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Rice prolamins: Heterogeneity of cDNAs and synthesis of precursors

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Abstract. Three cDNA clones, pX24, pS23 and pS18, were isolated from a λZAP cDNA library constructed from Poly(A)$^+$ RNA of developing rice (*Oryza sativa* L. cv Tainung 67) endosperm. All three of them showed a single open reading frame encoding 149, 150 and 158 amino acids, respectively. Analysis of *in vitro* translation products of hybrid-selected poly(A)$^+$ RNA revealed the synthesis of 16 kD and 12 kD polypeptides, presumably the precursors of the 14 kD and 10 kD prolamins, respectively. Deduced amino acid sequence homologous to other cereal prolamins were found in three regions of the 14 kD prolamin. Syntheses of the 16 kD and 12 kD prolamin began around 8 days after flowering (DAF) and increased markedly in 8-11 DAF.

Key words: *Oryza sativa*; Prolamin cDNAs; Prolamin precursors; Sequence homology.

Introduction

Alcohol-soluble prolamins are major storage proteins in most cereal seeds. It was indicated that prolamins as well as glutelins are major rice seed storage proteins (Sugimoto *et al.*, 1986). Rice prolamins accumulate within protein bodies which are formed by direct dilation of the endoplasmic reticulum membrane (Krishnan *et al.*, 1986; Yamagata and Tanaka, 1986). The major protein component in rice protein body type I (PB-I) is prolamin (Ogawa *et al.*, 1987), whereas that in protein body type II (PB-II) is glutelin (Tanaka *et al.*, 1980). The rice prolamins have molecular weights of about 12 to 17 kD and have a high content of glutamine but low levels of lysine, histidine, cysteine and methionine (Mandac and Juliano, 1978; Padhye and Salunkhe, 1979).

Analysis of *in vitro* translation products purified by immunoprecipitation revealed the formation of a 16 kD precursor form of prolamin (Krishnan and Okita, 1986; Yamagata and Tanaka, 1986). Synthesis of the 16 kD precursor prolamin is first detected at about 8 days after flowering (DAF), markedly increases at 8-11 DAF and then gradually reaches maximum levels during seed maturation (Shyur *et al.*, 1992).

Recently a number of rice prolamin cDNA and genomic clones have been described. Three prolamin cDNA clones and a genomic clone were isolated and sequenced by Kim and Okita (1988). These cDNA clones can be divided into two homology classes (classes I and II) based on cross-hybridization and restriction enzyme map analyses. A cDNA encoding a sulfur -rich 10 kD prolamin was characterized (Masumura *et al.*, 1989). The deduced mature polypeptide sequence shows that the rice 10 kD prolamin has high contents of methionine (20%), cysteine (10%), glutamine, and hydrophobic amino acid. In addition, several cDNA clones encoding the major rice prolamins were also reported (Masumura *et al.*, 1990; Shyur and Chen, 1990; Yamagata *et al.*, 1992; Shyur *et al.*, 1992). These cDNAs exhibit different degrees of homology in their

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coding regions. The sequence heterogeneity displayed by these genomic and cDNA clones, and the large gene copy number (Kim and Okita, 1988) indicate that the rice prolamins are encoded by a complex multigene family.

Various rice prolamin polypeptides were visualized by SDS-polyacrylamide gel electrophoresis and isoelectrofocusing (Hibino *et al.*, 1989). Two-dimensional electrophoresis of the two major components of purified rice prolamins revealed heterogeneity of prolamin polypeptides (Shyur and Chen, unpublished). These results substantiate the point of view that rice prolamin genes compose a complex multigene family. Post-translational modification of the prolamin molecules may also result in the polypeptide heterogeneity. Studies on the prolamin cDNAs and their corresponding individual prolamin polypeptides would provide insight into the molecular structures and heterogeneity of rice prolamins, and the evolutional relationship between cereal prolamins. This paper describes the characterization of three prolamin cDNA clones isolated from a λZAP cDNA library constructed from developing rice (*Oryza sativa* L. cv Tainung 67) endosperm poly(A)$^+$ RNA. The nucleotide sequences of these cDNA clones are compared with the previously described rice and other cereal prolamin cDNAs. Gene expression of rice prolamin during seed development is also reported.

Materials and Methods

Plant Materials and Reagents

Rice (*Oryza sativa* L. *japonica* cv. Tainung 67) was grown in the experimental field plot at the Institute of *al.*, 1979). The immature rice seeds were ground with an ice-cold mortar and pestle and then homogenized with Polytron (Kinematica) in a buffer containing 4 M guanidine thiocyanate. The homogenate was centrifuged in a JA-10 rotor (Beckman) at 8,000 rpm at 4°C for 40 min. The supernatant was centrifuged on top of 5.7 M CsCl cushion in 0.1 M Tris · HCl/25 mM EDTA, and RNAs were precipitated by the addition of 0.75 volume of ethanol containing 25 mM final concentration of acetic acid. The total RNAs were extracted with a small volume of sterile water, reprecipitated with two volumes of ethanol and 0.1 volume of 3 M potassium acetate and then redissolved in a small volume of sterile water. Poly (A)$^+$ RNA was obtained by oligo (dT)-cellulose chromatography (Aviv and Leder, 1972) except that NaCl in the buffer solution was replaced with LiCl.

Wheat germ S30 extract was prepared according to the method of Anderson *et al.* (1983). The optimal conditions for *in vitro* translation of rice poly(A)$^+$ RNA were determined by measuring the highest efficiency of [^{35}S]methionine incorporation into the total translation products.

Construction of cDNA Library

The poly(A)$^+$ RNA prepared from 8-11 DAF rice seeds was rechromatographed on the oligo (dT)-cellulose column and used for cDNA synthesis. The blunt-ended double stranded cDNA was synthesized by the method of Gubler and Hoffman (1983). EcoRI linker ligation and size fractionation of cDNA were carried out as described by Klickstein and Neve (1987). The cDNA library was constructed according to the instruction manual of Stratagene Cloning Systems

clones.

Single plaques of positive cDNA clones were isolated and cotransfected XL1-Blue host cells with f1 helper phage.

DNA Sequencing and Sequence Analysis

The cDNA inserts in the excised pBluescript KS(-) phagemid were used directly for sequencing by the dideoxy chain termination method (Sanger *et al.*, 1977) using supercoiled phagemide template (Chen and Seeburg, 1985) with either M13 (Stratagene) or synthetic sequencing primer. DNA sequences were analyzed by the computer programs DNASIS (Hitachi Software Engineering Co., Ltd.)

Hybrid-selection and Translation

Hybrid-selection was carried out as descibed (Mason and Williams, 1985). Denatured cDNA (pS18) was fixed onto a nitrocellulose membrane (Millipore, HATF) and hybridized with rice poly(A)$^+$ RNA. The poly(A)$^+$ RNA specifically hybridized to the fixed cDNA was eluted with water and then translated in an *in vitro* translation system using the aforementioned wheat germ extract. The translation products were separated by 5-20% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970) and detected by autoradiography.

Immunoprecipitation

To eliminate nonspecific binding of antigen, the *in vitro* translation products were first incubated with preimmune serum of the experimental rabbit and protein A-Sepharose CL-4B at room temperature for 30 min and centrifuged. The supernatant was then incubated with anti-prolamin serum and fresh protein A-Sepharose CL-4B. After extensively washing with 10 mM Tris · HCl (pH 7.5)/0.15 M NaCl/ 2 mM EDTA/ 10% Nonidet P-40, the precipitates were boiled in SDS sample buffer (Laemmli, 1970) and fractionated by [illegible]

20% SDS-PAGE. The [^{35}S]methionine-labeled translation products were detected by autoradiography.

Southern Blot Analysis

the gel was denatured and renatured. As size marker and negative control, DNA markers were run concomitantly on the same gel. The DNAs were transfered to GeneScreen Plus membrane (Dupont). The blot was then hybridized with ^{32}P-labeled cDNA probe by the recommended method of the manufacturer. After washing, the membrane was exposed with X-ray film.

Results

Identification of cDNA Clones Encoding 16 kD Prolamin

The synthetic 17-mer oligonucleotide probe was used for screening of the cDNA library. Eleven positive clones were obtained and three of them, designated pX 24, pS23 and pS18, were shown to consist of at least 620 bp in length which is approximately equivalent to a full length cDNA of 16 kD prolamin. Restriction maps of pX24, pS23 and pS18 were quite different from one another (Fig. 1). Analysis of *in vitro* translational products of hybrid-selected poly(A)$^+$ RNA complementary to pS18 revealed the synthesis of two polypeptides with molecular weights of approximately 16 kD and 12 kD (Fig. 2A). Immunoprecipitation of *in vitro* translational products of rice grain poly(A)$^+$ RNA with anti-prolamin serum also yielded 16 kD and 12 kD polypeptides (Fig. 2B). Similar results were obtained with pS 23 as the hybrid-selection probe (data not shown). Since the coding region of pX24 is highly homologous to that of pS23 (92%), it is reasonable to suggest that these three cDNA sequences encoded the 16 kD rice prolamins. The three prolamin cDNAs were further characterized by nucleotide sequence analyses (Shyur

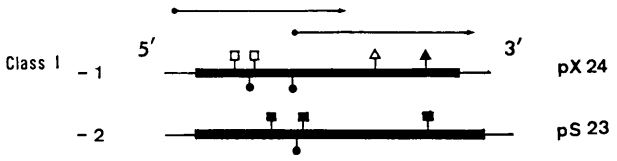


Fig. 1. Comparison of restriction maps of the class I and class

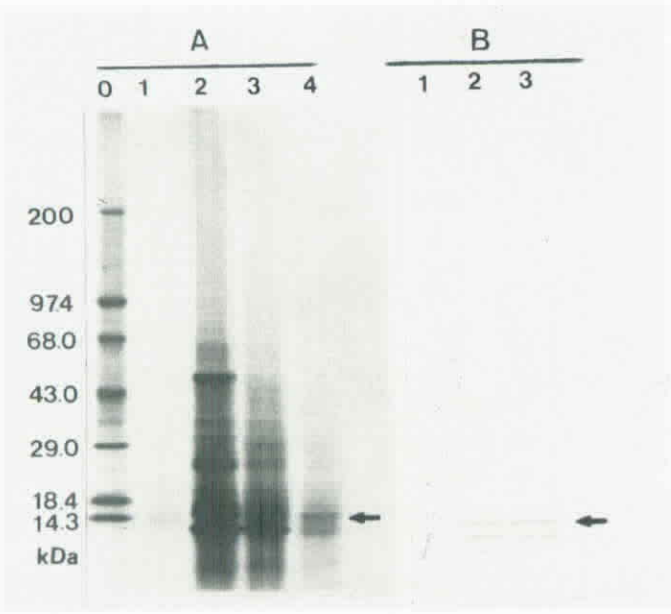


Fig. 2. A, Analysis of *in vitro* translation products of hybrid-selected poly(A)$^+$ RNA. Fifteen μg of pS 18 fixed onto a nitrocellulose membrane was hybridized with 20 μg of rice poly(A)$^+$ RNA. The translation products of the hybrid-selected poly(A)$^+$ RNA were separated by 5–20% SDS-PAGE and detected by autoradiography. Lane 0: ^{14}C-labeled molecular weight markers; land 1: no mRNA added in *in vitro* translation reaction; lane 2: total translation products; lane 3: products from non-selected poly(A)$^+$ RNA; lane 4: products from hybrid-selected poly (A)$^+$ RNA. B, Immunoprecipitation of *in vitro* translation products of rice grains poly (A)$^+$ RNA with rice anti prolamin serum. The mRNA was translated in the S30 wheat germ system and the ^{35}S-labeled products were separated on 5–20% mini SDS-PAGE as described in Materials and Methods. The 16 kD prolamin is marked by arrow. From lane 1 to lane 3: the total translation products immunoprecipitated with 10^4, 5×10^4, and 10^5 dilution of rice anti prolamin serum, respectively.

Table 1. *Comparison of coding sequence homology degree (%) of rice 16 kD prolamin cDNAs*

	pX24	pS23	pS18	λ RM7	pProl 7	pProl 14	pProl 17
pX24*	100						
pS23*	92.1	100					
pS18§	79.4	79.6	100				
λ RM7#	77.3	82.2	90.9	100			
pProl 7&	96.0	87.7	74.5	72.8	100		
pProl 14&	97.1	90.6	76.2	72.2	95	100	
pProl 17&	69.3	67.7	77.2	76.0	75	75	100

*: From Shyur and Chen, 1990.
§: From Shyur *et al.*, 1992
#: From Masumura *et al.* 1990.
&: From Kim and Okita, 1988.

Table 2. *Comparison of deduced amino acid homology degree (%) from cDNA clones of rice 16 kD prolamin*

	pX24	pS23	pS18	λ RM7	pProl 7	pProl 14	pProl 17
pX24*	100						
pS23*	84.5	100					
pS18§	65.8	64.1	100				
λ RM7#	66.7	66.7	89.7	100			
pProl 7&	89.7	76.8	62.6	63.5	100		
pProl 14&	94.9	83.9	67.1	67.9	87.7	100	
pProl 17&	57.1	55.8	71.9	71.3	54.8	63	100

*: From Shyur and Chen, 1990.
§: From Shyur *et al.*, 1992.
#: From Masumura *et al.* 1990.
&: From Kim and Okita, 1988.

and Chen, 1990; Shyur *et al.*, 1992).

Nucleotide Sequence of Rice Prolamin cDNAs

The coding regions of pX24 and pS23 were 92% and 84% homologous to each other at the nucleotide and amino acid levels, respectively (Tables 1 and 2). pX24 and pS23 are highly similar in nucleotide sequence (88–97%) to the class I rice prolamin cDNA

Table 3. *Amino acid composition of rice prolamin deduced from open reading frame of cDNA clone*

A. A.	pX24*		pS23*		pS18§	
	No	%	No	%	No	%
Ala	17	11.4	16	10.7	16	10.1
Val	10	6.7	11	7.3	14	8.9
Leu	20	13.4	22	14.7	18	11.4
Ile	9	6.0	10	6.7	11	6.9
Pro	7	4.7	5	3.3	7	4.4
Phe	9	6.0	8	5.3	7	4.4
Trp	1	0.7	0	0	1	0.6
Met	1	0.7	1	0.7	2	1.3
Lys	1	0.7	1	0.7	1	0.6
His	2	1.3	2	1.3	3	1.9
Gly	7	4.7	6	4.0	4	2.5
Thr	3	2.0	4	2.7	5	3.2
Cys	1	0.7	1	0.7	5	3.2
Tyr	10	6.7	10	6.7	7	4.4
Asp	4	2.7	4	2.7	2	1.3
Asn	6	4.0	7	4.7	5	3.2
Glu	1	0.7	1	0.7	2	1.3
Gln	25	16.8	25	16.7	30	19.0
Ser	8	5.4	8	5.3	13	8.2
Arg	7	4.7	8	5.3	5	3.2
Total	149	99.9	150	100	158	100

*From Shyur and Chen, 1990.
§: From Shyur *et al.*, 1992.

(Kim and Okita, 1988). pS18 was less homologous to

dues 34-39 are deleted in pProl 17 (Fig. 3).

The deduced amino acid sequences of those three cDNAs showed a typical prolamin composition except for a lower amount of proline (3.3～4.7 mol%) (Table 3). Those sequences are abundant in glutamine (17～19 mol%), leucine (13～15 mol%) and alanine (10～11 mol%), and low in tryptophan, methionine, lysine and glutamic acid. These data are consistent with the amino acid compositions obtained from purified rice prolamins (data not shown). The high content of hydrophobic amino acids (49%) might account for the solubility property of prolamin.

The codon usage in these three 16 kD prolamin cDNAs is shown in Table 4. The following amino acids have codon bias (the preferent codon is parenthesized): Ile (ATT), Thr (ACC), Ala (GCT), Asn (AAC), Arg (AGG) and Gly (GGT). Some codons were never used, such as CGT, CGC for Arg and GGA, GGG for glycine. The frequency of CGN usage (N=T, C, A or G) in the overall usage is only 0.7%, whereas GCN amounts to 7～11%. Thus, the frequency of codon usage in rice prolamin is very similar to that of maize zein (Kreis *et al.*, 1985). Our data also showed that rice prolamin had ending codon with either XXA/U (45.7-53.5%) or XXG/C (46.5-54.3%), suggesting that there is no bias of G/C over A/T at the wobble position in rice prolamin codons.

Gene Organization of Rice Prolamin

pX24 and pS23 respectively at the nucleotide level, and 65.8% and 64.1% homology at the amino acid level. On the other hand, the cDNA pS18 had high sequence

Gene copy numbers corresponding to the cDNAs of class I, pS23, and class III, pS18, were estimated by Southern blot analysis. Twenty μg of genomic DNA

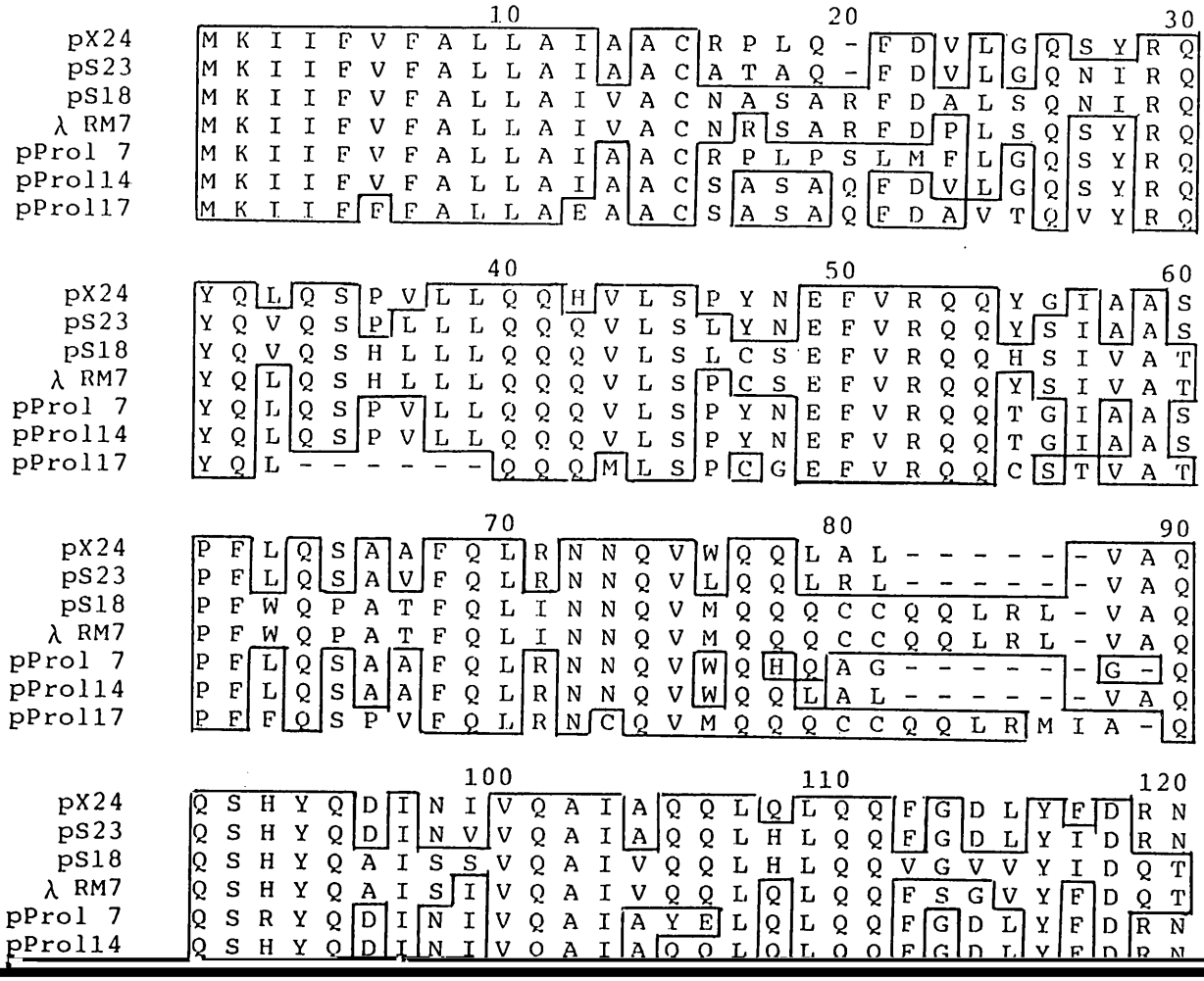

10 20 30
pX24 M K I I F V F A L L A I A A C R P L Q - F D V L G Q S Y R Q
pS23 M K I I F V F A L L A I A A C A T A Q - F D V L G Q N I R Q
pS18 M K I I F V F A L L A I V A C N A S A R F D A L S Q N I R Q
λ RM7 M K I I F V F A L L A I V A C N R S A R F D P L S Q S Y R Q
pProl 7 M K I I F V F A L L A I A A C R P L P S L M F L G Q S Y R Q
pProl14 M K I I F V F A L L A I A A C S A S A Q F D V L G Q S Y R Q
pProl17 M K I I F F F A L L A E A A C S A S A Q F D A V T Q V Y R Q
40 50 60
pX24 Y Q L Q S P V L L Q Q H V L S P Y N E F V R Q Q Y G I A A S
pS23 Y Q V Q S P L L L Q Q Q V L S L Y N E F V R Q Q Y S I A A S
pS18 Y Q V Q S H L L L Q Q Q V L S L C S E F V R Q Q H S I V A T
λ RM7 Y Q L Q S H L L L Q Q Q V L S P C S E F V R Q Q Y S I V A T
pProl 7 Y Q L Q S P V L L Q Q Q V L S P Y N E F V R Q Q T G I A A S
pProl14 Y Q L Q S P V L L Q Q Q V L S P Y N E F V R Q Q T G I A A S
pProl17 Y Q L - - - - - - Q Q Q M L S P C G E F V R Q Q C S T V A T
70 80 90
pX24 P F L Q S A A F Q L R N N Q V W Q Q L A L - - - - - - V A Q
pS23 P F L Q S A V F Q L R N N Q V L Q Q L R L - - - - - - V A Q
pS18 P F W Q P A T F Q L I N N Q V M Q Q Q C C Q Q L R L - V A Q
λ RM7 P F W Q P A T F Q L I N N Q V M Q Q Q C C Q Q L R L - V A Q
pProl 7 P F L Q S A A F Q L R N N Q V W Q H Q A G - - - - - - G - Q
pProl14 P F L Q S A A F Q L R N N Q V W Q Q L A L - - - - - - V A Q
pProl17 P F F Q S P V F Q L R N C Q V M Q Q Q C C Q Q L R M I A - Q
100 110 120
pX24 Q S H Y Q D I N I V Q A I A Q Q L Q L Q Q F G D L Y F D R N
pS23 Q S H Y Q D I N V V Q A I A Q Q L H L Q Q F G D L Y I D R N
pS18 Q S H Y Q A I S S V Q A I V Q Q L H L Q Q V G V V Y I D Q T
λ RM7 Q S H Y Q A I S I V Q A I V Q Q L Q L Q Q F S G V Y F D Q T
pProl 7 Q S R Y Q D I N I V Q A I A Y E L Q L Q Q F G D L Y F D R N
pProl14 Q S H Y Q D I N I V Q A I A Q Q L Q L Q Q F G D L Y F D R N

Table 4. *Codon usage in 16 kD prolamin cDNAs*

		pS18§	pX24*	pS23*			pS18§	pX24*	pS23*
Phe	TTT	4	6	5	Ala	GCT	9	8	6
	TTC	3	3	3		GCC	2	1	2
Lue	TTA	1	1	1		GCA	3	3	3
	TTG	4	3	5		GCG	2	5	5
	CTT	2	3	3	His	CAT	2	1	0
	CTC	4	4	5		CAC	1	1	2
	CTA	3	2	3	Gln	CAA	18	12	11
	CTG	4	7	5		CAG	12	13	14
Ile	ATT	6	5	7	Asn	AAT	0	2	3
	ATC	2	2	2		AAC	5	4	4
	ATA	3	2	1	Lys	AAA	0	0	0
Met	ATG	2	1	1		AAG	1	1	1
Val	GTT	4	2	2	Asp	GAT	2	3	2
	GTC	3	4	4		GAC	0	1	2
	GTA	3	1	1	Glu	GAA	1	0	0
	GTG	4	3	4		GAG	1	1	1
Ser	TCT	3	2	2	Cys	TGT	2	0	0
	TCC	1	0	0		TGC	3	1	1
	TCA	0	1	1	Trp	TGG	1	1	0
	TCG	1	1	1	Arg	CGT	0	0	0
	AGT	4	2	1		CGC	0	0	0
	AGC	4	2	3		CGA	0	0	1
Pro	CCT	1	3	2		CGG	1	1	1
	CCC	2	2	1		AGA	1	2	2
	CCA	3	2	1		AGG	3	4	4
	CCG	1	0	1	Gly	GGT	4	5	4
Thr	ACT	2	0	0		GGC	0	2	2
	ACC	2	3	2		GGA	0	0	0
	ACA	0	0	2		GGG	0	0	0
	ACG	1	0	0	Stop	TAA	1	1	0
	TAT	3	7	5		TAG	0	0	0
	TAC	4	3	5		TGA	0	0	1

From Shyur and Chen, 1990.
From Shyur *et al.*, 1992.

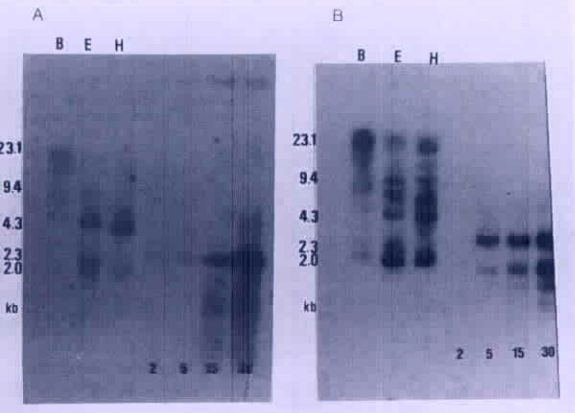
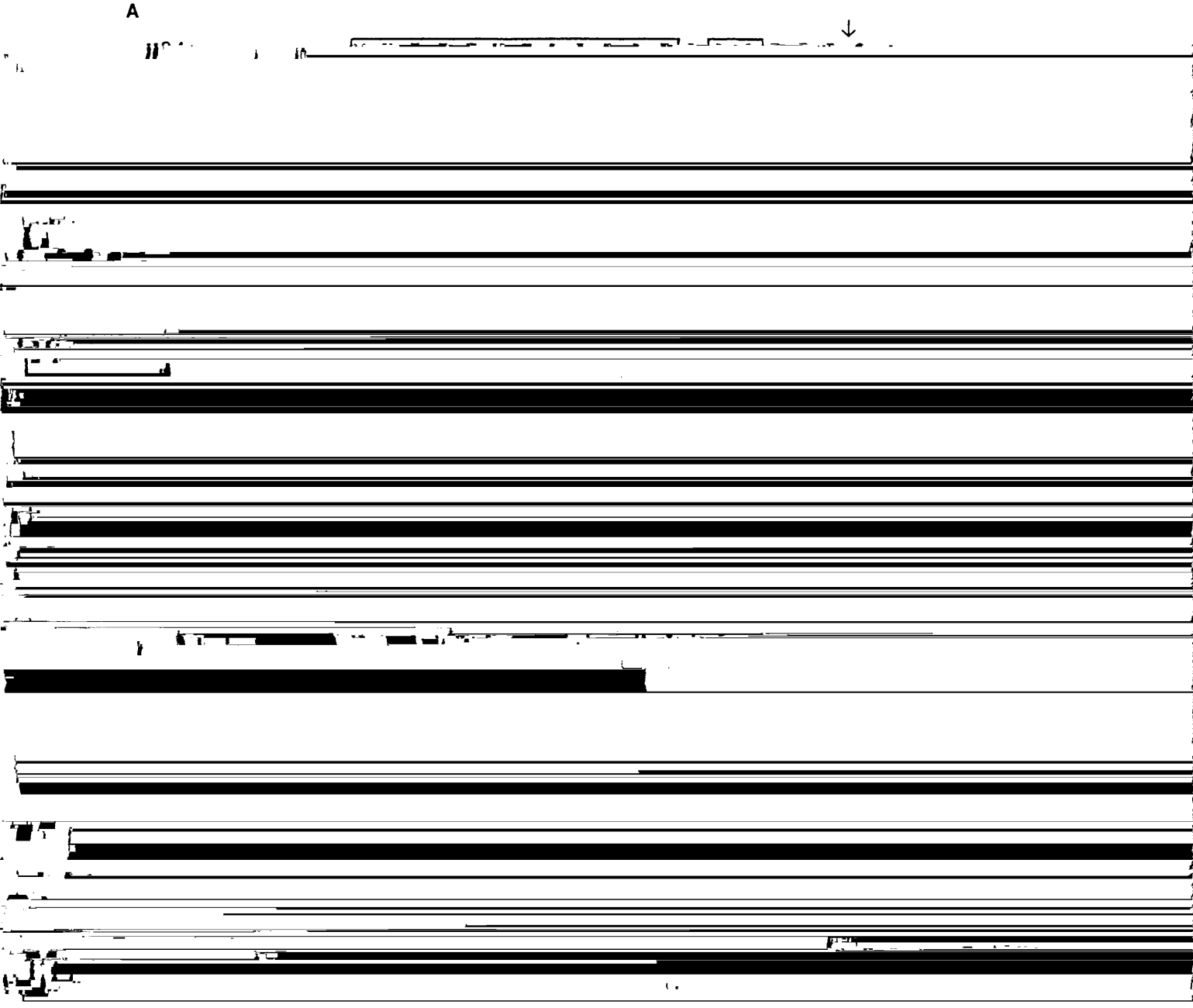


Fig. 4. Southern blot analysis of rice genomic DNA fragments. Twenty μg of rice genomic DNA, which had been digested with Bam HI (B), Eco RI (E) or Hind III (H), and different copy numbers of pS18 or pS23 inserts were run on 0.8% agarose gel. After electrophoresis, the DNA fragments on the gel were blotted to nylon membrane and then hybridized with either pS18 (A) or pS23 (B) probe. The size of DNA markers indicated in kb, and the numbers on the abscissa indicate the gene copy number of the prolamin insert sequence/haploid rice genome.

Conservation of Cereal Prolamin Gene

Seven prolamin cDNAs are compared for coding sequence homology (Table 1) and deduced amino acid sequence homology (Table 2). Kim and Okita (1988) divided pProl 7, pProl 14 and pProl 17 into two homology classes based on cross-hybridization and restriction enzyme map analyses. pProl 7 and pProl 14 are class I, and pProl 17 is class II. Both pProl 7 and pProl 14 have a deletion of a pentapeptide at the same position as pX24 and pS23. In addition, the coding sequence homology of these four cDNAs is in the range of 88-97% (Table 1), and deduced amino acid sequence homology is 77-95% (Table 2). Therefore, pX24 and pS23 are grouped into class I. The sequence homology between pS18 and λRM 7 is 91% (coding sequence) and 90% (amino acid sequence). These two cDNAs have fewer sequence homologies to class I and class II cDNAs. Homologies for nucleotide sequences are in the range of 72-77% and for amino acids are 63-72%. Accordingly, pS18 and λRM7 can be classified as class III rice prolamin cDNAs. Although rice prolamin polypeptides lack the repetitive sequences, polyproline, and polyglutamine, which are prevalent in other cereal prolamins, they do have homology to other cereal prolamins in several regions of the amino acid



sequences. Class III prolamins pS18 and λRM 7 share a unique octapeptide, Gln-Gln-Gln-Cys-Cys-Gln-Gln-Leu, with other cereal prolamins (Masumura *et al.*, 1990). In addition, high homology (~50%) was observed among rice prolamins, barley B1- and γ-hordein (Forde *et al.*, 1985; Cameron-Mills and Brandt, 1988), wheat α/β- and γ-gliadin (Okita *et al.*, 1985), and rye γ-secalin (Kreis *et al.*, 1985). These regions corresponded to the signal sequences (Fig. 5A), the residues 30–54 (Fig. 5B) and the residues 92–113 (Fig. 5C) of pS 18.

Gene Expression of Rice Prolamin During Seed Maturation

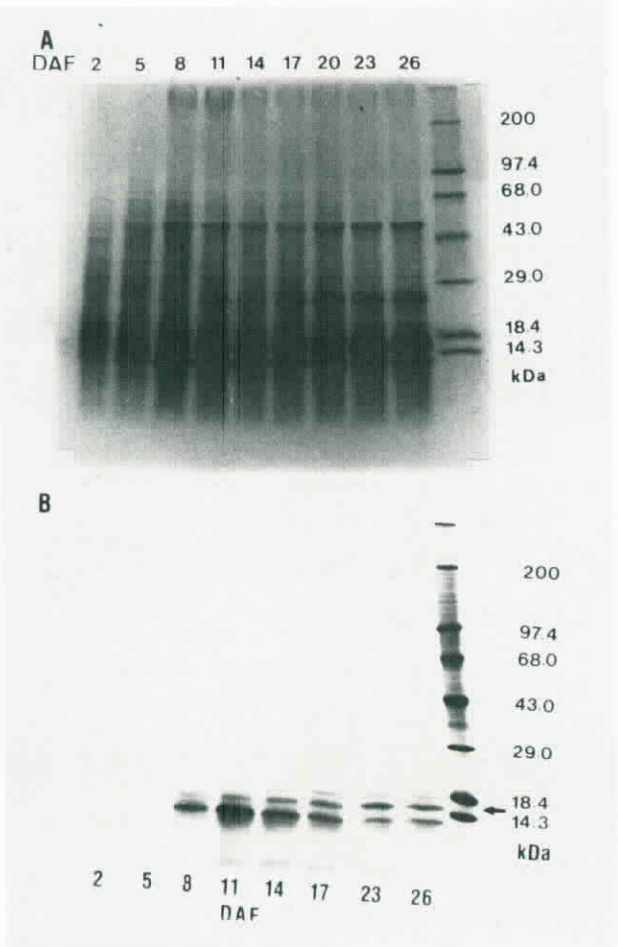


Fig. 6. Autoradiograms of *in vitro* translation products of poly (A)⁺ RNA from developing rice grains. One μg of poly (A)⁺ RNA from 2→26 DAF rice grains was subjected to *in vitro* translation as described in Fig. 1. The total translation products (A) and the alcohol-soluble polypeptides extracted from the total translation products (B) were analyzed by 5–20% mini SDS-PAGE and detected by autoradiography.

The poly(A)⁺ RNA fractions obtained in the aforementioned experiment were used for *in vitro* translation in the presence of radioactive amino acids. The total translation products were subjected to the serial extraction steps of the Osborne fractionation scheme (Shyur *et al.*, 1988). The total translation products and the 70% alcohol soluble polypeptides were separated by electrophoresis and detected by autoradiography (Fig. 6). Synthesis of the 16 kD prolamin (indicated by an arrow) began around 8 DAF and increased markedly during the 8–11 DAF. This is in good agreement with the results of Northern-blot analysis (Shyur *et al.*, 1992). Total proteins were also extracted from the developing rice seeds at 3 day intervals, separated by SDS-PAGE, and subjected to Western-blot analysis. The results also showed that the synthesis of prolamins began around 8–11 DAF and reached a maximum level about 35 DAF (data not shown).

The synthesis of 12 kD prolamin was first detected from poly(A)⁺ RNA obtained around 8 DAF, the maximal level of this synthesis was detected around 11 DAF and gradually decreased to a constant level (Fig. 6). A prolamin-like protein with a molecular weight of about 18 kD was also observed. Neither 14 kD prolamin nor 10 kD prolamin was found, suggesting that these two prolamin molecules were derived from precursor proteins, presumably the 16 kD and 12 kD prolamins, by post-translational modification. Experiments supporting this point of view were previously reported (Masumura *et al.*, 1990; Masumura *et al.*, 1989; Krishnan and Okita, 1986).

Discussion

The major storage proteins of most cereals have been extensively studied at the biochemical and molecular levels. Recent studies on rice prolamins have focused on the 16, 14 and 10 kD polypeptides. The heterogeneity of the major 16 and 14 kD prolamins have been revealed by cDNA cloning (Kim and Okita, 1988; Masumura *et al.*, 1990) and polypeptide analyses (Ogawa *et al.*, 1987; Hibino *et al.*, 1989). Hibino *et al.* (1989) reported that the 14 kD prolamin consisted of seven polypeptides. Our results of two-dimensional gel electrophoresis also showed that both 16 kD and 14 kD prolamins contained at least six polypeptides with similar molecular weight, but different isoelectric points

(data not shown). Several cDNA clones of rice prolamin were previously characterized (Kim and Okita, 1988; Masumura *et al.*, 1990; Masumura, 1989).

To our knowledge, four class I cDNAs (pProl 7, pProl 14 (Kim and Okita, 1988), pX24 and pS23 (Shyur and Chen, 1990)), one class II (pProl 17 (Kim and Okita, 1988)) and two class III (pS18 (Shyur *et al.*, 1992)) and λRM 7 (Masumura *et al.*, 1990)) have so far been characterized. The divergency of rice prolamin cDNAs appears to be the consequence of point mutations at codon positions 1 and 2 or the wobble position and the insertion/deletion of base(s) or short nucleotide stretches. It appears that the main differences in the primary structure of the three classes of prolamins are the insertion or deletion of a six amino acid oligopeptide. A comparison of their amino acid sequence homologies is shown in Fig. 3. The class I and class II cDNAs delete six consecutive amino acid residues at positions 82→87 and positions 34→39 from the amino terminus, respectively. Owing to these events in gene evolution of rice prolamin, restriction mapping, which is an useful method for characterization of DNA molecules, cannot be used to preliminarily characterize rice prolamin cDNA (Fig. 1).

Analysis of *in vitro* translational products of hybrid-selected poly(A)$^+$ RNA complementary to pS18 revealed the synthesis of two polypeptides with molecular weight of approximately 16 kD and 12 kD (Fig. 2). In light of the finding that the endosperm of *Oryza sativa* L. cv Tainung 67 contained two major prolamin proteins with molecular weights of 15.5 kD and 14.2 kD (data not shown), it is highly likely that 16 kD polypeptide is the precursor of 14.2 kD prolamin. Our results are in accord with the previous work by Masumura *et al.* (1990), and Krishnan *et al.* (1986). Rice endosperm also contains 10 kD prolamin (Ogawa *et al.*, 1987; Hibino *et al.*, 1989). The 12 kD polypeptide synthesized in the *in vitro* translation experiments was considered to be the precursor of the 10 kD prolamin (Masumura *et al.*, 1989). Immunological cross-reactivity between the 16 kD and 12 kD polypeptides (Fig. 2) indicated that these two prolamin molecules have conserved sequence or epitopes.

Rice prolamins are a group of low molecular weight alcohol soluble polypeptides lacking the typical cereal prolamin's repetitive sequences, polyglutamine and polyproline regions. However, in addition to the octapeptide, Gln-Gln-Gln-Cys-Cys-Gln-Gln-Leu (Masumura *et al.*, 1990), there are conserved amino acid sequences found in three non-repetitive regions of some cereal prolamins and rice prolamins as mentioned above (Fig. 3). The results indicated that these domain have a common evolutionary origin. The anti-serum raised against rice prolamin cross-reacted with prolamins of maize, sorghum and barley (data not shown). On the other hand, there was no immunological cross-reactivity and nucleotide sequence homology between prolamins and glutelins of rice and other cereals. These results also suggested that at least part of the prolamin structural genes have evolved from a similar ancestral gene.

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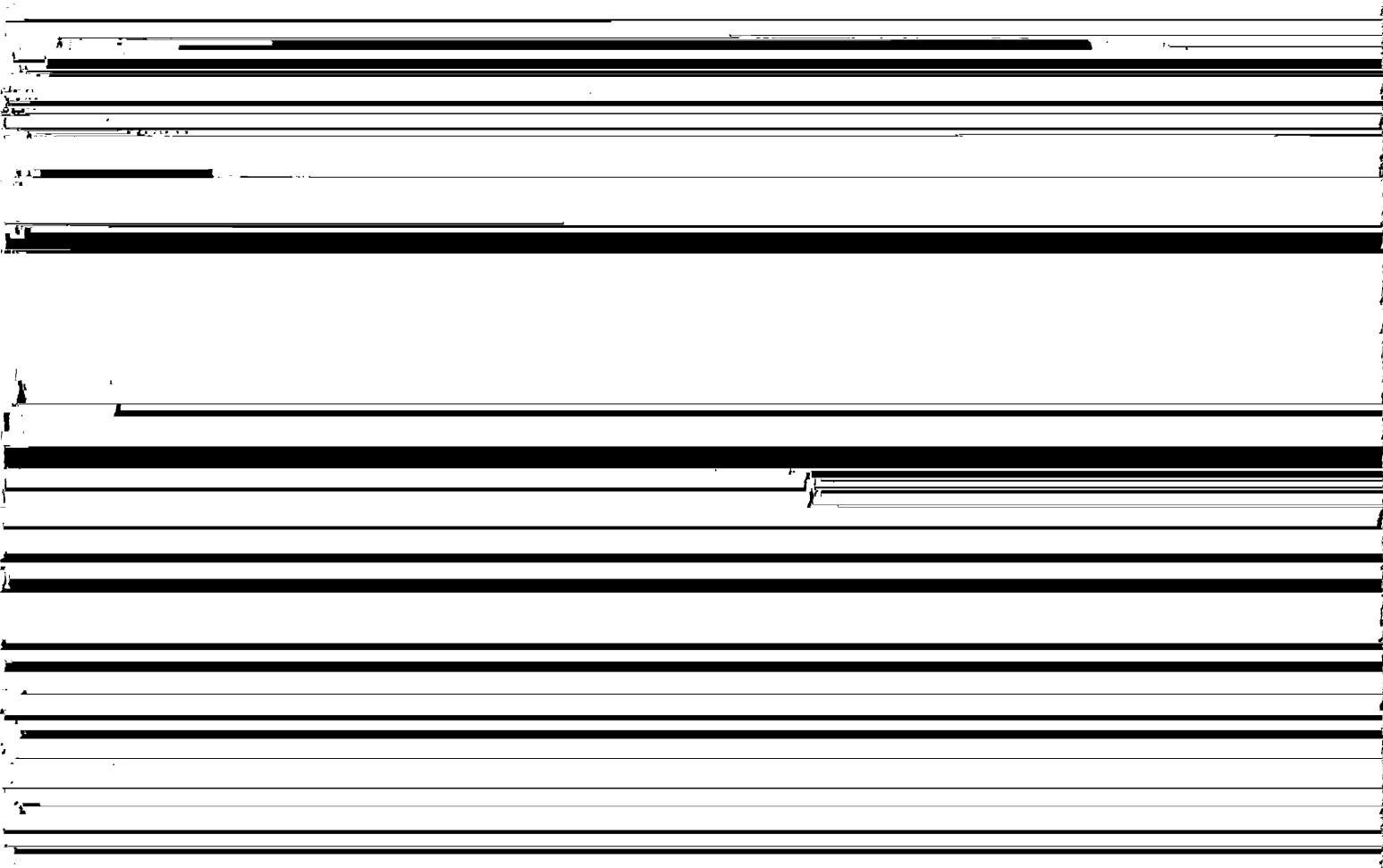

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水稻醇溶蛋白質：cDNA 異質性及前體的合成

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以水稻乳熟期胚乳的 poly(A)$^+$ RNA 構築 cDNA 庫。自此 cDNA 庫分離出三株 cDNA clones: pX24，pS23 及 pS18。此三株 cDNAs 都具有單一 open reading frame，可分別合成含 149、150 及 158 胺基酸的多肽。用 cDNA 雜交挑選的特異性 poly(A)$^+$ RNA 進行試管內轉譯試驗，結果顯示轉譯產物是 16 kD 及 12 kD 兩種多肽。16 kD 多肽可能是 14 kD 醇溶蛋白質的前體，而 12 kD 多肽是 10 kD 醇溶蛋白質的前體。比較水稻與其他穀類醇溶蛋白質之胺基酸序列，發現有三個具有同源性 (homology) 的區段，表示醇溶蛋白質基因至少有一部份可能是由類似基因演化而來。水稻約於開花後 8 天開始合成 16 kD 醇溶蛋白質前體，8-11 天間此前體迅速增加。12 kD 醇溶蛋白質前體約於水稻開花 8 天後出現於胚乳，11 天左右達到最高量，然後緩慢降低，最後維持一定含量到種子成熟。