Fonzo *et al.*, 1979)]. The reduction of the synthesis of the 21-kd zein polypeptides in the *opaque*-2 mutant is apparently due to a reduction in transcription of the zein genes (Pedersen *et al.*, 1980; Burr *et al.*, 1982; Langridge *et al.*, 1982; Marks *et al.*, 1985). Analyses of these mutants have suggested that more than one regulatory pathway is involved in zein gene expression and that the genes coding for the 19- and 21-kd zein proteins are at least, in part, independently regulated (Di Fonzo *et al.*, 1980). The above mutations map to different chromosomes from the zein structural genes which they affect (Valentini *et al.*, 1979; Soave *et al.*, 1978, 1981; Viotti *et al.*, 1982) and therefore the regulation of zein gene expression is expected to involve the interaction of the products of *trans*-active genes with sequence elements in the flanking regions of zein genes. In a comparison between the 5′ flanking sequences of zein genes coding for the two size classes of zeins the largest single stretch of homology was identified as a 15-bp region at −330 which was conserved in all zein genes (Brown *et al.*, 1986). This 15-bp sequence, CACATGTGTAAAGGT, contains part of the core sequence of viral, animal and human enhancers (Gillies *et al.*, 1983; Khoury and Gruss, 1983). In genes coding for zein polypeptides of a 19-kd class this sequence overlapped a 30-bp sequence capable of forming a stem–loop structure (Brown *et al.*, 1986). Furthermore, this 15-bp sequence overlapped the −300 box described for cereal storage proteins by Forde *et al.* (1985) and a similar sequence is also found in the maize sucrose synthetase gene which has a similar pattern of expression in the endosperm as zein genes (Werr *et al.*, 1985). These properties suggest that the 15-bp element may play a role in the regulation of zein gene expression and is, therefore, a candidate for the study of specific interactions between the zein 5′ regions and nuclear proteins.

In order to search for the specific binding of nuclear proteins to this region we have prepared crude nuclear extracts from endosperm tissue and studied their interaction with flanking sequences of the zein genomic clone, pMS1/7, coding for a 19-kd zein polypeptide. We have observed specific DNA–protein interactions in nitrocellulose filter binding and gel retention assays, and by DNase I footprinting, all of which methods have been used to identify sequence elements with nuclear protein binding ability in animals systems. The specific site of one such interaction was shown to occur at a 22-bp sequence which contained the 15-bp conserved sequence described above. This result is the first example of a specific interaction involving a plant gene upstream element.

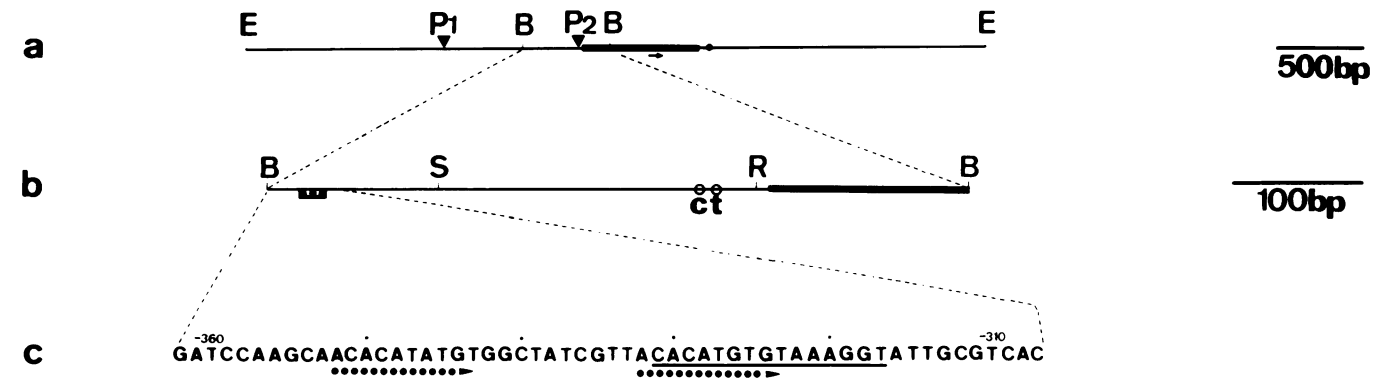

Fig. 1. Diagram of the genomic clone pMS1/7. (**a**) Restriction map of the 4.3-kb *Eco*RI genomic maize insert of pMS1/7. The zein structural gene is represented by thick black lines and the direction of transcription by an arrow below the gene. The solid circle shows the position of the polyadenylation signal. P1 and P2 indicate the zein P1 and P2 promoters. (**b**) Restriction map of the *Bam*−*Bam* fragment of pMS1/7. The open-circles, labelled c and t represent the position of the CAAT and TATA structures. The striped box represents a 15-bp consensus sequence. (**c**) DNA sequence of the region containing the 15-bp consensus sequence. The 15-bp consensus sequence is underlined with a solid line and two 10-bp direct palindromic sequences are underlined with dotted lines. E = *Eco*RI; B = *Bam*HI; R = *Rsa*I, S = *Sna*BI.

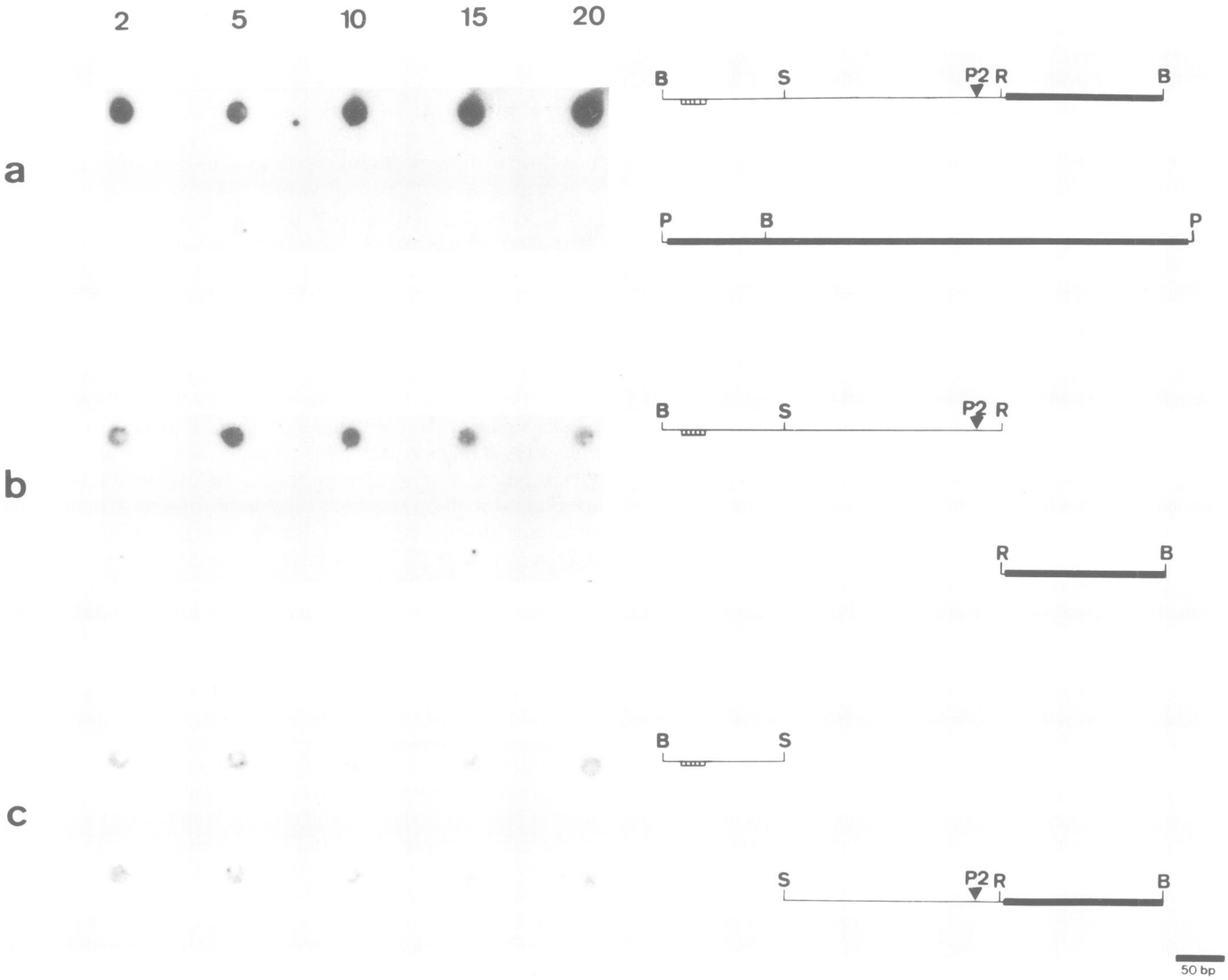

Fig. 2. Nitrocellulose filter-binding assays. A time course of the specific DNA-nuclear factor binding reaction is shown for each fragment. Samples were removed from the reaction mixture after 2, 5, 10, 15 and 20 min. (**a**) Nitrocellulose filter-binding results from the *Bam*−*Bam* fragment of pMS1/7 (**upper lane**) (Figure 1b) and a cDNA fragment containing the coding region of a zein gene of the 19 000-dalton protein class. (**lower lane**) (**b**) Nitrocellulose filter-binding results from the *Bam*HI−*Rsa*I fragment (**upper lane**) and the 170-b *Rsa*I−*Bam*HI fragment of pMS1/7 (**lower lane**) (Figure 1b). (**c**) Nitrocellulose filter-binding results from the 135-bp *Bam*HI−*Sna*BI fragment (**upper lane**) and the 217-bp *Sna*BI−*Bam*HI fragment (**lower lane**). B = *Bam*HI; S= *Sna*BI; R = *Rsa*I; P = *Pst*I P2 = P2 promoter. Zein coding regions are represented by thick black lines. The striped box indicates the 15-bp consensus sequence.

Results

Nitrocellulose filter-binding experiments

The binding of nuclear proteins to the 5′ flanking regions of zein genes was studied by incubating fragments of the zein genomic clone pMS1/7 with crude nuclear extracts from maize endosperm. The clone pMS1/7 is a deletion derivative of the genomic clone pMS1 (Wienand *et al.*, 1981) which codes for a protein of the 19-kd zein class. The distinctive features of the upstream region of pMS1 is the presence of two promoters, P1 and P2, lying ~900 bp apart (Figure 1a), each preceded by potential TATA and CAAT boxes (Brown *et al.*, 1986). In the deletion mutant, pMS1/7, bases −360 to −456 in the region between the two promoters have been removed thus creating a second *Bam*HI site at −362 (there is another *Bam*HI site at +171 in the zein coding region) (Figure 1b). Digestion with *Bam*HI therefore allows the isolation of a 533-bp fragment containing 362 bp of flanking sequence including the 15-bp consensus sequence (−317 to −331), a second moderately conserved region (−150 to −223), the conserved CAAT and TATA boxes, the P2 promoter region and 171 bp of the coding region (Figure 1c). Further digestion with *Sna*BI (at −225) or *Rsa*I (at −7) generates other fragments used in these studies (Figure 2).

The results of the nitrocellulose filter binding experiments are shown in Figure 2. A specific DNA−protein binding reaction is observed by the retention of labelled fragment on the nitrocellulose filter. Such a reaction could already be observed after incubation of labelled fragments with crude nuclear extracts for only 2 min. The DNA−protein binding reaction was shown to be specific for the pMS1/7 533-bp *Bam*HI fragment by the retention of label on the filter with this fragment while no label was retained with DNA from the coding region of a 19-kd zein gene [cDNA from clone pFW 13 (Wienand *et al.*, 1979)] (Figure 2a). The protein-binding ability was further shown to reside specifically in the 5′ flanking region since the *Rsa*I−*Bam*HI fragment (−7 to +171) containing mainly coding sequences was negative in the filter-binding assay (Figure 2b). Digestion of the pMS1/7 *Bam*HI fragment with *Sna*BI and incubation with nuclear

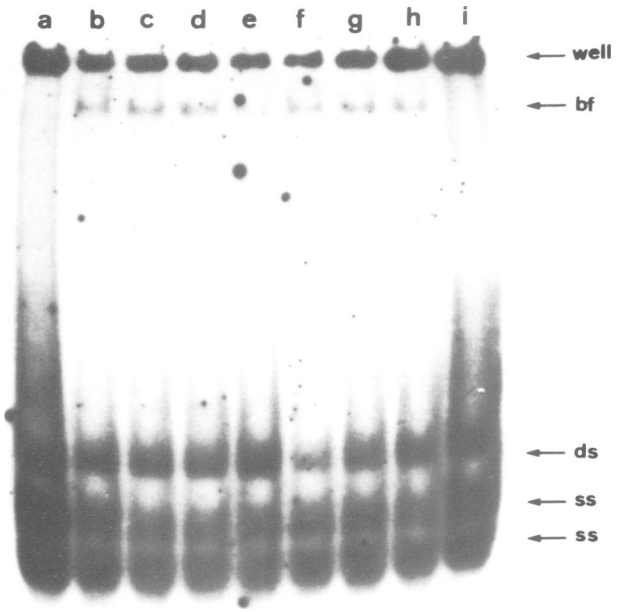

Fig. 3. Gel retention experiments. Gel retention experiments were performed with crude nuclear extracts from maize endosperm and a 21-bp oligonucleotide containing the 15-bp consensus sequence. Samples of the reaction mixture were electrophoresed in a 11% native polyacrylamide gel. The slower migrating bound fragment (bf) band resulting from the stable protein−DNA complex is indicated. The unreacted oligonucleotides are indicated by 'ds' (double strand) or 'ss' (single strand). (**a**) Migration behaviour of the ^{32}P-labelled oligonucleotide without incubation with crude nuclear extract. (**b**) Migration behaviour of the labelled fragment after incubation with crude nuclear extract. (**c, d** and **e**) Competition assay with increasing concentrations of the unlabelled specific oligonucleotide: (**c**) 1:1; (**d**) 1:10; (**e**) 1:100 molar ratios of labelled to unlabelled oligonucleotides. (**f, g** and **h**) Competition assay with increasing concentrations of an unspecific unlabelled fragment: (**f**) 1:1; (**g**) 1:10, (**h**) 1:100 molar ratios of unlabelled to labelled oligonucleotide. (**i**) Migration behaviour of the ^{32}P-labelled oligonucleotide after incubation with heat-treated nuclear extract.

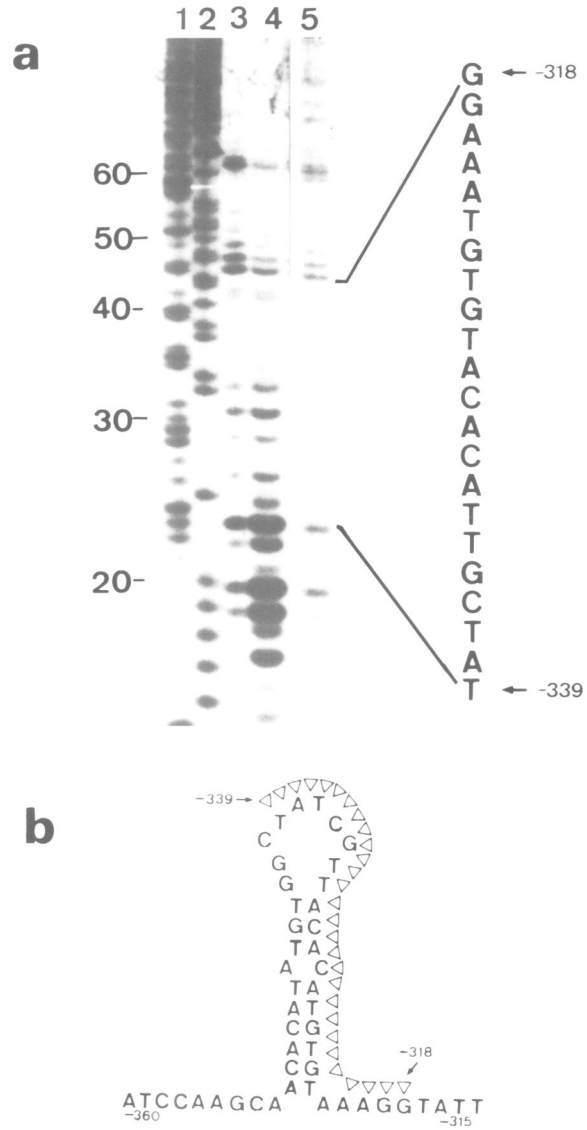

Fig. 4. Footprinting experiments. (**a**) DNase I footprinting experiments were carried out in the crude nuclear extract without purifying nuclear factors. After incubation of the labelled fragment (*Bam*HI−*Rsa*I, Figure 1b) in crude nuclear extract and DNase I treatment the resulting fragments were analysed in a 18.5% polyacrylamide−urea gel. **Lanes 1** and **2**: size marker lanes, G+A and C+T reactions, respectively of a fragment of known sequence. **Lane 3**: digestion with DNase I (1000 ng) in the absence of nuclear proteins. **Lanes 4** and **5**: digestion with DNase I−1000 ng (**lane 4**) and 10 ng (**lane 5**) − after incubation with nuclear extract. The protected sequence is written at the right. (**b**) Stem−loop structure of the two 10-bp direct repeats in the region of the 15-bp consensus sequence (Figure 1b). The bases of the stem−loop structure which are protected from the digestion of DNase I are indicated by triangles.

extract showed retention of label with both of the resulting fragments (Figure 2c) suggesting the presence of more than one protein binding site in the zein 5′ region.

Gel retention assays

In order to test if the protein-binding site of the *Bam*HI−*Sna*BI fragment was located at the 15-bp conserved sequence gel retention experiments (Schneider *et al.*, 1986) were conducted. Two 21-nt oligonucleotides representing the coding and non-coding strand in the region of the 15-bp consensus sequence were end-labelled, allowed to hybridize, and then incubated with nuclear extract. The results of such an experiment are shown in Figure 3. The labelled bands obtained without extract are shown in lane a and represent the labelled double-stranded and single-stranded oligonucleotides. Incubation with nuclear extract resulted in the appearance of a new band representing the bound fragment (bf) (Figure 3, lane b). Competition assays showed that a 100-fold excess of unlabelled, specific oligonucleotides would reduce the amount of binding to the labelled oligonucleotide (Figure 3, lane e). On the other hand, a 100-fold excess of non-specific oligonucleotide [a 30-nt poly(dA)/poly(dT) double-stranded oligonucleotide] did not reduce the intensity of the bound fragment band (lane h). The appearance of the bound fragment was shown to be protein dependent by its absence upon incubation of the double-stranded oligonucleotide with heat-treated nuclear extracts (Figure 3, lane i) or with proteinase K-treated nuclear extracts (data not shown).

Localization of binding site by DNA footprinting

Since nitrocellulose filter binding and gel retention assays did not allow an accurate localization of the specific binding site, footprinting experiments were performed (Figure 4a). The fragment used in the footprinting was the 355-bp *Bam*HI−*Rsa*I fragment labelled at the *Bam*HI site (Figure 1b). At a DNase I concentration of 10 ng per reaction volume a 22-nt sequence was specifically protected from digestion by DNase I (Figure 4a, lane 5). Use of much higher concentrations of DNase I (1000 ng/reaction volume) (Figure 4a, lanes 3 and 4) overcame the protection seen with 10 ng DNase I concentration (Figure 4a, lane 5). Furthermore, in the 1000-ng DNase I digestion, in the presence of nuclear extract (Figure 4a, lane 4), the intensity of the smaller fragments (15−23 nt) is greater and that of the larger fragments (45−65 nt) is lower than the same digestion without nuclear extract (Figure 4a, lane 3). This difference is presumably due to the effects of endogenous DNases in the nuclear extract. Comparison of the band intensities between the 1000-ng and 10-ng DNase I digestions with nuclear extract (Figure 4a, lanes 4 and 5) showed that in the 10-ng DNase I digestion the intensity of the fragments of sizes 15−23 nt was lower, fragments of 24−45 nt were absent (protected), fragments of 45−65 nt had similar intensities and fragments larger than 65 nt were clearly seen (these latter fragments were absent in the higher DNase I concentration digestion). These differences in intensity can be explained by the position of the protected region relative to the end label. The first protected base lines 23 nt downstream of the 5′ end label in the 355-bp fragment such that at low DNase I concentrations digestion within this short region would occur at a much lower frequency than at higher DNase I concentrations. The 22-nt protected region overlaps 14 bp of the 15-bp consensus sequence, and its position relative to the possible stem−loop structure in this region is shown in Figure 4b.

Discussion

DNA−protein binding studies with viral and animal genes using the filter-binding, gel retention and footprinting techniques have identified DNA sequence elements which specifically bind nuclear factors (Khoury and Gruss, 1983; Dynan and Tjian, 1985; Voss *et al.*, 1986; Sassone-Corsi and Borelli, 1986, for reviews). The binding of these *trans*-acting factors to these sequence elements can affect gene regulation in a number of ways. Transcription can be turned on as in the example involving the long terminal repeat of the murine mammary tumor virus (Chandler *et al.*, 1983) or in the heat-shock response (Parker and Topol, 1984). Transcription can be turned off by the binding of nuclear factors to blocker elements found in the upstream regions of, for example, the human 3-hydroxy-3-methylglutaryl coenzyme A reductase (Osborne *et al.*, 1985) or the large T-antigen of SV40 (Tjian, 1978). Transcription can be stimulated by the binding of nuclear factors to enhancer elements such as the binding of the Sp1 factor which is required for efficient early promoter transcription of SV40 (Dynan and Tjian, 1985). *Trans*-acting factors can also mediate tissue-specific expression, as in the case of IgH (Church *et al.*, 1985; Mercola *et al.*, 1985; Schöler and Gruss, 1985).

In this report we have used the filter-binding, gel retention and footprinting techniques and have identified a protein-binding site in the 5′ region of a zein gene. Nitrocellulose filter binding assays showed that at least two different regions of the zein 5′ region could be bound by nuclear factors. One of these binding sites lies in the region between positions −8 to −255 which contains a moderately conserved region (−150 to −223) and the P2 promoter. The binding site(s) in this region have not yet been investigated. The second binding site was defined as a 22-nt sequence (−318 to −339) containing 14 nt of the 15-bp sequence CACATGTGTAAAGGT (positions −331 to −317) which is conserved in sequence and position in all investigated zein genomic clones of both major protein size classes (Hu *et al.*, 1982; Langridge *et al.*, 1982; Langridge and Feix, 1983; Spena *et al.*, 1983; Kridl *et al.*, 1984; Langridge *et al.*, 1984, 1985; Brown *et al.*, 1986). The specificity of the binding reaction for this sequence was demonstrated in competition assays (Figure 3). In the 19-kd genes this sequence overlaps a potential stem−loop structure (Figure 4b) which, however, is not present in the genes for 21-kd zeins. The double-stranded oligonucleotide used in the gel retention experiments lacked the upstream repeat sequence (Figure 1c) and could not, therefore, form the stem−loop structure. Thus, the factor binds to the sequence rather than to the potential secondary structure and, therefore, this structure may be unimportant in terms of DNA−protein binding. The factor responsible for the retardation of the band in the gel retention assays was shown to be a protein by proteinase K or heat denaturation treatments which abolished the specific retention.

The zein genes of maize are coordinately expressed at high levels only in the endosperm tissue and are under the control of a number of regulatory loci. The sequence element described above may be involved in the turning-on of zein genes, in the enhancement of transcription rates or in the tissue specific expression of these genes, one or more of which may depend on the binding of nuclear proteins produced from the regulatory loci. The binding site may be relevant in terms of both tissue specificity and as an enhancer sequence. Firstly, a similar sequence is found in the upstream regions of endosperm-specific storage protein genes from other cereals (Forde *et al.*, 1985) and in the endo-

sperm-specific sucrose synthetase gene of maize (Werr *et al.*, 1985). Secondly, it contains a sequence virtually identical to the viral/animal enhancer core sequence TGTGG$^{AAA}_{TTT}$G (Sassone-Corsi and Borrelli, 1986).

The tissue-specific and coordinate expression of the zein genes is likely to be very complex involving multiple interactions between the flanking regions of the genes and nuclear factors and between different DNA−protein complexes. The demonstration in this paper of the binding of a nuclear factor to one region of the zein 5′ flanking sequence is a first step towards an understanding of zein gene expression. Further studies are under way to characterize the other nuclear factor binding sites and to isolate the nuclear factors themselves.

Materials and methods.

Materials

Restriction endonucleases were purchased from Biolabs (*Sna*BI) or Boehringer Mannheim (*Bam*HI, *Hpa*I, *Pst*I, *Rsa*I). *Bal*31 exonuclease, alkaline phosphatase and DNase I were from Boehringer Mannheim, radioactive, nucleotides and T4 polynucleotide kinase were purchased from Amersham.

Construction of pMS1/7 and preparation of fragments

The plasmid pMS1 contains a 4.4-kb *Eco*RI fragment of genomic maize DNA and carries one gene coding for a zein protein of the 19 000-dalton class (Brown *et al.*, 1986). pMS1/7 was constructed by linearizing pMS1 at the unique *Hpa*I-site lying between the two promoters P1 and P2 at position −470 relative to the translation start. Digestion with *Bal*31 removed 97 bp and religation generated a new *Bam*HI site. The size and extent of this deletion was confirmed by DNA sequencing by the base specific chemical cleavage method of Maxam and Gilbert (1980).

pMS1/7 was digested with *Bam*HI and the 533-bp *Bam*HI fragment, containing 362 bp of the 5′ flanking sequence and 171 bp of coding region, was isolated. This fragment was 5′ end-labelled with T4 polynucleotide kinase following alkaline phosphatase treatment. This labelled fragment was further digested with *Rsa*I or *Sna*BI and separated on 6% polyacrylamide gels. The resulting fragments were cut out of the polyacrylamide gel and eluted from the gel pieces onto DEAE-membrane (Schleicher and Schuell). The fragments were recovered by high-salt elution from the DEAE-membrane followed by ethanol precipitation.

Preparation of crude nuclear extracts

All steps were carried out at 4°C. Fresh or frozen maize endosperm (10 g) was ground in an electric mill (Moulinex) in 90 ml buffer A [25% (w/v) Ficoll 4000, 50% (w/v) dextran T40, 25 mM Tris pH 8.5, 25 mM EDTA, 0.5% (v/v) Triton X-100, 0.44 M sucrose, 10 mM β-mercaptoethanol, 2 mM spermine and 0.5 mM spermidine]. The solution was filtered through one layer of cheesecloth and then through one layer of miracloth and centrifugated for 5 min at 4000 r.p.m. in a Sorvall HB4 swing-out rotor. The pellet was dissolved in 10 ml buffer B [50 mM Tris pH 8.5, 5 mM $MgCl_2$, 25% (v/v) glycerol and 10 mM β-mercaptoethanol] and centrifuged in a Sorvall SS34 rotor at 13 000 r.p.m. for 20 min. The resulting pellet was dissolved in 0.5 ml buffer C [20 mM Hepes pH 7.5, 25% (v/v) glycerol, 0.42 M NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM PMSF, 0.5 mM DTT] and the nuclei were disrupted by ultrasonic treatment. Starch grains were removed by an additional centrifugation step in a Sigma centrifuge at 10 000 r.p.m. The clear supernatant was dialysed over night against 100 ml buffer D [20 mM Hepes pH 7.5, 20% (v/v) glycerol, 0.1 M KCl, 0.2 mM EDTA, 0.5 mM PMSF, 0.5 mM DTT] and this solution represented the crude nuclear extract and was stored in aliquots at −70°C. The protein concentration of the crude nuclear extracts was determined according to the method of Bradford (1976).

Filter-binding assays

The filter-binding assays were based on the method of Diffley and Stillman (1986). Aliquots of 30−50 μl crude nuclear extract (10−15 μg crude nuclear protein) were pre-incubated with 10 μg *Eco*RI digested pBR328 DNA or 10 μg sheared calf thymus DNA in binding buffer (10 mM Tris pH 7.5, 50 mM NaCl, 1 mM DTE, 1 mM EDTA and 5% glycerol) in a final volume of 500 μl. After 15 min 10 000 c.p.m. (~10^7 c.p.m./μg) of labelled fragment was added. Fractions (50 μl) were taken at 2, 5, 10, 15 and 20 min, filtered through nitrocellulose membranes (Schleicher and Schuell) in a dot-blot apparatus and washed three times with 200 μl of a solution containing 10 mM Tris pH 7.5, 50 mM NaCl and 1 mM EDTA. The filters were air-dried and autoradiographed.

Gel retention experiments

Gel retention experiments were carried out according to Schneider *et al.* (1986). Oligonucleotides were 5′-end labelled with T4 polynucleotide kinase. The complementary labelled oligonucleotides were mixed in an equimolar ratio in distilled water and heated for 60 s to 100°C followed by a cooling step for 15−30 min at room temperature to allow annealing of complementary oligonucleotides. The renatured DNA fragment (5 ng DNA) (10 000 c.p.m.) was incubated with 50 μl crude nuclear extract (10−15 μg crude nuclear protein) in binding buffer (see above). No pre-incubation step with unspecific DNA (e.g. pBR328 DNA, calf thymus DNA) was necessary. For competition analyses 5, 50 or 500 ng of unlabelled specific or unspecific double-stranded oligonucleotide were added to the crude nuclear extract along with 5 ng of labelled oligonucleotide. After 2 min 6 μl of loading buffer [100 mM DTT, 2% (w/v) bromophenol blue, 50% (v/v) glycerol] was added and the samples electrophoresed in an 11% polyacrylamide gel (acrylamide:bis = 29:1) in running buffer (10 mM Tris pH 8.0, 1 mM EDTA, 3 mM Na acetate). Following electrophoresis gels were dried and autoradiographed at −70°C with a screen.

DNase I footprinting experiments

DNase I footprinting experiments were carried out with the 355-bp *Bam*HI−*Rsa*I fragment (Figure 2) 5′-end labelled at the *Bam*HI site. The standard reaction mixture had a final volume of 100 μl. Ten μl of 10× binding buffer (see above), various amounts of crude nuclear extract and 10 μg *Eco*RI-digested pBR328 DNA were pre-incubated for 15 min at room temperature. After this 10 000 c.p.m. of end-labelled *Bam*HI−*Rsa*I fragment was added and incubated for 2 min at room temperature. The DNA fragment was digested with empirical DNase I concentrations at 37°C for 5 min following the addition of 5 mM $MgCl_2$. The digestion was stopped with 20 mM EDTA and by heating the mixture to 65°C for 15 min. The solution was extracted with phenol:chloroform (1:1) and chloroform:isoamylalcohol (24:1) and the DNA fragments were ethanol precipitated. These fragments were separated on 18.5% polyacrylamide urea gels (Ansorge *et al.*, 1984) and autoradiographed.

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References

Ansorge,W. and Barker,R.F. (1984) *J. Biochem. Biophys. Methods*, **9**, 33−47.
Bäumlein,H., Wobus,U., Pustell,J. and Kafatos,F.C. (1986) *Nucleic Acids Res.*, **14**, 2707−2720.
Bradford,M.M. (1976) *Anal. Biochem.*, **72**, 248−254.
Breathnach,R. and Chambon,P. (1981) *Annu. Rev. Biochem.*, **50**, 349−383.
Brown,J.W.S., Wandelt,C., Feix,G., Neuhaus,G. and Schweiger,H.G. (1986) *Eur. J. Cell Biol.*, **42**, 161−170.
Burr,B., Burr,F.A., John,T.P.St., Thomas,J.M. and Davis,R.W. (1982) *J. Mol. Biol.*, **154**, 33−49.
Chandler,V.L., Maler,B.A. and Yamamoto,K.R. (1983) *Cell*, **33**, 489−499.
Church,G.M., Ephrussi,A., Gilbert,W. and Tonegawa,S. (1985) *Nature*, **313**, 798−801.
Di Fonzo,N., Fornasari,E., Salamini,F. and Soave,C. (1977) *Maydica*, **22**, 77−88.
Di Fonzo,N., Fornasari,E., Salamini,F., Reggiani,A., and Soave,C. (1980) *Heredity*, **71**, 397−402.
Di Fonzo,N., Gentinetta,E., Salamini,F. and Soave,C. (1979) *Plant Sci. Lett.*, **14**, 345−354.
Diffley,J.F.X. and Stillman,B. (1986) *Mol. Cell. Biol.*, **6**, 1363−1317.
Dynan,W.S. and Tjian,R. (1985) *Nature*, **316**, 774−778.
Fluhr,R., Kuhlemeier,C., Nagy,F. and Chua,N.-H. (1986) *Science*, **232**, 1106−1112.
Forde,B.G., Heyworth,A., Pywell,J. and Kreis,M. (1985) *Nucleic Acids Res.*, **13**, 7327−7339.
Gillies,G.D., Morrison,S.L., Oi,V.T. and Tonegawa,S. (1983) *Cell*, **33**, 717−728.
Heidecker,G. and Messing,J. (1986) *Annu. Rev. Plant. Physiol.*, **37**, 439−466.
Hu,N.-T., Pfeifer,M.A., Heidecker,G., Messing,J. and Rubenstein,I. (1982) *EMBO J.*, **1**, 1337−1342.
Khoury,G. and Gruss,P. (1983) *Cell*, **33**, 313−314.
Kridl,J.C., Vieira,J., Rubenstein,I. and Messing,J. (1984) *Gene*, **28**, 113−118.
Langridge,P. and Feix,G. (1983) *Cell*, **34**, 1015−1022.
Langridge,P., Pintor-Toro,J.A. and Feix,G. (1982) *Planta*, **156**, 166−170.
Langridge,P., Eibel,H., Brown,J.W.S. and Feix,G. (1984) *EMBO J.*, **3**, 2467−2471.
Langridge,P., Brown,J.W.S., Pintor-Toro,J.A., Feix,G., Neuhaus,G. and Schweiger,H. (1985) *Eur. J. Cell Biol.*, **39**, 257−264.

Marks,M.D., Lindell,J.S. and Larkins,B.A. (1985) *J. Biol. Chem.*, **260**, 16451–16459.
Maxam,A.M. and Gilbert,W. (1980) *Methods Enzymol.*, **65**, 499–560.
Mercola,M., Goverman,J., Mirell,C. and Calame,K. (1985) *Science*, **227**, 266–270.
Messing,J., Geraghty,D., Heidecker,G., Hu,N.-T., Kridl,J. and Rubenstein,I. (1983) In Kosuge,T., Meredith,C.P. and Hollaender,A. (eds), *Genetic Engineering of Plants*. Plenum, New York, pp. 211–227.
Morelli,G., Nagy,F., Fraley,R.T., Rogers,S.G. and Chua,N.-H. (1985) *Nature*, **315**, 200–204.
Osborne,T.F., Goldstein,J.L. and Brown,M.S. (1985) *Cell*, **42**, 203–212.
Parker,C.S. and Topol,J. (1984) *Cell*, **37**, 273–283.
Pedersen,K., Bloom,K.S., Anderson,J.N., Glover,D.V. and Larkins,B.A. (1980) *Biochemistry*, **19**, 1644–1650.
Sassone-Corsi,P. and Borrelli,E. (1986) *Trends Genet.*, **8**, 215–219.
Schneider,R., Gander,I., Mueller,U., Mertz,R. and Winnacker,E.L. (1986) *Nucleic Acids Res.*, **3**, 1303–1317.
Schöler,H.R. and Gruss,P. (1985) *EMBO J.*, **11**, 3005–3013.
Spena,A., Viotti,A. and Pirotta,V. (1983) *J. Mol. Biol.*, **169**, 799–811.
Soave,C., Righetti,P.G., Lorenzoni,C., Gentinetta,E. and Salamini,F. (1976) *Maydica*, **21**, 61–75.
Soave,C., Suman,N., Viotti,A. and Salamini,F. (1978) *Theor. Appl. Genet.*, **52**, 263–267.
Saove,C., Reggiani,R., Di Fonzo,N. and Salamini,F. (1981) *Genetics*, **97**, 363–377.
Soave,C., Reggiani,R., Di Fonzo,N. and Salamini,F. (1982) *Biochem. Genet.*, **20**, 1027–1038.
Tjian,R. (1978) *Cell*, **13**, 165–179.
Timko,M.P., Kausch,A.P., Castresana,C., Fassler,J., Herrera-Estrella,L., Van den Broeck,G., Van Montagu,M., Schell,J. and Cashmore,A.R. (1985) *Nature*, **318**, 579–582.
Valentini,G., Soave,C. and Ottaviano,E. (1979) *Heredity*, **42**, 33–40.
Viotti,A., Abildsten,D., Pogna,N., Sala,E. and Pirrotta,V. (1982) *EMBO J.*, **1**, 53–58.
Voss,G., Schlokat,U. and Gruss,P. (1986) *Trends Biochem. Sci.*, **11**, 287–289.
Werr,W., Frommer,W.-B., Maas,C. and Starlinger,P. (1985) *EMBO J.*, **4**, 1373–1380.
Wienand,U., Brüschke,C. and Feix,G. (1979) *Nucleic Acids Res.*, **6**, 2707–2719.
Wienand,U., Langridge,P. and Feix,G. (1981) *Mol. Gen. Genet.*, **182**, 440–444.

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