Reduced Synthesis of Zein In Vitro by a High Lysine Mutant of Maize.

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SUMMARY

Membrane-bound polyribosomes from normal maize and the opaque-2 mutant synthesized proteins in vitro which were similar to native zein in ethanol solubility and mobility in sodium dodecyl sulfate polyacrylamide gels. Unique size classes of large membrane-bound polyribosomes in normal maize were absent in the mutant. Correspondingly, there was a marked reduction in the synthesis of one of the major zein components by the mutant.

INTRODUCTION

Zein, a major storage protein in the endosperm of maize, has been separated into two major components (1). This protein comprises approximately 60% of the total endosperm protein (2). Mutation at the opaque-2 locus dramatically reduces zein levels while permitting secondary increases in non-zein proteins which are nutritionally superior (3). This finding has stimulated interest in the selection of mutants which enhance the protein quality of cereal grains. Although changes in the endosperm protein fractions resulting from this mutation have been extensively characterized, the mechanism controlling the suppression of zein accumulation is unknown.

Recently we demonstrated the preferential synthesis of zein by membrane-bound polyribosomes isolated from normal maize (4, 5). The opaque-2 mutant is deficient in one of the major zein components, Z1 (1). It is not evident whether the opaque-2 locus inhibits the synthesis of the Z1 component or affects its turnover. Attempts were made to establish whether

the mutant polyribosomes can support the synthesis of zein components in vitro. This communication is the first report of the localization and characterization of a regulatory effect of the opaque-2 gene on zein synthesis.

MATERIALS AND METHODS

Intact kernels of normal maize inbred line (W22) and its isogenic opaque-2 mutant (W22o_2) were obtained from ears initially frozen in liquid nitrogen to retain polyribosome integrity (5). Free and membrane-bound polyribosomes were prepared according to the procedure of Larkins et al. (4, 5).

In vitro protein synthesis by maize polyribosomes was carried out in a cell-free system derived from wheat germ (6). The incubation mixture contained in a final volume of 0.28 ml: 20 ug tRNA; 0.12 ml wheat germ S100[1]; 48 mM KCl; 4 mM Mg-acetate; 1 mM ATP; 35 uM GTP; 11 mM creatine phosphate; 16 mg creatine phosphate kinase; 35 mM Tris-acetate, pH 8.0; 3 mM dithiothreitol; 45 uM each of 19 amino acids: 0.125 uCi of [^{14}C] -leucine (364 mCi/mM, Schwartz Bioresearch, Inc.); and 1 to 2 A260 units of polyribosomes. Assays were incubated at 30° C for 20 min. The total protein (hot 5% CCl_3COOH-insoluble) and zein (hot 70% ethanol-soluble) were prepared according to the procedures of Mans and Novelli (7) and Dalby (8), respectively.

The proteins synthesized in vitro were further characterized by Na Dod SO_4-polyacrylamide gel electrophoresis (1). Total protein and the zein fraction were solubilized in 1% Na Dod SO_4 and dialyzed against Na Dod SO_4 buffer (50 mM Tris-Hcl, pH 6.9; 0.5% NaDod SO_4; and 1% 2-mercaptoethanol) for 12 hr prior to electrophoresis. Zein was purified from mature kernels of normal maize and the opaque-2 mutant for use as standards.

RNA extractions were made by homogenizing subcellular fractions in buffer (pH 9.4) containing 0.1M glycine, 0.3M NaCl and 50 mM K_2HPO_4 (9). The homogenate was centrifuged at 37,000 x g for 15 min and the supernatant layered over a linear 7.5% to 30% sucrose gradient in the same buffer. Gradients were centrifuged at 189,000 x g (avg) for 4 hr in a Beckman SW50.1 rotor, then scanned at 254 nm. The amount of ribosomal material present was determined by planimetry.

RESULTS AND DISCUSSION

Representative density gradient profiles of free polyribosomes from normal (Fig. 1A) and from opaque-2 (Fig. 1B) are similar, with a maximum absorbance peak at the 9 or 10-mer. Profiles of membrane-bound polyribosomes (bound polyribosomes) from normal maize contain several unique size-classes

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[1] Abbreviations: S100 is a post 100,000 x g supernatant from wheat germ; membrane-bound polyribosome, bound polyribosome.

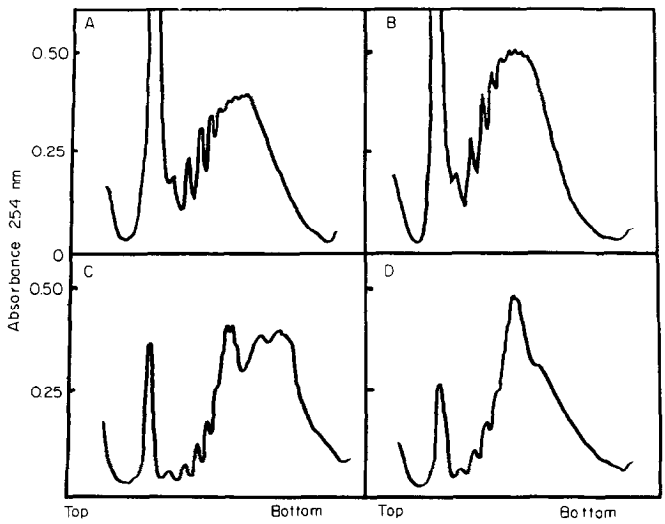

Figure 1.

Free and initially membrane-bound polyribosomes from 22-day post-pollinated kernels of normal maize (A, 3.17 A260 units of free polyribosomes; C, 2.85 A260 units of membrane-bound) and opaque-2 mutant (B, 4.15 A260 units of free polyribosomes; D, 2.25 A260 units of membrane-bound polyribosomes).

(5). One major polyribosome peak is at the 8 to 9-mer, while two other large polyribosome size-classes are present which sediment deeper into the gradient (Fig. 1C). The mutant bound polyribosome profile (Fig. 1D), while similar to normal in having a major polyribosome peak at the 8-mer, is strikingly reduced in the larger polyribosome size-classes (Fig. 1D). The large size-classes of bound polyribosomes isolated from normal were retained when normal and opaque-2 kernels were co-extracted or when the normal bound polyribosomes were incubated in the post ribosomal supernatant from opaque-2 (data not shown). Therefore, the large size-classes appear to have a genetic basis.

The free and bound polyribosomes from normal and opaque-2 synthesized protein in vitro (Table 1). The bound polyribosomes are about 2 to 3 times more active than the free polyribosomes in the incorporation of [^{14}C] -leucine

TABLE I. [^{14}C]-Leucine Incorporation into Protein Synthesized by Free and Membrane-Bound Polyribosomes from Kernels of Normal Maize and Opaque-2 Mutant.

Genotype	Polyribosome	Total Acid-Insoluble	Ethanol-Soluble	Ethanol-Soluble / TCA-insoluble
		CPM/A260/hr	CPM/A260/hr	Ratio
Normal	Free	8318	1249	0.15
	Membrane-bound	16054	7961	0.50
Mutant	Free	5869	1162	0.20
	Membrane-bound	13056	4439	0.34

The radioactive values are expressed as the mean of triplicate assays from three experiments.

into acid-insoluble protein. Approximately 50% of the radioactive protein synthesized by the bound polyribosomes of normal maize was ethanol soluble, while only 15% of the protein synthesized by the normal free polyribosomes was ethanol soluble. In terms of absolute amounts, ethanol soluble protein synthesized by the free polyribosomes made a minor contribution relative to that synthesized by the bound polyribosomes. We do not know whether the ability to synthesize small amounts of ethanol soluble protein by free polyribosomes is a reflection of the *in vivo* state or an artifact of the polyribosome isolation procedure. In contrast, on an equal A250 unit basis, only 34% of the total protein synthesized by the bound polyribosomes of *opaque*-2 was ethanol soluble. This represents a 30% reduction in ethanol soluble protein synthesized by the mutant from that of the normal control. Furthermore, the mutant reduces the amount of membrane-bound ribosomal

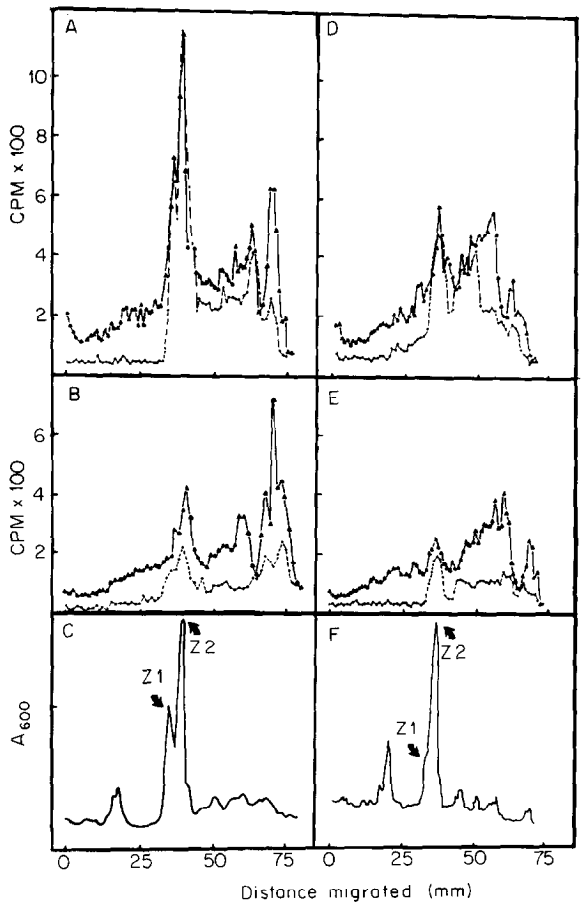

Figure 2.

Na Dod SO_4-disc gel electrophoresis of ^{14}C -leucine labelled products synthesized by membrane-bound (A, D) and free polyribosomes (B, E) isolated from normal (A, B) and opaque-2 (D, E): (▲), hot acid-insoluble; and (●), hot 70% ethanol-soluble protein. Figures 2-C and F represent the zein standards prepared from normal maize and the mutant, respectively, which were stained with coomassie blue and scanned at 600 nm.

material by 27% on a kernel basis (20.2 planimeter units versus 27.8 units for normal). On a kernel basis, the opaque-2 mutant synthesized approximately 37% of the normal zein levels in vitro, which agrees with the levels of zein in vivo at this developmental stage (10).

Na Dod SO_4-disc gel electrophoresis of the [^{14}C]-leucine labelled protein determined whether the opaque-2 mutant reduced the in vitro synthesis of the zein components proportionately, or reduced a particular component. For comparison, purified zein preparations were electrophoresed as standards (Fig. 2-C and F). The bound polyribosomes of normal maize predominantly synthesize the two major zein components (Fig. 2A). Ethanol soluble protein was synthesized to a lesser extent by free polyribosomes (Fig. 2B).

The opaque-2 mutant, which is deficient in the Z1 component (Fig. 2-F), also reduced the synthesis of this component in vitro (Fig. 2-D and E). The reduction of zein synthesis in vitro by the mutant polyribosomes suggests that the 'availability' of zein mRNA is decreased. The fact that zein is synthesized predominantly by bound polyribosomes in normal and opaque-2, while the mutant dramatically reduced the large bound polyribosomal size-classes, suggests that these classes contain zein mRNA. The possibility that the large, unique polyribosomal size-class might specifically support the synthesis of the Z1 component is being investigated.

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