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Synthesis and processing of maize storage proteins in *Xenopus laevis* oocytes

(signal peptide/seed protein/zein mRNA)

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ABSTRACT *Xenopus* oocytes injected with zein mRNAs efficiently synthesized maize storage proteins for prolonged periods. Under optimal conditions, zein was synthesized at 3 ng/hr and represented approximately 10% of the total protein synthesized in the oocyte. The mRNA from the normal maize inbred line directed synthesis of all the major zein components; however, products of mRNA from the *opaque*-2 mutant did not contain the largest zein component. Zein proteins synthesized in the oocyte were 2000 daltons smaller than proteins synthesized by cell-free translation of mRNAs in the wheat germ and reticulocyte systems. This result, which suggested that the oocyte processed prezein polypeptides into native zein proteins, was confirmed by amino-terminal sequence analysis of zein proteins from the oocytes. Cyanogen bromide cleavage of translation products from oocytes and the wheat germ system confirmed the existence of several proteins within each of the major zein components. However, we were unable to detect the presence of signal sequences on zein peptides by this technique.

Zein, the major storage protein fraction in maize (*Zea mays* L.) seed, consists of a group of ethanol-soluble proteins that are localized in protein bodies. Sodium dodecyl sulfate ($NaDodSO_4$)/polyacrylamide gel analysis of zein proteins reveals two major components of M_r 22,000 and 19,000 and small amounts of 15,000 and 10,000 M_r proteins (1, 2). When these proteins were analyzed by isoelectric focusing between pH 5 and 9, as many as 28 different species were resolved (3), indicating charge heterogeneity among zein polypeptides. A structural basis for at least part of this heterogeneity was demonstrated by amino-terminal sequence analysis of the major zein components from the inbred line W64A (4), where each of the major components gave a minimum of two different amino-terminal sequences.

During development of the maize seed, zein proteins are synthesized in the endosperm, where they form protein bodies within the rough endoplasmic reticulum (RER) (5). mRNAs that direct the synthesis of zein proteins have been isolated from polysomes of the RER, and in the wheat germ cell-free system these mRNAs direct the synthesis of proteins that are 2000 daltons larger than native zein proteins (5–7). However, when intact RER vesicles are placed in the wheat germ system, proteins of the same molecular weight as native zein proteins are synthesized (5). These observations suggest that these plant storage proteins, like animal secretory proteins, contain signal peptides that direct their synthesis into the RER (8).

Xenopus laevis oocytes efficiently translate heterologous mRNAs (9–11) and can process signal sequences when mRNAs from animal cells that direct the synthesis of preproteins are microinjected (12–14). To our knowledge similar studies with plant preprotein mRNAs have not been reported nor, for that matter, have any plant proteins with signal peptides been identified. In this communication we report experiments to determine whether maize seed storage proteins can be synthesized in *Xenopus* oocytes and whether the signal peptides associated with them are processed.

MATERIALS AND METHODS

Preparation of mRNA. Zein mRNA was isolated from frozen kernels of the maize inbred line W64A and its *opaque*-2 mutant as described (5). Polyadenylylated RNA from membrane-bound polysomes of kernels 22 days after fertilization was fractionated on linear logarithmic sucrose gradients to remove contaminating 18S rRNA, and RNA sedimenting at 13 S was precipitated with 2 vol of absolute ethanol. The RNA was later pelleted by centrifugation and dissolved in sterile H_2O.

Preparation and Injection of Oocytes. *X. laevus* were obtained from South Africa and maintained as described (15). Ovaries were removed from frogs anesthesized by hypothermia, and oocytes were defolliculated manually in sterile Ringer's solution. Stage-six oocytes were transferred to Eppig and Dumont's medium (16) and, after "equilibration" for 1–2 hr, were microinjected individually with 20 nl of mRNA dissolved in sterile H_2O or Ringer's solution. After an additional 24 hr of incubation, the mRNA-injected oocytes were injected with 20 nl (0.4 μCi; 1 Ci = 3.7×10^{10} becquerels) of [^{3}H]leucine (Amersham) or [^{3}H]leucine plus [^{14}C]isoleucine (0.04 μCi, Amersham). The label was prepared by addition of 0.25 μmol of carrier leucine or isoleucine to 1 ml of [^{3}H]leucine (1 mCi/ml). After evaporation to dryness in a desiccator, the sample was dissolved in 50 μl of sterile water.

Measurement of Protein Synthesis and Amino Acid Pool Size. Incorporation of [^{3}H]leucine into total protein was determined by placing two oocytes in hot (60°C) 0.5 M perchloric acid for 30 min, followed by a 30-min rinse with distilled H_2O (17). The acid-extracted oocytes were then dissolved in NCS solubilizer (New England Nuclear) and radioactivity was determined in a Beckman LS 230 liquid scintillation counter after addition of 10 ml of Omnifluor (New England Nuclear). For measurement of incorporation into zein proteins, two oocytes were homogenized in 0.25 ml of 70% ethanol containing 1% 2-mercaptoethanol. After extraction at 60°C for 10 min, insoluble proteins were precipitated by centrifugation in a Sorvall-SS 34 rotor at 6000 rpm for 10 min. Aliquots of the ethanol-soluble proteins were spotted on Whatman 3MM filter papers and hot trichloroacetic acid-insoluble radioactivity was determined (18).

The endogenous pool of leucine in oocytes incubated for 24 hr in Eppig and Dumont's medium (16) was determined by analyzing the cold 30% trichloroacetic acid-soluble extract of 400 manually defolliculated oocytes. After the acid was removed by ether extraction, the homogenate was lyophilized and

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Abbreviations: $NaDodSO_4$, sodium dodecyl sulfate; RER, rough endoplasmic reticulum.

analyzed on a Beckman model 120C amino acid analyzer. Based on the average of four separate determinations, the endogenous leucine pool was determined to be 160 ± 25 pmol/oocyte.

Rates of total protein and zein synthesis were determined by the method of Shih *et al.* (17), assuming an endogenous leucine pool of 160 pmol/oocyte and an injected leucine concentration of 100 pmol/oocyte. Rates of synthesis were calculated assuming a 20% leucine content for the maize zein polypeptides (1, 2) and 10% leucine for the endogenous oocyte proteins. No correction was made for zein content among the total oocyte proteins.

***In Vitro* Protein Synthesis.** A standard cell-free protein-synthesizing system (19) was prepared from wheat embryos as described (5). After translation of zein mRNA, the reaction mixture was brought to 70% with absolute ethanol and held at 60°C for 10 min. Ethanol-insoluble proteins were removed by centrifugation at 6000 rpm for 10 min. An mRNA-dependent cell-free translational system was also prepared from a rabbit reticulocyte lysate by micrococcal nuclease digestion (20). After a 60-min translation, zein proteins were extracted by adding absolute ethanol to a final concentration of 70%, and insoluble proteins were removed by centrifugation. Radioactive protein samples were lyophilized and analyzed on 12.5% $NaDodSO_4$/polyacrylamide gels (acrylamide/bisacrylamide, 75:1) as described (5). Proteins cleaved with cyanogen bromide were analyzed on 25% $NaDodSO_4$/polyacrylamide gels (acrylamide/bisacrylamide, 150:1). Radioactivity was detected by fluorography with pre-exposed Kodak RP X-Omat film (21). Native zein proteins were labeled with ^{125}I by the chloramine-T method (22).

Amino-Terminal Sequence Analysis of Zein Proteins. Zein proteins synthesized in oocytes or the wheat germ system were double-labeled with [^{3}H]leucine and [^{14}C]isoleucine and mixed with small amounts of dansylated native zein proteins. After electrophoresis on 12.5% $NaDodSO_4$/polyacrylamide gels, the positions of the 19,000 and 22,000 M_r zein proteins were visualized with a long-wave UV lamp and excised with a razor blade. Labeled proteins were recovered by electrophoretic elution and dialyzed extensively against 70% ethanol. The amino-terminal sequence of proteins was determined with a Beckman 890 C sequencer, and the recovery of [^{14}C]isoleucine and [^{3}H]leucine was compared with the positions of these amino acids in the native polypeptides (4).

RESULTS

Analysis of Zein Proteins Synthesized in Oocytes. In order to determine whether *Xenopus* oocytes could synthesize maize storage proteins, we injected oocytes with zein mRNAs and several hours later injected [^{3}H]leucine to label the proteins. After 6 hr of incubation, the oocytes were extracted in 70% ethanol and the ethanol-soluble proteins were analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis (Fig. 1). Native zein proteins labeled with ^{125}I (Fig. 1, samples 1 and 4) and [^{3}H]leucine-labeled mRNA translation products from the wheat germ system (Fig. 1, samples 3 and 6) were used as M_r standards. Oocytes injected with zein mRNAs from normal maize synthesized two major ethanol-soluble proteins (Fig. 1, samples 2, 5, and 8) that had the same M_r as the ^{125}I-labeled native zein proteins. The oocytes also synthesized small amounts of protein with a M_r similar to the ^{125}I-labeled 15,000 M_r component. No labeled proteins were detectable in 70% ethanol extracts of oocytes that were not injected with zein mRNA (Fig. 1, sample 7), although stained gels revealed the presence of a high M_r oocyte protein.

Rates of Zein Synthesis in mRNA-Injected Oocytes. As shown in Fig. 2, injection of [^{3}H]leucine at relatively low specific activity (4 Ci/mol) expanded the endogenous pool sufficiently to generate linear incorporation into both total protein and zein proteins for at least 90 min. This allowed accurate estimation of the absolute rates of protein synthesis as described (17). In this particular experiment, the oocytes were injected with zein mRNA (20 ng) 24 hr before isotope injection; during

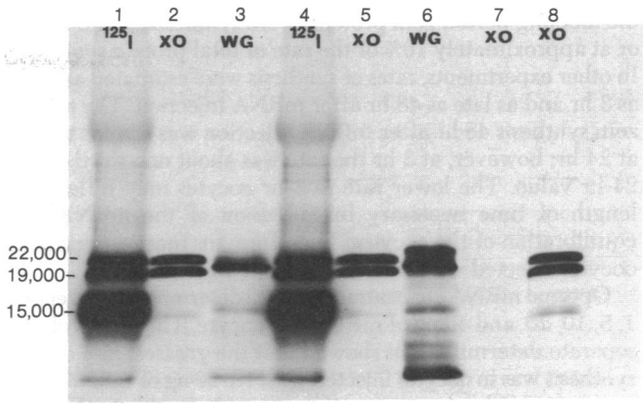

FIG. 1. $NaDodSO_4$/polyacrylamide gel analysis of 70% ethanol-soluble proteins synthesized in *Xenopus* oocytes. ^{125}I-Labeled native zein proteins as well as normal and *opaque*-2 mRNA translation products from a wheat germ cell-free system were analyzed for comparison. Although the tyrosine content of the different zein proteins is similar (1, 2), the 15,000 M_r protein was preferentially labeled by ^{125}I. Samples 1 and 4, ^{125}I-labeled normal zein, 190,000 cpm; samples 2, 5, and 8, [^{3}H]leucine-labeled ethanol-soluble proteins from oocytes injected with normal zein mRNA, 50,000 cpm; samples 3 and 6, [^{3}H]leucine-labeled products of *opaque*-2 and normal zein mRNA translation, respectively, from a wheat germ cell-free system, 30,000 cpm; sample 7, [^{3}H]leucine-labeled ethanol-soluble proteins from *Xenopus* oocytes without injected mRNA, number of oocytes equivalent to samples 2, 5, and 8.

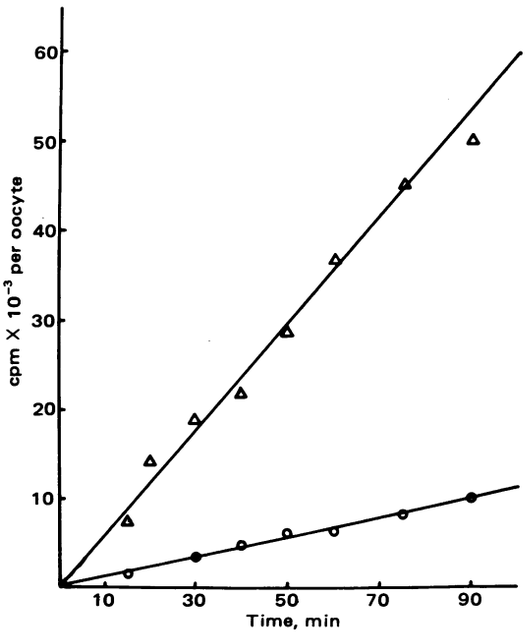

FIG. 2. Kinetics of [^{3}H]leucine incorporation in mRNA-injected oocytes. Oocytes were injected with 20 ng of zein mRNA and incubated in Eppig and Dumont's medium for 24 hr. After 0.4 μCi of [^{3}H]leucine (4 Ci/mol) was injected, oocytes were sampled at appropriate times to determine incorporation into total protein or protein soluble in 70% ethanol. △, Incorporation into total protein; ○, incorporation into 70% ethanol-soluble protein.

the labeling period zein proteins were synthesized at 3 ng/hr or at approximately 10% of the rate of total protein synthesis. In other experiments, rates of synthesis were estimated as early as 3 hr and as late as 48 hr after mRNA injection. The rate of zein synthesis 48 hr after mRNA injection was similar to that at 24 hr; however, at 3 hr the rate was about one-third of the 24-hr value. The lower rate in 3-hr oocytes may reflect the length of time necessary for diffusion of the mRNA and equilibration of the oocytes. Therefore, for most experiments oocytes injected with mRNA for 24 hr were used.

Optimal mRNA concentrations were determined by injecting 1, 5, 10, 20, and 40 ng of mRNA per oocyte. The results of two separate determinations showed that the greatest rate of zein synthesis was in oocytes injected with 10–20 ng of mRNA (data not shown). These concentrations are comparable to those reported to be optimal for maximum rates of protein synthesis in experiments with other mRNAs (23, 24).

Table 1 summarizes the results obtained in four separate experiments in which the rates of zein and total protein synthesis were determined from the incorporation kinetics shown in Fig. 2. Because zein represented the only ethanol-soluble protein synthesized in mRNA-injected oocytes (Fig. 1), the rates are an accurate measurement of the synthesis of the two protein fractions. Rates of total protein synthesis, as well as zein synthesis, varied among oocytes from different females, but were within the ranges previously reported for oocytes. Such variation has frequently been noted in studies with amphibian oocytes (17) and presumably reflects variability in the physiological status of different females. Although the rate of zein synthesis varied among groups of oocytes, on the average it represented about 10% of the total protein synthesized in the oocyte.

Comparison of Zein Proteins Synthesized in Oocytes and in *In Vitro* Translation Systems. When zein mRNAs from normal maize were translated in the wheat germ cell-free system, the major zein polypeptides were 2000 daltons larger than the native proteins (5–7). Similarly, mRNA from the *opaque*-2 mutant, which does not direct the synthesis of the 22,000 M_r zein component (25), produced a precursor of the 19,000 M_r zein component (26).

Fig. 3 shows a comparison of zein proteins synthesized by normal and *opaque*-2 mRNAs in oocytes and two cell-free protein-synthesizing systems. The translation products of *opaque*-2 and normal mRNA in either wheat germ or reticulocyte systems had similar M_r (Fig. 3, samples 3–6) and were 2000 daltons larger than the native zein proteins (5). In both cell-free systems, *opaque*-2 mRNA directed the synthesis of predominantly the smaller major zein component, whereas both components were synthesized by mRNA from the normal maize inbred line. The 15,000 M_r component, which seemed to be more efficiently labeled in the reticulocyte system, was a minor product in both cell-free systems. When oocyte proteins were labeled with [^{3}H]methionine rather than [^{3}H]leucine (Fig. 3, compare samples 10 and 9), the 15,000 M_r protein was more extensively labeled than the two larger zein proteins, as predicted from its higher methionine content (1, 2).

The two major zein components synthesized in oocytes were

Table 1. Comparison of zein synthesis with endogenous protein synthesis in mRNA-injected oocytes

Exp.	Zein synthesis, ng/hr	Total synthesis, ng/hr
1	0.72	16
2	3.8	21.4
3	3.9	36.8
4	7.0	98.8

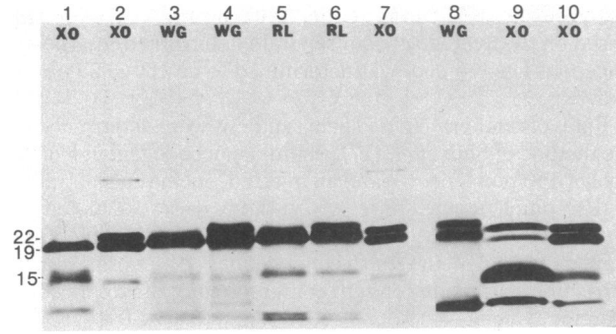

FIG. 3. NaDodSO$_4$/polyacrylamide gel analysis of zein mRNA translation products from heterologous translation systems. Samples 1 and 2, *opaque*-2 and normal zein mRNA translation products from *Xenopus* oocytes, [^{3}H]leucine, 50,000 cpm; samples 3 and 4, *opaque*-2 and normal zein mRNA translation products from a wheat germ cell-free system, [^{3}H]leucine, 60,000 cpm; samples 5 and 6, *opaque*-2 and normal zein mRNA translation products from a reticulocyte lysate cell-free system, [^{14}C]leucine, 30,000 cpm; sample 7, normal zein mRNA translation products from *Xenopus* oocytes, [^{3}H]leucine, 50,000 cpm; sample 8, [^{3}H]leucine-labeled zein from a wheat germ cell-free system, 50,000 cpm; samples 9 and 10, [^{3}H]methionine and [^{3}H]leucine-labeled zein, respectively, from *Xenopus* oocytes, 50,000 cpm. Values on left are $M_r \times 10^{-3}$.

each 2000 daltons smaller than the corresponding proteins from either wheat germ or reticulocyte lysates (Fig. 3, samples 1 and 2), suggesting that the proteins were processed in the oocyte. This reduction in M_r was also apparent for the 15,000 M_r protein. To determine whether zein proteins synthesized in the oocyte had the same amino-terminal sequence as native polypeptides, mRNA-injected oocytes were double-labeled with [^{3}H]leucine and [^{14}C]isoleucine. After the oocytes were extracted in 70% ethanol, the 19,000 and 22,000 M_r proteins were separated and isolated by NaDodSO$_4$/polyacrylamide gel electrophoresis. Fig. 4 shows an analysis of the [^{3}H]leucine and [^{14}C]isoleucine distributions from the amino-terminal sequence of the 22,000 M_r zein proteins. Radioactive isoleucine and leucine were recovered at precisely the same positions they occupied in the native polypeptides, with [^{14}C]isoleucine in the

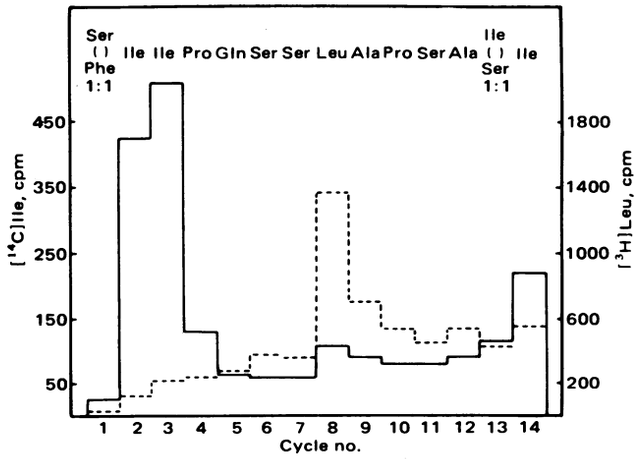

FIG. 4. Amino-terminal sequence analysis of the 22,000 M_r zein polypeptides synthesized in *Xenopus* oocytes. Proteins synthesized in mRNA-injected oocytes were double-labeled with [^{14}C]isoleucine and [^{3}H]leucine. After the oocytes were extracted with 70% ethanol, the 22,000 M_r polypeptides were isolated by NaDodSO$_4$/polyacrylamide gel electrophoresis and their amino-terminal sequences were analyzed with a Beckman 890 C sequencer. The histograms show the recovery of [^{14}C]isoleucine (——) and [^{3}H]leucine (- - -) compared with the sequence of these amino acids in the native polypeptides.

positions 2, 3, and 14 and [^{3}H]leucine in position 8. The recovery of [^{14}C]isoleucine in position 13 was lower than expected, but this could have resulted from differential mRNA translation in the oocyte. These results verified the authenticity of the zein polypeptides synthesized in the oocyte and demonstrated that their amino-terminal sequences were identical to those of the native polypeptides.

A similar analysis was made of [^{3}H]leucine- and [^{14}C]isoleucine-labeled zein proteins synthesized in the wheat germ cell-free system. [^{3}H]Leucine was recovered within several of the first 10 amino acid cycles. Although this result indicated sequence differences from the native polypeptides, the distribution of radioactivity was sufficiently heterogeneous that a single amino-terminal sequence could not be determined.

Burr *et al.* (6) reported that cyanogen bromide cleavage of the 22,000 M_r zein component yielded three fragments, one of which contained the signal peptide. They also reported that the 19,000 M_r component was not cleaved by cyanogen bromide. To further analyze the zein proteins synthesized in the oocytes and the wheat germ system and determine which fragments of the *in vitro* products were associated with signal peptides, we treated the translation products from both systems with cyanogen bromide.

Zein proteins treated with cyanogen bromide showed a complex pattern on 25% NaDodSO$_4$/polyacrylamide gels, indicating as many as seven different bands. From these data alone it was difficult, if not impossible, to determine the origin of various peptides. However, products of *opaque*-2 mRNA translation, which do not include the 22,000 M_r zein component, should be less complex than those of normal mRNA. Indeed, by comparing the cleavage products of *opaque*-2 with those of normal mRNA it should be possible to identify the peptides derived from the 19,000 and 22,000 M_r components. The results shown in Fig. 5 demonstrate that the cleavage products from *opaque*-2 mRNA translation in either oocytes or wheat germ (Fig. 5, samples 4 and 8, respectively) were less complex than those of their normal counterparts. Some of the 19,000 M_r polypeptide in the *opaque*-2 samples was not cleaved by cyanogen bromide, but in contrast to previous results (6, 7), some was cleaved into smaller M_r polypeptides. Two bands at positions a and b resulted from cleavage of the wheat germ products; bands at positions a, c, and d were formed by cleavage of the oocyte products.

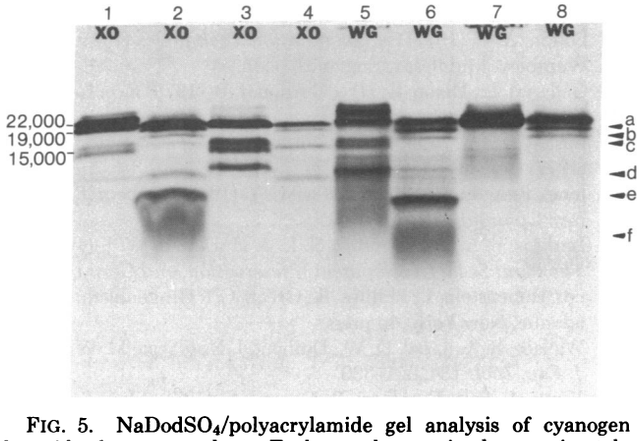

FIG. 5. NaDodSO$_4$/polyacrylamide gel analysis of cyanogen bromide cleavage products. Each sample contained approximately 75,000 cpm of [^{3}H]leucine. Samples 1 and 2, normal zein mRNA translation products from oocytes before and after cyanogen bromide cleavage; samples 3 and 4, *opaque*-2 mRNA translation products from oocytes before and after cyanogen bromide cleavage; samples 5 and 6, normal zein mRNA translation products from wheat germ before and after cleavage; samples 7 and 8, *opaque*-2 mRNA translation products from wheat germ before and after cleavage.

The cyanogen bromide cleavage products from normal mRNA translation (Fig. 5, samples 2 and 6) contained the peptides found in the *opaque*-2 samples as well as several others. In addition to the 19,000 M_r protein that was not cleaved, peptides at positions a, b, e, and f were among products from both oocytes and wheat germ. Bands at positions c and d were present in the oocyte cleavage products, but absent from the wheat germ products.

Because fragments e and f were present only among cleavage products resulting from translation of normal mRNA, they must be derived from the 22,000 M_r component. Fragments c and d were unique to the cleavage products of normal and *opaque*-2 mRNA from oocytes. Although these fragments may have been associated with signal peptides, the presence of larger amounts of 15,000 M_r subunits in oocyte samples makes such a conclusion untenable. Aside from these fragments, all others from translation of normal zein mRNA in either oocytes or wheat germ were of similar size, suggesting they did not contain signal sequences.

DISCUSSION

Reports that zein mRNAs direct the synthesis of precursor polypeptides in the wheat germ cell-free system (5, 6) prompted us to analyze zein mRNA translation products from *Xenopus* oocytes. Oocytes efficiently translate a number of heterologous animal mRNAs and can cleave signal peptides from secretory proteins (12–14) and secrete proteins into membrane vesicles (13). The results presented here clearly demonstrate that *Xenopus* oocytes will translate plant mRNAs and also indicate that they will process plant preprotein polypeptides.

In our initial experiments, we incubated oocytes in Eppig and Dumont's medium to maintain them for prolonged periods. Under these conditions, oocytes actively synthesized zein proteins for several days without any obvious decrease in rate. It was also found that oocytes incubated in a basal salts medium, OR$_2$ (27), synthesized zein very efficiently (data not shown). Because the zein proteins were the only 70% ethanol-soluble proteins synthesized in the oocytes, we were able to measure the rate of zein synthesis accurately relative to endogenous proteins. There was some variation in rates of protein synthesis among different groups of oocytes, but when optimal concentrations of zein mRNA were injected, zein represented approximately 10% of the total protein synthesized.

Zein proteins synthesized in oocytes had M_r similar to ^{125}I-labeled native zein proteins (Fig. 1) and were approximately 2000 daltons smaller than the translation products from either wheat germ or reticulocyte cell-free systems (Fig. 3). This alteration in protein size was not restricted to the major zein polypeptides, but also included the 15,000 M_r component. This result supports the recent report of Melcher (7) that the 15,000 M_r protein is synthesized as a precursor in the wheat germ cell-free system. We were unable to detect zein precursor proteins in the oocytes during short labeling times (30 min), which suggests that the processing occurs during elongation of nascent polypeptides (8).

Additional evidence that the oocyte processed prezein polypeptides was provided by amino-terminal sequence analysis. The pattern of [^{14}C]isoleucine and [^{3}H]leucine labeling of the 22,000 M_r polypeptides was entirely consistent with the sequence of these amino acids in the native polypeptides. Furthermore, zein proteins synthesized in the oocyte were accumulated in membrane vesicles, which were stable for several days (unpublished data), indicating that the proteins were compartmentalized within the oocyte.

We were unable to obtain definitive sequence information for the zein proteins synthesized *in vitro* in the wheat germ

system. The recovery of [^{3}H]leucine in the first several sequence cycles was different from that of the native polypeptides; however, the data were too complex to derive sequence information. Although we do not know the source of this heterogeneity, it may result from sequence variation among the prezein polypeptides or exopeptidase activity in the wheat germ extract.

A comparison of the peptides generated by cyanogen bromide cleavage of zein proteins synthesized in the oocyte and wheat germ systems showed both similarities and differences between the two. Although the origin of each polypeptide could not be unequivocally demonstrated, by comparing those of *opaque*-2 with normal it was possible to determine the origin of some of the peptide fragments. Analysis of the cleavage products of *opaque*-2 mRNA translation (Fig. 4, samples 4 and 8) indicated that some of the 19,000 M_r protein was cleaved by cyanogen bromide. This result, which contradicts the conclusion of Burr *et al.* (6) and Melcher (7), is supported by the methionine labeling pattern of the proteins (Fig. 3, sample 9) and the amino acid analysis indicating an average of two methionine residues per polypeptide (1). As reported by Burr *et al.* (6), the 22,000 M_r zein component was cleaved by cyanogen bromide. However, we could account for only two polypeptides resulting from its cleavage and neither of these appeared to be associated with a signal sequence.

The analysis of mRNA translation products and their cleavage by cyanogen bromide provided additional evidence of the heterogeneity among zein polypeptides. The production of several large peptides, as well as some uncleaved 19,000 M_r protein, indicated that this component must consist of at least three different proteins. Based on our analyses, we could account for five different polypeptides within the zein fraction, but whether all the different charged forms of zein resolved by isoelectric focusing (3) represent unique gene products remains to be established.

There was some evidence for differences in the efficiency of synthesis of the 15,000 M_r protein by the three translational systems. When labeled with [^{3}H]leucine, this protein was clearly evident among the translational products of the oocyte and reticulocyte lysate (Fig. 3, samples 1, 2, 5, and 6), but it was barely discernable among the wheat germ translation products. This variation in labeling made it difficult to determine the origin of some of the lower M_r cyanogen bromide cleavage products. The 15,000 M_r protein contains approximately 14 leucine residues compared to 35 and 41 in the 19,000 and 22,000 M_r components, respectively, and it contains 7 methionine residues compared to 2 and 4 in the 19,000 and 22,000 M_r proteins (1). This accounts for the more intense labeling of this protein with [^{3}H]methionine (7, 28). Because of its higher methionine content, this protein would be cleaved into lower M_r polypeptides by cyanogen bromide.

Our observation that zein proteins are processed in oocytes is not entirely surprising because signal sequences from other heterologous proteins are known to be cleaved in oocytes (12–14). Although the storage proteins are not secreted outside the cell, they are vectorally discharged into the RER, where they accumulate and form protein bodies (5). Our results add additional support to the conclusion that the enzymes cleaving signal sequences in oocytes are not highly specific (11, 13) and demonstrate the existence of identical posttranslational modifications in frog eggs and maize seeds.

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