Plant Molecular Biology 7: 71–79, 1986

Specific transcription of a 15-kilodalton zene gene in HeLa cell extracts†

Rebecca S. Boston & Brian A. Larkins*
Dept. of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907, U.S.A.

Summary

A maize genomic clone containing a 15 kilodalton zein gene was used as a template in an *in vitro* transcription system for HeLa cells. A runoff assay indicated transcription was initiating 5′ to the map position of the open reading frame for the protein. Fine-structure mapping of RNAs synthesized *in vitro* showed two transcription start sites separated by 24 bases. One start site is 27 bases downstream of a consensus TATA sequence; the other is 30 bases downstream of a TATG sequences. The initiation sites for RNA synthesized *in vitro* map to the same region of the genomic clone as zein RNA isolated from developing maize kernels.

Introduction

Zeins, the alcohol-soluble storage proteins of maize, exhibit tissue-specific expression in the endosperm of developing seeds. These proteins are localized in membrane-bound vesicles termed protein bodies and represent approximately 50% of the protein in maize kernels. Separation of zeins by two-dimensional gel electrophoresis reveals multiple proteins that fall into major subgroups of 10, 15, 19, 22 and 27 kDa based on apparent molecular weights (16). Genes encoding the 27, 15, and 10 kDa polypeptides are present in relatively few copies (1–3) (27, 29), whereas genes encoding the 19 and 22 kDa proteins are present in multigene families (12, 29).

The expression of these gene families is temporally coordinated during seed development. Messenger RNA transcripts can be detected by 10 days after pollination and increase to maximum levels by around 20 days after pollination before gradually decreasing with seed maturation. Although the transcription of these genes is coordinately regulated, the level of expression among gene families varies. Certain gene families account for higher levels of stable mRNA than others but, in general, there is a good correlation between mRNA levels and the apparent number of genes in each gene family (21, 22). One exception is the gene(s) encoding the 15 kDa polypeptide. The level of transcripts produced by this single or low copy number gene is as high as that produced by multiple genes of the 22 and 19 kDa families (21). This increased expression of the 15 kDa zein gene may be due to transcriptional regulation – perhaps by multiple effectors. Thus, at one level, the mode of regulation would be common among all zeins to coordinately initiate transcription during seed development. At another level, a higher rate of transcription for the 15 kd zein gene could account for its increased expression relative to that of the other zeins.

Transcription of a 15 kDa zein gene using a soluble *in vitro* system prepared from HeLa cell nuclei is presented here. The ability to mimic *in vivo* events with defined systems may help identify and define regulatory loci controlling transcription and, in addition, provide a means of directly comparing transcriptional activities of various classes of zein genes.

* Author to whom correspondence should be addessed.

† Journal paper number 10347 of the Purdue Agricultural Experiment Station.

Materials and methods

Preparation of DNA templates

Plasmid DNA was isolated as described by Clewell (5) and cleaved with restriction enzymes according to the manufacturer's instructions. Following cleavage, DNA was extracted with phenolchloroform-isoamyl alcohol (50:48:2, v/v/v) and concentrated by ethanol precipitation. Some experiments required purification of zein sequences from vector sequences. This was accomplished by separating the restriction fragments on agarose gels, locating the band of interest by staining with ethidium bromide, transferring the DNA band onto NA-45 paper (Schleicher and Schuell, Keene, NH) by electrophoresis and eluting the DNA according to manufacturer's instructions. The eluted DNA was extracted first with isobutanol to remove the ethidium bromide. This was followed by phenol-chloroform extraction and concentration by ethanol precipitation.

Preparation of extracts

Nuclei were isolated from $1-2\times10^9$ HeLa cells maintained in suspension cultures at $2-5\times10^5$ cells/ml in Eagle's minimal essential medium (Gibco Laboratories, Grand Island, NY) supplemented with 10% calf serum. Extracts were prepared according to Dignam *et al.* (9) with the following modifications: The hypotonic buffer contained 10 mM Tris-HCl pH 8 at 25 °C, 6 mM $MgCl_2$, 0.5 mM DTT,[1] 10 mM KCl and 0.1 mM PMSF and crude nuclei were resuspended in a buffer of 20 mM Tris-HCl pH 8 at 25 °C, 6 mM $MgCl_2$, 0.5 mM DTT, 25% glycerol (v/v), 0.5 mM EDTA and 0.1 mM PMSF.

In vitro *transcription and purification of RNA*

Assays were carried out at 30 °C for 30 min in 20 μl reactions with final concentrations of various components as follows: 50 mM KCl, 10 mM HEPES pH 7.9 at 25 °C, 10% glycerol (v/v), 0.25 mM DTT, 0.1 mM EDTA, 7.5 mM $MgCl_2$, 200 μM CTP, 200 μM UTP, 400 μM ATP, 15 μM GTP, 20 μCi α-^{32}P-GTP, 10 mM creatine phosphate, 2% polyvinyl alcohol (w/v), 15 mM $(NH_4)_2SO_4$, 5–100 μg/ml DNA (as indicated in figure legends) and HeLa nuclear extract at protein concentrations of 6–7 mg/ml. When α-amanitin was used to inhibit RNA polymerase II it was present at 1 μg/ml. RNA for S1 mapping and primer extension analysis was synthesized as described above except that GTP was present at 400 μM and no α-^{32}P GTP was included. Following incubation, transcription was terminated and RNAs purified as described by Murphy et al. (25). When RNAs were to be used in S1 mapping and primer extension analyses, the transcription reactions were treated with 40 μg/ml of RNase-free DNase (Worthington, DPRF) for 5 min immediately prior to the organic extractions. DNase was further purified by UDP-agarose chromatography before use (24).

Maize RNAs were isolated from 50 W64A kernels harvested at 16 days after pollination according to Langridge and Feix (14), except that 200 μg/ml proteinase K (Beckman, Palo Alto, CA) was included in the grinding buffer (10). RNAs were further purified by resuspending pellets from the first ethanol precipitation in a guanidine thiocyanate solution and pelleting the RNA through a CsCl pad according to Kaplan *et al.* (13).

S1 nuclease analysis

End labeled DNA probes were prepared from cloned zein DNA as described in figure legends. Terminal phosphates were removed with calf intestine alkaline phosphatase (Boehringer Mannheim). Fragments were then 5′ end-labeled with α-^{32}P-ATP (ICN) and T4 polynucleotide kinase (BRL) to specific activities of 5×10^5 to 1×10^6 cpm per picomole of 5′ ends. After labeling, the restriction fragments were cleaved with a second restriction enzyme and the fragments to be used as probes were purified by polyacrylamide gel electrophoresis.

Strand-specific probes (approximately 1×10^5 cpm per annealing reaction) were heat denatured and allowed to anneal with RNA for 8–12 h at 60 °C in 0.4 M NaCl, 40 mM PIPES pH 6.4 at 25 °C, 0.2 mM EDTA, 80% (v/v) deionized formamide. Reactions were then incubated with 1000

[1]Abbreviations used: DTT, dithiothreitol; HEPES, 4-(2-Hydroxyethel)-1-piperazineethanesulfonic acid; PIPES, piperazine-N, N′-bis[2-ethanesulfonic acid]; 1, 4-piperazine-diethanesulfonic acid; PMSF, phenylmethylsulfonyl fluoride.

U/ml of S1 nuclease (BRL) according to Berk and Sharp (1977). After 30 min at 37 °C, reactions were extracted with phenol-chloroform and the nucleic acids precipitated with two volumes of ethanol. Products were analyzed on 5% polyacrylamide gels containing 8 M urea.

Primer extension analysis

RNAs were hybridized with a 5′ end-labeled Dde I-Hae III restriction fragment from pZG15A (see Fig. 4a) as described for S1 analysis except that the temperature during annealing was reduced to 52 °C. Hybrids were recovered by ethanol precipitation and extended by the method of Ghosh *et al.* (11) as modified by Carlson and Ross (4), except that extension reactions also contained 10 U RNasin (Promega Biotec, Madison, WI).

Results

Accurate transcription initiation of the zein gene

DNA fragments from the zein genomic clone pZG15A were used as templates in the *in vitro* transcription assay. Figure 1 shows the DNA sequence of the 5′ end of pZG15A. Details of the isolation, characterization and sequence analysis of this clone will be published elsewhere[2]. Based on the DNA sequence an open reading frame begins at position + 1. The DNA sequence of pZG15A agrees with that determined for two cDNA clones whose 5′ ends terminate at positions − 158 and + 108 respectively.[2] A schematic representation of the locations of several restriction endonuclease cleavage sites on pZG15A is shown in Fig. 2A. These restriction sites were used either to truncate the DNA template for *in vitro* runoff assays (19) or to provide specific radiolabeled probes for S1 mapping or primer extension analysis (2, 11).

DNA fragments cleaved at the Dde I site directed synthesis of discrete RNAs when incubated with the HeLa cell nuclear extract (Fig. 2B, lanes 1–6). These RNAs migrated as a species of approximately 220 bases as compared to DNA markers on denaturing polyacrylamide gels. When DNA fragments cleaved with Hinf I or Bal I were used as templates, RNAs with lengths of approximately 520 and 450 bases, respectively, were generated (Fig. 2B, lanes 7–10). Synthesis of these RNAs was dependent upon exogenous DNA (data not shown) and was inhibited by 1 g/ml α-amanitin (Fig. 2B, lanes 4, 6, 8 and 10), indicating RNA polymerase II-dependent transcription.

Several other discrete RNAs were also produced by this assay. Some of these appeared to originate from pBR322 DNA since they are not detected when purified maize inserts were used as templates (Fig. 2B, lanes 1–3 cf. lane 5). Other transcripts may result from initiations at the ends of restriction fragments producing transcripts of the same size as the initial restriction fragments. Incubation of tran-

[2]Pedersen K, Argos P, Nayarana SVL, Larkins BA, manuscript in preparation.

```
5′
  GAATTCATTG ACAACCCTTG ACATGTAAAG TTGATTCATA TGTATAAGAA   50
                                     *
  AGCTTAATGA TCTATCTGTA CATCCAAATC CATGTACTAT GTTTCCAGCT  100
  CATGCAACGC AACATTCCAA AACCATGGAT CATCTATAAA TGGCTAGCTC  150
  CCACATATGA ACTAGTCTCT ↓ATCATCATCC AATCCAGATC AGCAAAGCGG  200
                                    +1
  CAGTGCGTAG AGAGGATCGT CGAACAGAAC AGCATG
```

Fig. 1. DNA sequence of 5′ end of pZG15A. The DNA sequence of 1300 bases of a 6500 base genomic subclone encoding a 15 kDa zein gene was previously determined.[2] The clone contains the entire coding sequence for the 15 kDa zein as well as 5′ and 3′ flanking DNA. An asterisk denotes the 5′ end of a minor *in vivo* transcript. The closed arrow denotes the 5′ end of the major *in vivo* transcript. The position of the first nucleotide encoding the initiating methionine for the zein signal sequence is at position + 1. The mature protein initiates at + 61. The canonical 'TATA' sequences are underlined.

scription products with 40 μg/ml DNase did not affect the runoff pattern while addition of DNase at the initiation of the transcription assay resulted in no RNA synthesis (data not shown). Synthesis of specific transcripts indicates that this zein gene is transcribed *in vitro* by the heterologous extract and suggest that at least some signals controlling specific transcription of plant genes are correctly recognized by factors in the HeLa nuclear extract.

S1 mapping of In vitro *transcripts*

To determine whether or not the RNAs produced *in vitro* had the same 5′ ends as RNAs isolated from developing maize kernels, the RNAs were mapped using S1 nuclease (2). RNAs synthesized in the runoff assay in the absence of radiolabeled triphosphates, or RNAs isolated from maize kernels 16 days after pollination, were allowed to anneal with DNA probes radiolabeled at their 5′ ends. Regions of the probes protected from nuclease degradation were analyzed on denaturing polyacrylamide gels alongside a sequencing ladder.

One major *in vivo* transcript and two *in vitro* transcripts were detected (Fig. 3B). The *in vivo* transcript and one of the *in vitro* transcripts had initiation sites corresponding to position − 63 to − 69 on the genomic clone; the second *in vitro* site aligned with position − 47. None of the protected fragments were observed in control experiments when RNA and S1 nulcease were omitted from the hybridization reactions. As observed in the runoff assay, both *in vitro* transcripts required zein DNA sequences and disappeared when the transcription assay was carried out in the presence of α-amanitin.

In addition to the major band position − 63, several minor bands were produced when RNA from maize endosperm was used in the S1 protection assay. These may be the result of incomplete digestion by the nuclease or by hybridization with partially degraded RNAs in the preparation leading to protection of only a small fraction of the probe. Furthermore, the detection of these species varied among different RNA preparations (data not shown).

Protection of two DNA fragments by RNA synthesized *in vitro* reflects a protection pattern common to zein genes. Evidence indicating the presence of double starts, separated by 12−14 bases, has been observed by us and others when endosperm RNA is used to protect probes for 19 kDa and 22 kDa zein genes[3] (14, 15, 23). Also, S1 protection assays of some preparations of endosperm RNA indicate a double start for the 15 kDa gene probe used here.[4]

[3]Boston RS, unpublished data.

[4]Goldsbrough PB, Gelvin SB, Larkins BA, manuscript in preparation.

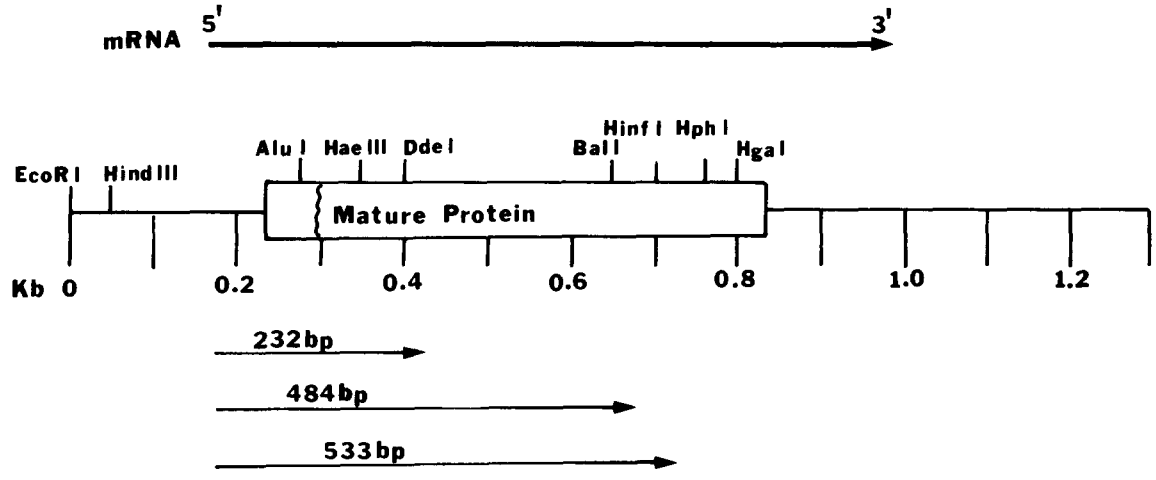

Fig. 2A. Schematic representation of pZG15A showing the gene organization and expected products of the runoff assay. Map units are in kilobase pairs. The Hae III site corresponds to map position + 117, the Dde I site + 166, the Bal I site + 419 and the Hinf I site + 468.

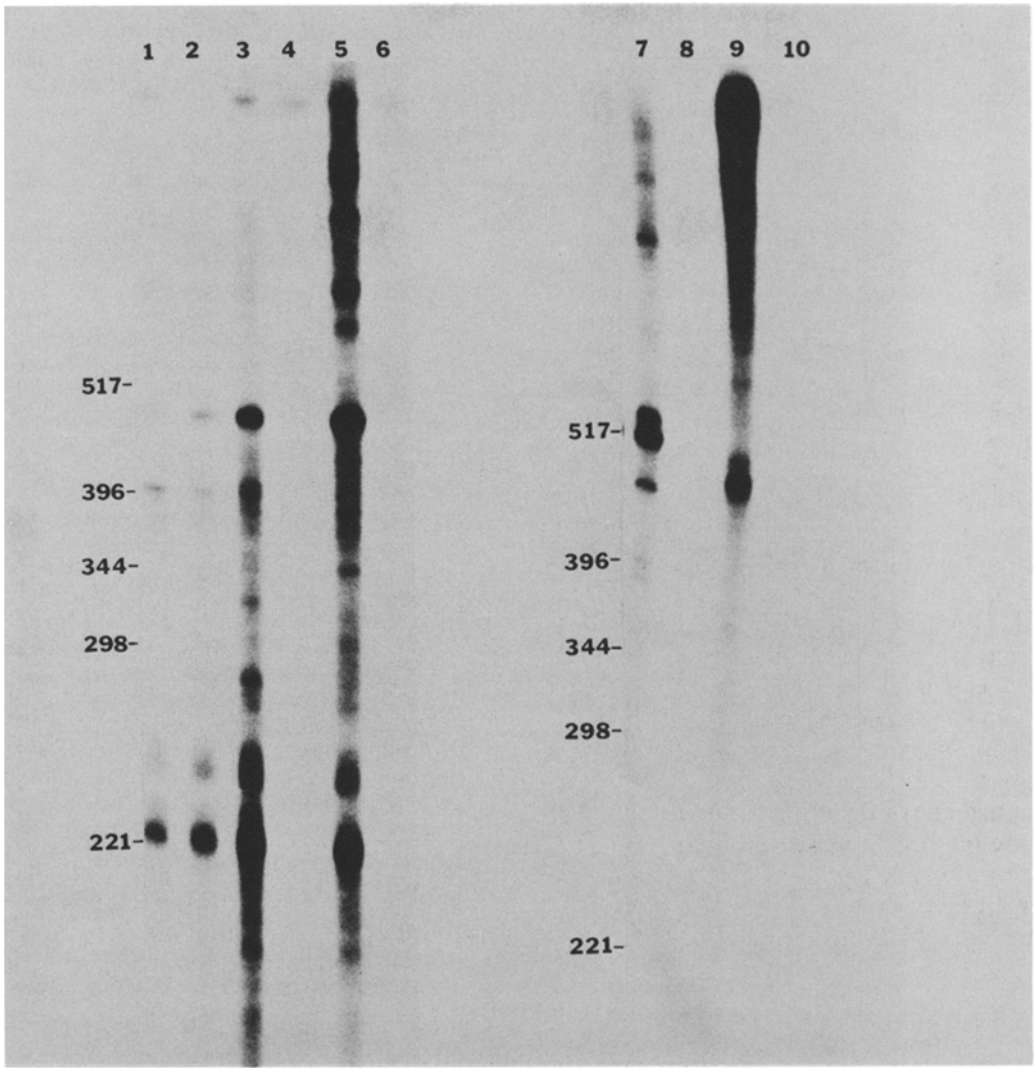

Fig. 2B. Electrophoretic analysis of RNAs synthesized *in vitro* from truncated zein DNA templates. RNAs were made and purified as described in Methods. (A.) Autoradiograms of RNAs resulting from transcription from 100 ng (Lane 1), 200 ng (Lane 2), or 400 ng (Lane 3) of the 6.5 kb EcoRI fragment containing the zein insert of pZG15A cleaved with Dde I. Transcription from the Dde I fragments of the entire pZG15A is shown in Lane 5. Transcription of the zein insert and transcription of the entire plasmid in the presence of α-amanitin yields the RNA patterns shown in Lanes 4 and 6 respectively. Size of expected runoff products are shown in Fig. 2A. The molecular weight markers shown at left denote the migration of pBR322 DNA cleaved with Hinf I, radiolabeled with polynucleotide kinase and denatured by heating in formamide. Analysis of the runoff transcription pattern of pZG15A cleaved with Hinf I (Lanes 7 and 8) or Bal I (Lanes 9 and 10) is shown in the right panel. α-Amanitin was added to the transcription reactions analyzed in Lanes 8 and 10.

As shown in Fig. 1, the sequence TATGAAC occurs 33 bases upstream from the second transcriptional start. Transcription of Adenovirus major late promoter using a HeLa cell extract demonstrated a requirement for a TATA sequence (11). Thus, it seems likely that the TATGAAC sequence is acting as the transcriptional initiation signal *in vitro* resulting in the second start.

Primer extension analysis

Since transcription of the 15 kDa zein gene by a

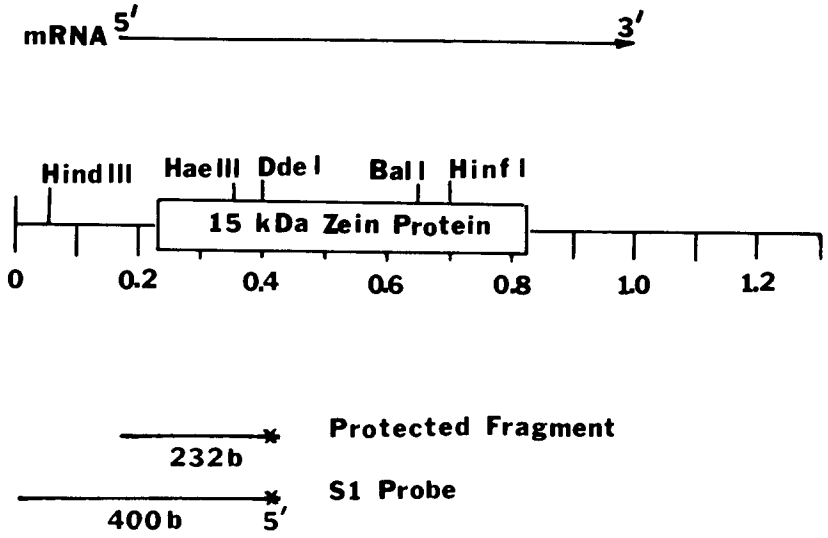

Fig. 3A. Schematic representation of pZG15A showing the radiolabeled probe and expected S1 protection product.

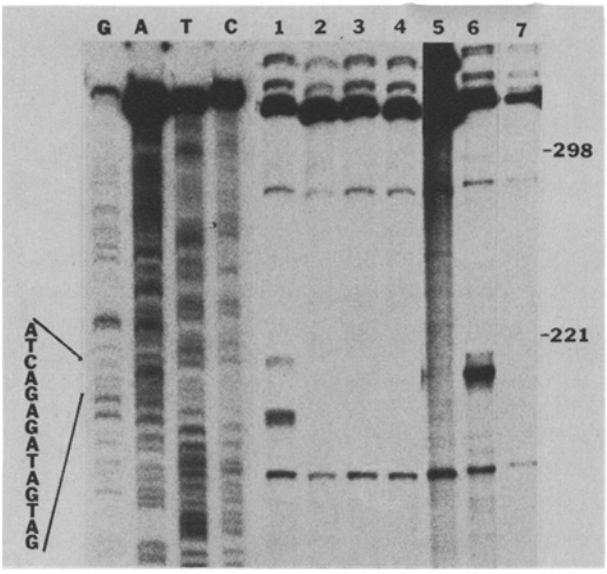

Fig. 3B. S1 Nuclease Analysis of RNAs synthesized *in vitro* and *in vivo*. RNAs were synthesized as in Fig. 2 using Hinf I cleaved pZG15A as a template. Purified RNAs were hybridized to the radiolabeled EcoRI-Dde I fragment shown in Fig. 3A and incubated with S1 nuclease as described in Materials and Methods. Lanes GATC depict a sequencing ladder generated by Maxam-Gilbert sequencing of the radiolabeled fragment. This sequence is complementary to the sequence in Fig. 1. This ladder shows the sequence of the complementary strand of DNA in Fig. 1A. Lanes 1 and 3 show the RNA pattern from the *in vitro* transcription in the presence (Lane 3) and absence (Lane 1) of α-amanitin. In Lane 2, pBR322 DNA cleaved with Hinf I was used and in Lane 4, no exogenous DNA was added to the transcription reaction. RNA (1μg) isolated from maize kernels was used in the analysis in Lanes 5 and 6. Lane 5 was not subjected to treatment with nuclease. Lane 7 contained no RNA during the hybridization. The numbers on the right refer to the migration of denatured, radiolabeled DNA markers. The dark band migrating just slower than the 298 marker is reannealed probe DNA.

HeLa extract indicated the presence of a second start site not present in the endosperm RNA preparation used as a control, transcripts were further analyzed with a primer extension assay. *In vitro*- and *in vivo*-synthesized RNAs were hybridized to a heat denatured 56 base pair restriction fragment 5' radiolabled at a Dde I site (position + 169). The radiolabeled primer was then extended using the RNA as a template for reverse transcriptase, and the resulting cDNAs were analyzed on a denaturing polyacrylamide gel. Multiple 5' ends were generated with RNAs transcribed both *in vitro* and *in vivo* (Fig. 4B). The RNA termini mapped to the same region of DNA with this assay as with the S1 nuclease protection assay. In the primer extension assay, however, several start sites were detected with both *in vivo*- and *in vitro*-synthesized RNAs. These data suggest that while in general, the HeLa extract seems to correctly initiate transcription, precise comparison of *in vitro* and *in vivo* start sites is limited by the mapping techniques available.

In addition to the major cDNA synthesized from the endosperm mRNA template, a minor species was also detected. This cDNA maps to a position approximately 100 bases upstream of the major start and corresponds to the end of a cDNA clone that has been isolated and sequenced (21). This start is not found among *in vitro* transcripts even upon extensive overexposures of the denaturing gel. Attemps to reproduce this transcript by transcribing DNA fragments containing only sequences upstream from the major start site have also been unsuccessful (data not shown). Thus the absence of *in*

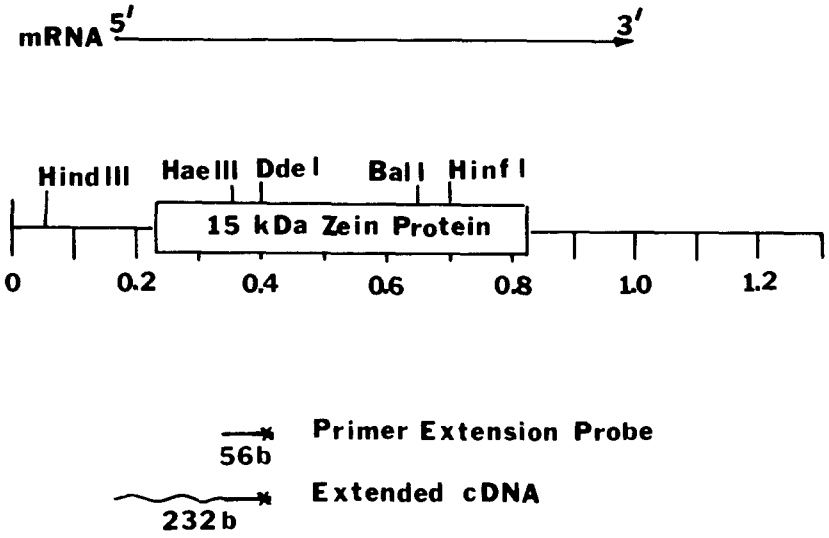

Fig. 4A. Schematic representation of pZG15A showing probe and expected products of primer extension assay.

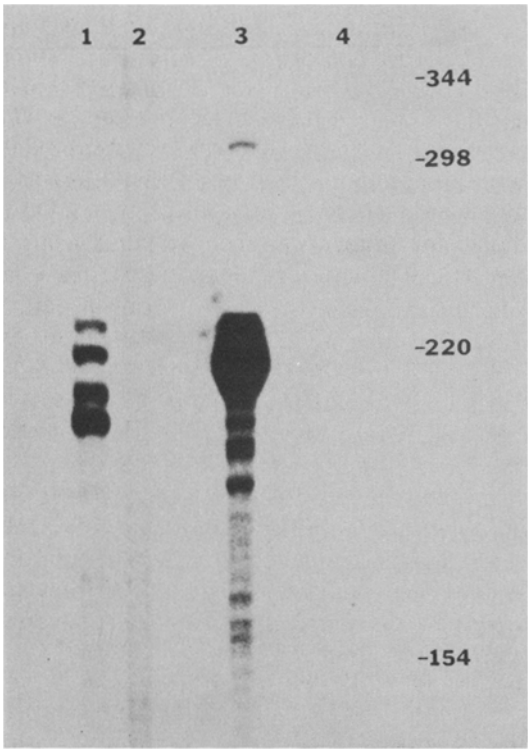

Fig. 4B. Primer Extension analysis of *in vivo* and *in vitro* transcripts. RNAs synthesized as in Fig. 3 were annealed to a radiolabeled Dde I-Hae III restriction fragment. Complementary DNA was extended from the primer using the RNA as a template (see Materials and Methods for details). An autoradiogram of cDNA synthesized from the *in vitro* made template is shown in Lanes 1 and 2 and cDNA from *in vivo* RNA in Lane 3. The reaction products in Lane 2 were made in the presence of α-amanitin. tRNA was used as a template in Lane 4. The position of radiolabeled molecular weight markers is shown at right.

vitro initiation at this site is apparently not due to saturation of transcription specificity factors by the stronger downstream promoter.

Discussion

Specific transcription of plant genes *in vitro* using a nuclear extract from HeLa cells indicates a cross-kingdom conservation of at least some elements required for specificity of initiation. Both plants and animals have consensus CAAT and TATA sequences near the 5′ ends of most genes analyzed to date. The TATA sequence is considered to be necessary for specific initiation *in vitro* as determined by specific mutagenesis or deletion of certain bases within this sequence (3, 6, 7, 18, 28). At least one exception has been noted, however, in the case of the Adenovirus IVa transcript (26, 17).

The data presented here indicate the presence of correct but not unique start sites for transcription in an *in vitro* system of a genomic clone encoding a 15 kDa zein protein. Others have reported initiation of transcription for a gene encoding a 22 kDa zein using a whole-cell extract from HeLa cells (14) but their assay would not have distinguished two or more starts in close proximity to each other. They did, however, observe two similarly-sized bands protected by S1 nuclease using RNA isolated from maize kernels.

From the data shown here, one cannot distinguish between separate initiation events that produce multiple 5′ ends of a mRNA and multiple ends artifactually generated as a result of S1 map-

ping or primer extension. Detection of double start sites for several zein mRNA classes certainly strengthens the argument against the double starts being artifacts of the mapping technique. In other experiments zein DNAs have been introduced into *A. tumifaciens* by T. DNA vectors and the resulting bacteria inoculated onto sunflower stem sections. S1 analysis of tumor RNAs have indicated double starts for a 19 kDa zein gene[4] (23). Likewise, our results indicate that transcription *in vitro* is due to initiation events in very close proximity to the map positions of the 5′ ends of RNA found *in vivo*.

It has been reported that genes encoding a 22 kDa and a 19 kDa zein protein are transcribed from two widely separated promoter regions – one near the 5′ end of the coding sequences and the other approximately 1000 bases upstream (14, 15). The double promoter activity for the 22 kDa zein gene was observed both *in vitro* and *in vivo*, with the upstream promoter being the more active *in vitro*. Although a minor RNA species encoding a 15 kDa zein maps approximately 90 bases upstream of the major 5′ end in a primer extension assay (Fig. 4B), this species was not detected *in vitro* even in the absence of the downstream promoter region. In addition, no RNA products able to protect DNA further upstream of the major promoter have been found by S1 analysis using probes extending beyond the zein sequence.[3] This suggests that transcription of the 15 kDa zein gene both *in vitro* and *in vivo* is not analogous to that reported for a 2 kDa gene (14).

A simple explanation for the different transcriptional start sites of the 15 kDa zein is the presence of two copies of similar but not identical genes in the maize genome. Reconstruction analyses of genomic Southern hybridization indicated that 1–3 genes encoding 15 kDa zeins are present per haploid maize genome (29). The assays used in this study would not distinguish between the possibility of two similar genes that are homologous within the coding region but diverged beyond the main start site and one in which are two starts from a single gene. Absence of correct transcription signals upstream of the minor start could be responsible for the lack of transcription *in vitro* as the genomic clone used in these studies contains only 75 base pairs of maize DNA sequence 5′ to the minor start. Several additional genomic clones encoding 15 kDa zeins have now been isolated and further characterization of these may prove the existence of a second gene.

Although the 15, 19, and 22 kDa zeins show similar temporal regulation of RNA and protein accumulation, they differ in other ways. The 19 and 22 kDa size classes are encoded by large multigene families of approximately 50 and 25 genes, respectively. They also have regions of homology and contain a repeated nucleotide sequence (1, 20). The 15 kDa zeins have no sequence homology to either of the 19 and 22 kDa classes and do not have regions with a repeated DNA sequence (20). In addition, the coding sequence of the 15 kDa zein is more GC-rich (55%) than the 19 and 22 kd zeins (38% GC).

The 15 kDa zeins are encoded by only one to three gene copies, and the corresponding mRNA accounts for 10% of the mRNA in the endosperm at 18 days after pollination (21). Like the 15 kDa zeins, the other two classes of zeins also have increased levels of RNA during endosperm development, but the amount per gene is less than the 15 kDa zein class. The higher level of mRNA for the 15 kDa protein implies enhanced transcription, although an increased stability of mRNA for 15 kDa zeins cannot be ruled out.

Mutations in strains of maize show differential effects on expression of various zein groups. In *opaque*-2 mutants the 22 kDa component is greatly reduced while *opaque*-7 mutants are deficient in the 19 kDa component (8). Floury-2 mutants have decreased levels of all three zeins groups (16). It seems likely that the three classes of zeins have common and unique modes of regulation. More refined *in vitro* transcription assays may provide further insight into regulation of zein gene transcription since such analyses provide a direct means of comparing transcriptional activities of various classes of zein genes.

Acknowledgements

We thank P. Gutay for excellent technical assistance; J. Lindell, C. Logan and M. Coffey for help in preparing the manuscript; N. Jones and J. Williams for their generous gift of HeLa cells; and A. Jackson, R. Cuellar, R. Hanau and P. Goldsbrough for critically reading the manuscript.

Note added in proof

Pedersen *et al.*, manuscript in preparation is now in press in J. Biol. Chem. Goldsbrough *et al.* is now in press in Mol. Gen. Genet.

References

1. Argos P, Pedersen K, Marks MD, Larkins BA: A structural mold for maize zein proteins. J Biol Chem 257:9984 – 9990, 1982.
2. Berk AJ, Sharp PA: Sizing and mapping of early adenovirus mRNAs by gel electrophoresis of S1 endonuclease-digested hybrids. Cell 12:721 – 732, 1977.
3. Brady J, Radonovich M, Vodkin M, Natarajan V, Thoren M, Das G, Janik J, Salzman NP: Site-specific base substitution and deletion mutations that enhance or suppress transcription of the SV40 major late RNA. Cell 31:625 – 633, 1982.
4. Carlson DP, Ross J: Human β-globin promoter and coding sequences transcribed by RNA polymerase III. Cell 34:857 – 864, 1983.
5. Clewell DB: Nature of ColE, plasmid replication in *Escherichia coli* in the presence of chloramphenicol. J Bacteriology 110:667 – 676, 1972.
6. Concino M, Goldman RA, Caruthers MH, Weinmann R: Point mutations of the adenovirus major late promoter with different transcriptional efficiencies *in vitro*. J Biol Chem 258:8493 – 8496, 1983.
7. Concino M, Lee RF, Merryweather JP, Weinmann R: The adenovirus major late promoter TATA box and initiation site are both necessary for transcription *in vitro*. Nuc Acids Res 12:7423 – 7433, 1984.
8. DiFonzo N, Fornesari E, Salamini F, Reggiani R, Soave C: Interaction of maize mutants *floury*-2 and *opaque*-7 with *opaque*-2 in the synthesis of endosperm proteins. J Heredity 71:397 – 402, 1980.
9. Dignam JD, Lebovitz RM, Roeder RG: Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. Nuc Acids Res 11:1475 – 1488, 1983.
10. Fourcroy, P: Isolation of undegraded polysomes from radish cotyledons: use of protease K and cycloheximide. Phytochem 19:7 – 10, 1980.
11. Ghosh PK, Reddy VB, Swinscoe J, Choudary PV, Lebowitz P, Weissman SM: The 5′-terminal leader sequence of late 16S mRNA from cells infected with Simian virus 40. J Biol Chem 253:3643 – 3647, 1978.
12. Hagen G, Rubenstein I: Complex organization of zein genes in maize. Gene 13:239 – 249, 1981.
13. Kaplan BB, Bernstein SL, Gioio AE: An improved method for the rapid isolation of brain RNA. Biochem J 183:181 – 184, 1979.
14. Langridge P, Feix G: A zein gene of maize is transcribed from two widely separated promoter regions. Cell 34:1015 – 1022, 1983.
15. Langridge P, Eibel H, Brown JWS, Feix G: Transcription from maize storage protein gene promoters in yeast. EMBO Journal 3:2467 – 2471, 1984.
16. Lee KH, Jones RA, Dalby A, Tsai CY: Genetic regulation of storage protein synthesis in maize endosperm. Biochem Genetics 14:641 – 651, 1976.
17. Leong K, Flint SJ: Specific transcription of an adenoviral gene that possesses no TATA sequence homology in extracts of HeLa cells. J Biol Chem 259:11527 – 11533, 1984.
18. Luse DS, Haynes JR, VanLeeuwen D, Schon EA, Cleary ML, Shapiro SG, Lingrel JB, Roeder RG: Transcription of the β-like globin genes and pseudogenes of the goat in a cell-free system. Nuc Acids Res 9:4339 – 4354, 1981.
19. Manley JL, Fire A, Cano A, Sharp PA, Gefter ML: DNA-dependent transcription of adenovirus genes in a soluble whole-cell extract. Proc Natl Acad Sci USA 77:3855 – 3859, 1980.
20. Marks MD, Lindell JS, Larkins BA: Nucleotide sequence analysis of zein mRNAs from maize endosperm. J Biol Chem 260:16451 – 16459, 1968a.
21. Marks MD, Lindell JS, Larkins BA: Quantitative analysis of the accumulation of zein-mRNA during maize endosperm development. J Biol Chem 260:16445 – 16450, 1986b.
22. Marks MD, Pedersen K, Wilson DR, DiFonzo N, Larkins BA: Molecular biology of the maize seed storage proteins. In: Current Topics in Plant Biochemistry and Physiology 3:9 – 18, 1984.
23. Matzke MA, Susani M, Binns AN, Lewis ED, Rubenstein I, Matzke AJM: Transcription of a zein gene introduced into sunflower using a Ti plasmid vector. EMBO Journal 3:1525 – 1531, 1984.
24. Maxwell IH, Maxwell F, Hahn WE: Removal of RNase activity from DNase by affinity chromatography on agarose-coupled aminophenyl-phosphoryl-uridine2′(3′)-phosphate. Nue Acids Res 4: 241 – 246, 1977.
25. Murphy JT, Burgess RR, Dahlberg JE, Lund E: Transcription of a gene for human U1 small nuclear RNA. Cell 19:265 – 273, 1982.
26. Natarajan V, Madden MJ, Salzman NP: Preferential stimulation of transcription from Simian virus 40 late and Adeno IVa_2 promoters in a HeLa cell extract. J Biol Chem 258:14652 – 14655, 1983.
27. Salamini F, Soave C: Zein: Genetics and biochemistry. In: Sheridan WF (ed) Maize for Biological Research. 1982, pp 155 – 160.
28. Wasylyk B, Chambon P: A T to A base substitution and small deletions in the conalbumin TATA box drastically decrease specific in vitro transcription. Nuc Acids Res 9:1813 – 1825, 1981.
29. Wilson D, Larkins BA: Zein gene organization in maize and related grasses. J Mol Evol 20:330 – 340, 1984.

Received 14 January 1986; in revised form 18 April 1986; accepted 23 April 1986.