Accumulation and subcellular localization of glutelin-2 transcripts during maturation of maize endosperm

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The expression of glutelin-2 genes has been studied during maize endosperm maturation and compared to the expression of two different zein genes. Glutelin-2 mRNA in endosperm accumulates in parallel to zein mRNAs, nevertheless, the opaque-2 mutation does not affect glutelin-2 transcription. Subcellular fractionation of endosperm tissue shows that glutelin-2 mRNA is accumulated in the protein body fraction indicating that these organelles are the place of translation of glutelin-2 mRNA. The results indicate that the levels of glutelin-2 transcripts in maize endosperm increase following a temporal pattern similar to that displayed by zein transcripts, the typical maize storage protein.

RNA (Maize endosperm) Glutelin Northern blot analysis Protein body Cereal storage protein

1. INTRODUCTION

Glutelins are the second largest group of proteins in maize endosperm, contributing from 35 to 45% of its protein content [1]. A subfraction named glutelin-2 (G2) and defined by its solubility in alkaline buffers, contains a major component running in electrophoresis as a broad band with an apparent molecular mass of 28 kDa [2]. Our laboratory has recently reported the complete sequence of a 28 kDa G2 protein as derived from the nucleotide sequence of its cDNA clone [3]. Unlike zeins, its amino acid sequence shows homology with storage proteins from other cereals.

It is generally accepted that zeins constitute the maize storage proteins but little is known about the function of the glutelin fraction. In this respect, the possibility that glutelins also fulfil a structural function in protein bodies (storage vesicles) has been indicated [4]. To establish the relations between these two types of proteins it is interesting to compare them at the level of the accumulation of their mRNA during endosperm development.

We present the analysis of glutelin-2 expression during endosperm maturation. Two types of measurements have been made. First, the localization of glutelin-2 mRNA in the protein body subcellular fraction. Second, a comparative study of G2 mRNA accumulation in endosperm with relation to several zein mRNAs. We show that, although the synthesis of the two groups of mRNA occurs in a coordinate way during endosperm maturation, the opaque-2 mutation has no effect on the transcription levels of glutelin-2.

2. MATERIALS AND METHODS

2.1. *Biological material and subcellular fractionation*

Maize kernels were collected in the field at several stages after pollination, frozen immediately in liquid air and stored at −70°C. The maize lines studied were the W64A inbred line and its corresponding opaque-2 mutant W6402.

Subcellular fractionation of immature maize endosperm (20 days after pollination from E-10 variety) was carried out by centrifugation in 20–70% sucrose gradients as described by Ludevid et al. [4]. 1 ml fractions were pooled according to their cytoplasmic content [5]. RNA was extracted

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as detailed in the next section and dissolved in 0.2 ml of sterile water.

2.2. *Preparation and analysis of RNA*

RNA was isolated as described in [6]. The RNA obtained was cleaned of DNA and polysaccharides by precipitation in 2 M LiCl and 4 M urea. RNA concentration was determined by alkaline hydrolysis.

For Northern blot analysis RNA (15 μg) was separated by electrophoresis in 1.5% agarose and 2.2 M formaldehyde gels as described [7]. RNA was transferred to nitrocellulose (BA85, Schleicher and Schuell) membranes [8]. For dot blot analysis 5 and 10 μl RNA from the different subcellular fractions were dotted onto nitrocellulose membranes using a multidot device (BRL, Bethesda) [8]. Immobilized RNA was hybridized with ^{32}P-labelled DNA probes essentially as described by Alwine et al. [9] for 24 h at 65°C in 25 mM phosphate buffer, pH 7.5, 3 × SSC, 3 × Denhardt's and 0.1% SDS. The filters were washed in 0.3 × SSC and 0.1% SDS, dried and exposed at −70°C to MAFE RPA film with intensifying screen. Autoradiographs of equivalent contrast were scanned in a Joyce Loebl Chromoscan 3 microdensitometer. The integral value of the signal peak was plotted in the accumulation graphics. The units in the *y* axis correspond to relative amounts taking as 100% the maximal value obtained for each probe.

3. RESULTS AND DISCUSSION

3.1. *Measurement of glutelin-2 mRNA during endosperm development*

The accumulation of G2 transcripts in maize seed development was examined by Northern blot hybridization using clone pME 119 as a probe [3]. This clone corresponds to the cDNA of a 28 kDa glutelin as observed by hybrid selection and sequencing. Fig.1 shows the results obtained in such an analysis running total and poly(A)$^+$ endosperm RNA. The glutelin-2 probe hybridizes to a band migrating at 1140 nucleotides. When the same analysis is carried out in an opaque-2 variety of the same line (W6402) no difference in the size of the transcript can be observed (fig.1).

Glutelin-2 (28 kDa) mRNAs are detectable in maize endosperm from day 10 postpollination.

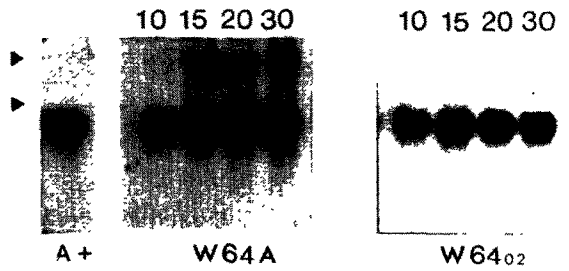

Fig.1. Hybridization of glutelin-2 cDNA probe to electrophoretically fractionated RNA from maize endosperm extracted at different days postpollination. The arrows mark the position of maize rRNAs. The results for W64 inbred line and its opaque-2 mutant are shown. A sample of poly(A)$^+$ RNA from day 20 postpollination is included.

Their concentration increases reaching a maximum at 20 days and it decreases when the process of seed drying begins. To provide a more quantitative analysis of the G2 mRNA accumulation the results obtained are presented in graphic plots elaborated as described in section 2. Fig.2 shows these results, including also those obtained for the two major storage proteins mRNAs: the 19 kDa zein (A20 cDNA probe) and the 22.5 kDa zein (B49 cDNA probe, both probes kindly provided by Drs F. and B. Burr, Brookhaven) [10]. Taking into account the similar specific activities of our labelled probes and the exposure times we have estimated that the level of G2 messages is of the order of 20% that of the 19 kDa zein messages in normal W64A endosperms.

The three species of mRNA studied show a similar rate of accumulation in the first stages of endosperm development, with the maximum levels observed at days 15–20 postpollination. This observation agrees with the rate of accumulation of the protein as measured by analysis of the different components by protein gel electrophoresis [4], indicating that the main control mechanism in the expression of these genes in endosperm is transcriptional. The level of 22 kDa zein mRNA remains significantly high at 30 days after pollination. The different behaviour of these mRNAs could be interpreted as a consequence of specific hydrolysis of some transcripts associated with seed maturation [11], however we have not detected any sign of this activity in the Northern analysis since

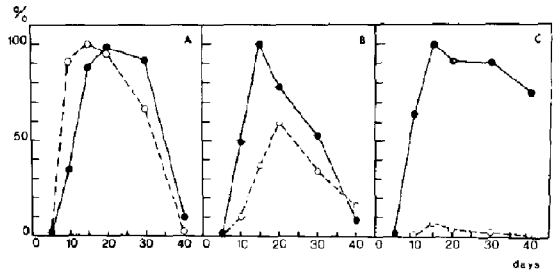

Fig.2. Accumulation of mRNA for three major endosperm polypeptides during seed development. The bottom line represents days postpollination, and the relative amount of each transcript as percentage of the maximum level is represented in ordinates. (A) 28 kDa glutelin-2 mRNA, probe pME119. (B) 19 kDa zein mRNA, probe A20. (C) 22 kDa zein mRNA, probe B49. Normal (●) and opaque-2 (○) genotypes are shown for each probe.

no differential splitting or spreading of the hybridization bands was ever observed for the different probes used.

The transcription of 28 kDa G2 mRNA is not affected by the opaque-2 mutation. The same amount of mRNA is present in both genotypes, the only difference is that in the opaque there is already 90% of the maximal level at day 10 after pollination. The overall accumulation profile is similar for the normal and the opaque-2 genotypes except that the latter is advanced for 5 days. This is probably due to the major relative abundance of G2 messages in the early stages of opaque-2 endosperm development since zein mRNAs are underrepresented. Our results suggest that zein and glutelin genes share common regulatory features because both are expressed in the same tissue and at the same stage of endosperm maturation. However other regulatory levels differ in both genes as mutations such as opaque-2 produce an effect in at least some zein genes but not in glutelin-2.

3.2. *Subcellular localization of G2 mRNA*

Sucrose gradient sedimentation analyses of cellular homogenates were performed to locate the G2 mRNAs at the subcellular level. The different fractions (fractions 1–5) were pooled following the described distribution of cytoplasmic materials [6]. Aliquots of the different pooled fractions were screened for the presence of G2 messages by dot blot analysis (table 1). More than 70% of the signal was detected in the protein body fraction (fraction 5). The free polyribosome, membrane and mitochondrial fractions give low hybridization signals attributed to broken or different sized protein bodies. The same result was obtained for the zein messages (A20) considered as protein body markers [5]. We can therefore conclude that G2 mRNA is attached to polyribosomes bound to the protein body membrane. This result is consistent with the fact that the G2 mRNA contains in its coding region an N-terminal signal peptide characteristic of secreted proteins [3] and the localization of glutelin-2 polypeptides in the inner part of the protein body membrane [4].

Table 1

Distribution of specific RNA sequences in subcellular fractions of maize endosperm

Fraction number	Nucleic acid content (%)	28 kDa glutelin RNA (%)	19 kDa zein RNA (%)
1	30	2	9
2	21	10	16
3	28	6	8
4	5	4	7
5	16	78	60

Fractions: 1, soluble cytoplasm; 2, membrane fraction; 3, mitochondrial fraction; 4, endoplasmic reticulum; 5, protein bodies. 28 kDa glutelin-2 mRNA was probed with pME119 and 19 kDa zein mRNA with A20 cDNA clones. The values are expressed as percentage of the total signal observed

In a recent paper we reported the sequence homologies between glutelin-2 and other cereal storage proteins as wheat a/b-gliadins and b-hordeins from barley [3]. These homologies point to maize glutelin-2 as a member of the typical cereal storage protein family. In maize, zeins have displaced this type of polypeptides as the main storage proteins. In this context the results presented above will sustain this view since glutelin-2 transcripts are synthesized during maize endosperm development, they are not sensitive to the opaque-2 mutation that reduces the levels of zein mRNAs, and present a subcellular localization typical of cereal storage products. The advantage of zeins over glutelins in evolution might have

come either from structural features of the proteins favouring a better packing in the protein body or from the existence of several starting signals for transcription in zeins [13] that have not been found in glutelin-2 mRNAs.

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