ZEIN SPECIFIC RESTRICTION ENZYME FRAGMENTS OF MAIZE DNA

Udo WIENAND and Günter FEIX
Institut für Biologie III der Universität, Schänzlestr. 1, D-7800 Freiburg i.Br., FRG

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1. Introduction

Zein is the major storage protein of maize endosperm and is synthesized in great amounts at defined times of endosperm development [1]. It consists primarily of two size classes of M_r ~19 000 and 21 000 with considerable charge heterogeneity [2]. The two major protein classes are coded for by separate non-homologous mRNA [3,4]. CDNA clones constructed from zein mRNAs have revealed 3 classes of mRNAs which can be distinguished on the basis of hybridisation experiments [5]. However, 15 non-crosshybridizing mRNA were suggested [6] as a result of reassociating zein mRNA with cDNA. At the DNA level, by nucleic acid reassociation studies, the presence of 120 zein genes was proposed [6].

To allow a more direct analysis of zein specific sequences in the maize genome, an investigation of zein specific restriction enzyme fragments was undertaken by applying the Southern technique [7]. The results obtained can be taken as an indication for the presence of a multigene system.

2. Materials and methods

2.1. *Materials*

Restriction enzymes, *Escherichia coli* DNA polymerase I, and deoxyribonucleotide triphosphates were obtained from Boehringer. d[α-^{32}P]ATP was from Amersham Buchler. Salts were reagent grade from Merck. Nitrocellulose was from Schleicher and Schüll.

2.2. *Isolation of DNA from maize seedlings*

Eight day old frozen seedlings (250 g) of the inbred line A619 (obtained from Mike Brayton seeds/Ames) were ground in a cold mortar and, after suspension in 350 ml cold buffer (50 mM Tris–HCl (pH 7.6), 100 mM NaCl, 50 mM EDTA, 0.5% sarcosyl), were stirred at low speed for 1 h at 4°C. The supernatant, obtained by centrifugation for 10 min at 10 000 rev./min, was phenol-extracted by gently stirring with 100 ml of a 1:1 mixture of phenol and chloroform saturated with 50 mM Tris–HCl (pH 8). The nucleic acids precipitated from the aqueous phase with ethanol were washed twice with ethanol and once with ether and redissolved in 100 ml 10 mM Tris–HCl (pH 7.6) and 10 mM EDTA. After adding NaCl to 2 M, the solution was left overnight at 4°C and the precipitated RNA removed by centrifugation. DNA was then precipitated by adjusting the solution to 0.5 M NaCl with 10 mM Tris–HCl (pH 7.6) and 10 mM EDTA, and adding 40 ml 2% cetyltrimethyl ammonium bromide at room temperature. The DNA was collected by centrifugation, washed first for 3 h with 70 ml 70% ethanol containing 0.2 M Na-acetate and then with pure ethanol and finally ether before being dissolved in 10 mM Tris–HCl (pH 7.6).

2.3. *Preparation of ^{32}P-labelled inserts from recombinant plasmids*

Preparation of DNA from plasmids pFW13 and pFW19, the digestion of these DNAs with restriction enzyme *Pst*I and the isolation of the zein specific inserts were done as in [9]. Aliquots (1 μg) of the isolated inserts were labelled by nick translation with d[α-^{32}P]ATP according to [10].

3. Results and discussion

Maize DNA was isolated from 8 day old seedlings by phenol extraction followed by a modified fractionation procedure with cetyltrimethyl ammonium

bromide according to [8]. The DNA preparation contained <10% RNA and when electrophoresed in 0.5% agarose migrated as a single band of M_r ~60 × 10^6. Restriction enzyme fragments obtained from this DNA by digestion with *Eco*RI or *Bam*HI were electrophoresed in 0.8% agarose and stained with ethidium bromide to reveal a distinct banding pattern for both restriction enzymes with fragment sizes varying from M_r 1–~20 × 10^6 as demonstrated in fig.1. Digestion was complete as indicated by the fragmentation pattern of phage λDNA added to a maize DNA containing incubation mixture. The functional significance of the DNA sequences present in these bands is unknown. The DNA fragments in the gel were transferred to nitrocellulose as in [7] and then hybridized to ^{32}P-labelled zein specific DNA sequences. Zein specific sequences coding for the M_r 19 000 protein or the M_r 21 000 protein were obtained by excision of the zein specific inserts from recombinant plasmids constructed recently from zein mRNAs [9]. As shown [9], the two inserts, 580 nucleotides (pFW 13) and 450 nucleotides (pFW 19) long, do not cross hybridize. The hybridizations of the inserts to the separated DNA fragments revealed the banding patterns depicted in fig.2. It can be seen that maize DNA contains at

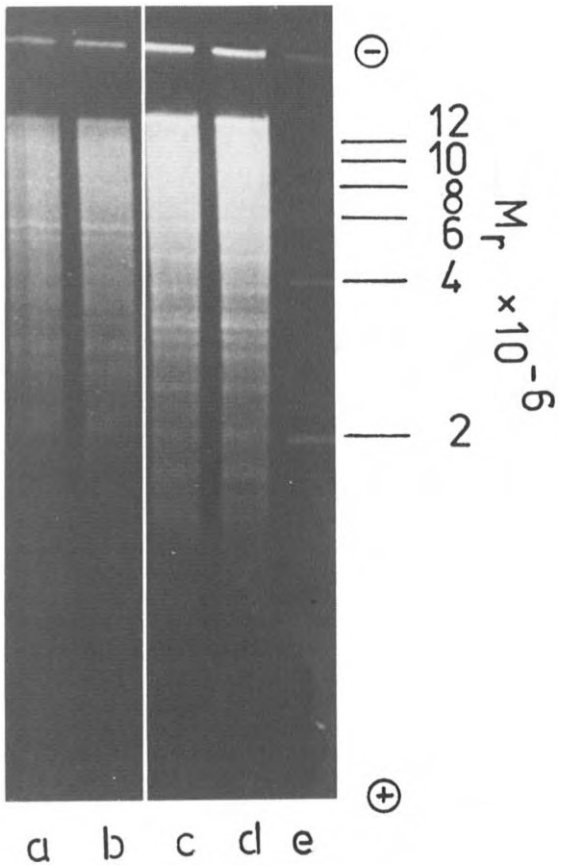

Fig.1. Agarose gel electrophoresis of restriction fragments of maize DNA. Maize DNA (250 μg) was digested for 8 h at 37°C with 500 units *Eco*RI or with 100 units *Bam*HI. 15 μg each of the digested DNA were electrophoresed for 18 h in 0.8% agarose gels. The gels stained with ethidium bromide, were photographed under UV light. Lanes a,b represent duplicate experiments with *Eco*RI fragments, lanes, c,d duplicate experiments with *Bam*HI fragments. Lane e shows *Eco*RI fragments of λdv DNA used as size markers [12].

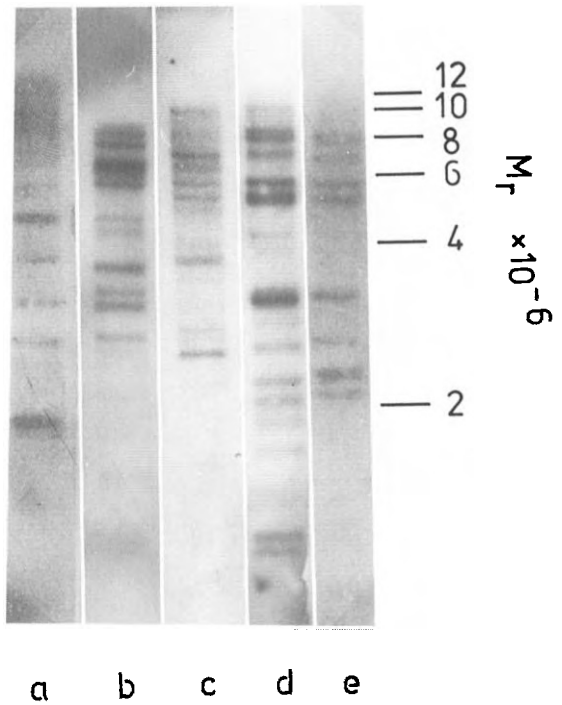

Fig.2. Electrophoretic separation pattern of zein specific restriction enzyme fragments of maize DNA. DNA fragments corresponding to the various lanes of fig.1 were transferred to nitrocellulose paper as in [7]. The nitrocellulose strips were hybridized with 0.5–1 μg (1–6 × 10^6 cpm/μg) insert fragments for 18 h at 65°C in 15 ml (lanes a–d) or 3 ml (lane e) of hybridization solution (3 × SSPE, 0.02% Ficoll, 0.02% polyvinylpyrrolydone, 10% dextran sulfate, 0.1% SDS, 50 μg/ml salmon sperm DNA). After washing for 90 min at 70°C with 2 × SSPE containing 1% SDS the nitrocellulose strips were exposed for 20 days to X-ray film Kodak RP5. Lanes a,b represent *Eco*RI fragments, lanes c–e *Bam*HI fragments. Lanes a,c were hybridized with insert from plasmid pFW19, lanes b,d with insert from plasmid pFW13, lane e with the large *Bam*HI fragment of the insert from plasmid pFW13 [9]. SSPE contains 0.15 M NaCl, 0.01 M Na_2HPO_4 and 0.001 M EDTA adjusted to pH 7.0.

least 13 *Eco*RI fragments in the M_r range $2-12 \times 10^6$ and 19 *Bam*HI fragments in the M_r range $1.5-12 \times 10^6$ that hybridize with zein specific sequences. It is evident that the *Eco*RI hybridization patterns differ from the *Bam*HI patterns (lanes a,b versus c,d). Furthermore, most of the DNA fragments specific for the M_r 19 000 protein differ from those specific for the M_r 21 000 protein in both the *Eco*RI digests (lane a,b) and the *Bam*HI digests (lane c,d). The 580 nucleotide insert was also used to provide a 500 nucleotide subfragment both fragments being specific for the coding sequence of the M_r 19 000 protein [9]. When used as a hybridization probe to the *Bam*HI digests this shortened fragment failed to label 2 previously strong hybridization bands of $M_r \sim 1.5 \times 10^6$ and possibly also some weaker bands (lane e, fig.2).

It is assumed that different intensities of the various hybridization bands result from varying amounts of zein specific sequences contained in the bands. The low intensity bands are thought to comprise only one copy of a zein specific fragment since one copy of insert DNA processed under identical conditions showed hybridization of comparable intensity.

The varying intensities and numbers of hybridization bands indicate the presence of a multigene system coding for zein proteins although this cannot be correlated, at present, to specific proteins. Similar hybridization patterns have also been obtained recently for the keratin multigene system [11].

However, correlation of the multiple hybridization bands to a defined number of zein genes will only be possible with knowledge of the restriction enzyme map of the zein specific genomic structures and after an analysis of the genes for a possible split gene structure. Work is in progress to analyse these features.

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