71

Expressed Sequence Tags in Developing Anthers of Rice (Oryza sativa L.)

Yukako HIHARA*.[†], Keiko SHODA*, Qiang LIU*.**, Chikage HARA*, Masaaki UMEDA*, Kinya TORIYAMA*** and Hirofumi UCHIMIYA*

*Institute of Molecular and Cellular Biosciences, University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113, Japan

**Institute of Life Sciences and Biotechnology, Tsinghua University, Beijing 100084, China

*** Faculty of Agriculture, Tohoku University, Tsutsumi-dori amemiya-cho 1-1, Aoba-ku, Sendai 981, Japan

Received 3 October 1996; accepted 26 November 1996

In the early stages of microspore development, dramatic changes in nuclear phase and cell morphology occur [1]. The pollen mother cells undergo meiosis, giving rise to a tetrad of haploid cells. After microspores are released to the anther locule, they grow rapidly and cell walls are actively synthesized. These successive changes are not caused by the male gametophyte alone but are consequences of the cooperative works of gametophyte and surrounding sporophytic tissues such as the tapetum. For example, tapetal cells secrete β -1, 3–glucanase (callase) at the time of microspore release to dissolve the callose wall which surrounds the tetrads, and provide nourishment and structural components to the microspores at the appropriate time of development.

A large number of proteins may participate in the intricate process of anther development showing a strictly controlled expression pattern both temporally and spatially. Further, it is remarkable that their gene expression seems to be controlled differentially or in a coordinated manner between sporophytic and gametophytic tissues. To gain insight into the molecular processes underlying anther development, several anther-specific genes have been obtained by differential screening. The isolation of anther-or pollen-specific genes have been reported for some plant species (reviewed by [2, 3]). The roles of their gene products have been elucidated by sequence similarity, immunolocalization and the introduction of antisense constructs using transgenic techniques. However, for the systematic analysis of gene expression, it is necessary to isolate many homologous probes from one plant species and to characterize their expression patterns in terms of developmental stages and cell types.

Random cDNA sequencing is a powerful approach for the isolation of many functional genes which are expressed in a particular tissue or under specific environmental conditions [4]. Recently, a large number of cDNA clones were identified by random sequen-

[†] Present address: Department of Biology, University of Tokyo, Komaba 3-8-1, Meguro-ku, Tokyo 153, Japan cing from human [5, 6], *Caenorhabditis elegans* [7, 8], mouse [9], maize [10], *Arabidopsis thaliana* [11], *Brassica napus* [12] and rice [13–15]. Now it is clear that these studies (the expressed sequence tags, ESTs) have greatly contributed to increased gene resources in each organism. Moreover, the cDNA clones thus identified can be used for expression analysis of genes engaged in specific metabolic pathways such as glycolysis, alcohol fermentation and the TCA cycle [15, 16]. These results showed that ESTs provide us the opportunity to analyze the regulation of gene expression under specific conditions.

In order to survey the ESTs of early stages of anther development, we identified a number of putative genes from cDNA clones of rice anthers at the uninucleate microspore stage. Further, by using the isolated cDNA clones as probes, the transcript levels during anther development were investigated.

Oryza sativa L. var. Hayayuki plants were grown in a greenhouse. Poly(A)+ RNA was extracted from anthers at the uninucleate microspore stage ($\leq 2 \text{ mm}$ in length) using the Fast Track mRNA isolation kit (Invitrogen). cDNAs synthesized using cDNA synthesis kit (Pharmacia) were ligated with EcoRI adapters to introduce them into the EcoRI site of the $\lambda ZAPII$ vector (STRATAGENE). In vivo excision of pBluescript plasmids was performed in the Escherichia coli K-12 strain XL1-Blue. Partial nucleotide sequences of cDNA inserts were determined by the fluorescence detection method [17] using either dye-labeled T3 or T7 primer. A Model 373A sequencer (Applied Biosystems) was used for automated sequence analysis. The homology of each cDNA sequence averaging 300 bases was searched in the recorded nucleic acid sequences of GenBank and EMBL databases using the FASTA program. Homology scores (>160) were searched for amino acids [13, 15].

From this analysis, partial nucleotide sequences of 653 cDNA clones were determined. Seventy-seven cDNA clones, which occupy 11.8% of the total cDNAs, had significant similarity to nucleotide sequences registered in the databases (**Table 1**). The

Table	1.
-------	----

Summary of ESTs isolated from cDNA library of rice anthers at uninucleate microspore stage.

No. compared (p) matched (p) compared (p) matched (p) compared (p) D21130 Acidic ribosonal protein P2 113 7.9 37 7.8.4 TCP2BNRNA D29690 Acjdic ribosonal protein P2 113 7.9 37 7.8.4 TCP2BNRNA D29690 Acjdic ribosonal protein P2 113 7.9 9.7 9.3.5 ATHADPRFA D29692 Calrondulin 178 82.5 47 9.3.5 ATHADPRFA D29692 Calrondulin 178 87.5 91.6 OSCALM D29695 Cancin kinase I- γ 392 62.1 44 68.2 DIOCARS D29695 Chackone synthase 179 83.4 50 84.7 S03393 D29695 Chalcone synthase 179 84.4 89 7.9.5 M2CCYP D29700 Chicroplast genome (ORF109) 306 9.7 9.1 10.0 RICOSX D29700 Chicroplast genome (ORF109) 306 9.7 7.0	Accession	Gene	Length	%	Length	%	Source of
(bp) (bp) (a) (a) D21130 Acidic ribosonal protein P2 113 696 45 57.3 HUMPPARP1 D29690 Acyl carrier protein II 178 77.4 55 BLYACL2 D29690 Acyl carrier protein IC 178 78.4 58 T5.9 BLYACL2 D29690 Achier inbosonal protein Cato* 138 89.3 115 94.4 TOMCDHB D29693 Calmodulin* 178 97.8 59 100 OSCALM D29694 Casein kinase I-* 177 84.4 68.2 TMACK2 DMACK2 D29695 Calacone synthase 179 88.8 59 84.7 Xa3339 D29699 Chioroplast genome (DRF109) 36 99.7 91 100 RICCPOSXX D29699 Chioroplast genome (pabb) 171 94.4 55 98.2 CHOSXX D29700 Choroplast genome (pabb) 171 94.4 50 90.7 1100 RICCPOSXX	No.		compared	matched	compared	matched	comparison
			(bp)	(bp)	(aa)	(aa)	
D29869 Acidic ribosomal protein P2 113 77.9 37 78.4 TCP2DMTNA D29600 Acyl carrier protein II 178 73.6 58 75.9 BLYACL2 D29609 ADP-ribosylation factor 137 78.2.5 74 93.6 ATHADPFFA D29605 Candoulin* 178 97.8 59 100 OSCALM D29665 CARSR12 132 62.1 44 68.2 DINCARSR12 D29666 Carsin kinase I+γ 342 56.7 111 44.0 BOVCKIG D29669 Chalcone synthase 179 88.8 59 84.7 X3333 D29699 Chloroplast genome (DRF109) 366 99.7 91 100 RICCPOSX D29700 Choroplast genome (psbb) 174 99.4 55 98.2 CHOSX D29701 Cyclophilin* 29 81.4 89 79.8 MZECYP D29702 Choroplast genome (psbb) 140 70.0 47	D21130	Acidic ribosomal protein P0	135	69.6	45	57.8	HUMPPARP 1
D29560 Acyl carrier protein II 178 736 58 75.9 FNLACL2 D29681 ADP ribosylation factor* 137 82.5 47 93.6 ATHADPRA D29692 ATP dependem protease 348 80.5 115 94.8 TOMCDAB D29695 CARSN12 132 62.1 44 68.2 DINCARSN12 D29694 Casein kinase 1-7 342 56.7 111 44.0 BONCKIG D29695 Chalcone synthase 176 81.4 87 72.1 SPICTNICK D29696 Choroplast genome (DF100) 36 99.7 91 100 RICCPOSX X D29700 Cyclophilin* 259 81.4 89 78.8 MZCYP D29701 Cyclophilin* 264 93.9 86 100 SIG4689 D29703 Elongation factor 1-4* 22 84.4 34 79.4 ZMGYP D29704 Ghytome b-5* 160 67.3 97.7 FIG508	D29689	Acidic ribosomal protein P2	113	77.9	37	78.4	TCP2BMRNA
D29691 ADP-rhosplation factor 137 92.5 47 93.6 ATTHADPFFA D29692 ATP dependent protease 348 80.5 115 94.8 TOMCD4B D29696 Calmodulin* 178 97.8	D29690	Acyl carrier protein II	178	73.6	58	75.9	BLYACL2
D29692 ATP-dependent protease 348 90.5 115 94.8 TOMCABB D29693 Calmodulin* 178 97.8 59 100 OSCALM D29666 CARSN12 132 62.1 44 68.2 DINCARSN12 D29664 Casein kinase 1-\$\sigma\$ 177 81.4 56 87.5 ZMACK2 D29667 Chalcone synthase 179 88.8 59 84.7 X58339 D29668 Chaperonin 10 132 73.5 43 72.1 SPICPN10X D29709 Choroplast genome (DRF109) 366 99.7 91 100 RICCPOSX X D29702 Cyclophilin* 264 93.9 86 100 SIL6499 D29703 Elongation factor 1-\$\scimash 264 93.9 86 100 SIL6499 D29704 GranknA (cloneF) 140 70.0 47.4 PSINS D29705 GAmRNA (cloneF) 213 75.2 43 90.7 TOHSINS	D29691	ADP-ribosylation factor*	137	82.5	47	93.6	ATHADPRFA
D29903 Calmoduln* 178 97.8 59 100 OSCALM D29905 CARSR12 132 62.1 44 68.2 DINCARSR12 D29904 Casein kinase I-γ 342 56.7 111 44.0 BOVCKIG D29967 Chalcone synthase 177 81.4 56 87.5 X3339 D29698 Chalcone synthase 179 88.8 59 84.7 X3339 D29699 Chloroplast genome (ORF109) 306 96.7 91 100 RICCCOSXX D29700 Choroplast genome (DRF109) 306 96.7 91.0 BNACYTESA D29701 Cyclophilin* 264 93.9 86 100 S116489 D29705 GAmRNA (cloneF) 141 60.3 25 80.0 CREGELP D29705 GAmRNA (cloneF) 122 84.4 34 74.4 PSPHSP1 D29706 Gutamate I-semialdebyde amintransferase 129 75.2 43 90.7 78.4	D29692	ATP-dependent protease	348	80.5	115	94.8	TOMCD4B
D29665 CARSEL2 132 62.1 44 66.2 DINCARSEL2 D29666 Casein kinase I $-x$ 177 81.4 66 87.5 ZMACK2 D29667 Chalcone synthase 179 88.8 59 84.7 XS3339 D29668 Chaperonin 10 132 73.5 84.3 72.1 SPICPNIOX D29699 Chloroplast genome (pBIB) 174 99.4 55 98.2 CHOSX D29700 Cytochrome b-5* 150 67.3 51 71.0 BNACYTB5A D29702 Cytochrome b-5* 150 67.3 51 71.0 BNACYTB5A D29704 Goratin factor 1-x* 264 93.9 66 100 St16489 D29705 GAMRNA (cloneF) 140 70.0 47 77.9 PEAGAMEF D29706 Givtinc rich protein* 122 84.4 34 79.4 ZMGCHY D29707 Givtinc rich protein* 123 69.3 97 78.4	D29693	Calmodulin*	178	97.8	59	100	OSCALM
D29694 Case in kinase II - α 342 56.7 111 44.0 BOVCKIG D29697 Chalcone synthase 179 88.4 56 87.5 ZMACK2 D29698 Chaperonin 10 132 73.5 43 72.1 SPICPNI0X D29699 Chioroplast genome (psbB) 174 99.4 55 98.2 CHOSXX D29700 Choroplast genome (psbB) 174 99.4 53 98.2 CHOSXX D29702 Cyclophilin* 264 93.9 86 10.0 SI16449 D29705 GAnRNA (choeF) 140 70.0 47.7 PEAGAMFF D29706 Glutamate 1-semialdehyde-aminotransferase 129 75.2 43 90.7 BLYGSA D29706 Glutamate 1-semialdehyde-aminotransferase 129 75.1 95 84.2 THEHSP91 D29709 Heat shock protein 70* 293 69.3 97 78.4 PSPHSP1 D29709 Heat shock protein 70* 293 75.1 95 84.2 THEHSP90 D29710 High mobility group protein	D29695	CARSR12	132	62.1	44	68.2	DINCARSR12
D29566 Casein kinase II - a 177 81.4 56 87.5 ZMACK2 D29697 Chalcone synthase 179 88.8 59 84.7 X38333 D29698 Chaorenin 10 132 73.5 43 72.1 SPICCPN10X D29699 Chloroplast genome (0RF109) 306 99.7 91 100 RICCPOSXX D29709 Chloroplast genome (0RF109) 306 99.7 91 100 RICCPOSXX D29704 Cyclophilin* 269 81.4 89 9.8 MZCCYP D29704 G-protein β -subunit-like polypeptide 141 60.3 25 80.0 CREGPLP D29704 Glycine-rich protein* 122 84.4 34 79.4 ZMGLYR D29704 Glycine-rich protein* 122 84.4 34 79.4 ZMGLYR D29704 Glycine-rich protein* 123 63.0 65 79.0 TOBHSP2 D29704 Heat shock protein 80* 233 75.1	D29694	Casein kinase $1-\gamma$	342	56.7	111	44.0	BOVCKIG
D28697 Chalcone synthase 179 88.8 59 84.7 X58339 D28989 Charperini 10 132 73.5 43 72.1 SPICPN10X D28989 Chloroplast genome (0RF109) 306 99.7 91 100 RICCPOSXX D29700 Chloroplast genome (psbB) 174 99.4 55 98.2 CHOSXX D29701 Cytochrome 5-5* 150 67.3 51 71.0 BXACTTBSA D29703 Elongation factor 1-a* 264 93.9 86 100 S116489 D29704 G-protein f-subuit-like polypeptide 141 60.3 25 80.0 CREGPLP D29705 Glutamate 1-semialdehyde-aminotransferase 129 75.2 43 90.7 78.4 PSPHSP1 D29709 Heat shock protein 70* 293 65.3 97 78.4 PSPHSP1 D29709 Heat shock protein 82* 30 65.0 66 70.0 SPHISP1 D29710 Histat shock protein 90* <td>D29696</td> <td>Casein kinase II-α</td> <td>177</td> <td>81.4</td> <td>56</td> <td>87.5</td> <td>ZMACK2</td>	D29696	Casein kinase II- α	177	81.4	56	87.5	ZMACK2
D23698 Chaperonin 10 132 73.5 43 72.1 SPICPN10X D23699 Chloroplast genome (ORP109) 306 99.7 91 100 RICCPOSXX D23700 Chloroplast genome (pbB) 174 99.4 55 98.2 CHOSXX D23701 Cyclophilin* 269 81.4 89 79.8 MZECYP D23702 Cyclorme b-5* 150 67.3 51 71.0 BXACYTB5A D23703 Elongation factor 1-a* 264 93.9 86 100 STI6489 D23704 G-protein A-subunit-like polypeptide 141 60.3 25 80.0 CREGPLP D23707 Glycine-rich protein* 212 84.4 34 79.4 ZMGLYR D23708 Heat shock protein 72* 203 66.3 97 78.4 PSPHSP1 D29709 Heat shock protein 82* 30.6 6 70.0 TDBHSP2 D29710 Heat shock protein 82* 195 87.1 95	D29697	Chalcone synthase	179	88.8	59	84.7	X58339
D2869 Chloroplast genome (ORF109) 306 99.7 91 100 RICCPOSXX D29700 Chloroplast genome (psbB) 174 99.4 55 98.2 CHOSXX D29701 Cyclophilin* 269 81.4 89 70.8 MZECYP D29702 Cyclophilin* 264 93.9 86 100 SIB6489 D29704 G-protein factor 1-a* 264 93.9 86 100 CRECPLP D29705 GAmRNA (clonef) 140 70.0 47 77.9 PEAGAMRF D29706 Glutamate 1-semialdehyde-aminotransferase 123 86.3 97 78.4 PSPHSP1 D29709 Heat shock protein % 293 65.3 97 78.4 PSPHSP1 D29709 Heat shock protein 80° 293 75.1 95 84.2 THENSP80 D29711 High mobility group protein* 163 76.4 97.0 SPHISP1 D29712 Histone H2* 204 81.4 65	D29698	Chaperonin 10	132	73.5	43	72.1	SPICPN10X
D29700Chloroplast genome (psbB)17499.45598.2CHOSXXD29701Cyclophilin*26981.48979.8MZECYPD29702Cyclophrome b 5*15067.35171.0BNACYTBSAD29703Elongation factor 1- a^* 26493.986100S116489D29704G-protein β -subunit-like polypeptide14160.32580.0CRECPLPD29705GAmRNA (cloneF)14070.04777.0PEAGAMRFD29706Glutamate 1-semialdehyde- aminotransferase12975.24390.7BLYGSAD29709Heat shock protein 70*29363.06679.0TOBHSP82D29709Heat shock protein 70*29375.19584.2THEHSP90D29710Heat shock protein 70*29375.19584.2THEHSP90D29711High mobility group protein*16377.45068.0ZMHMCPMRD29712Histone H25*19587.76590.8TAHISTH2BD29714Hydroxyprolinc-rich glycoprotein13310040100S86024D29714Hydroxyprolinc-rich glycoprotein13310040100S86024D29714Hydroxyprolinc-rich glycoprotein13310040100RCEIF4AD50570Initiation factor (eIF -4C)16365.64663.0200633AD29716NADPH HC-toxin reductase22680	D29699	Chloroplast genome (ORF109)	306	99.7	91	100	RICCPOSXX
D23701 Cyclophilin* 269 81.4 89 79.8 MZECYP D29702 Cytochrome b-5* 156 67.3 51 71.0 BNACYTB5A D29703 Elongation factor 1-a* 264 93.9 86 100 S116489 D29705 GAmRNA (cloneF) 140 70.0 47 77.0 PEAGAMRF D29706 Glutamate 1-semialdehyde-aminotransferase 129 75.2 43 90.7 BLAGAMRF D29707 Glycine rich protein* 122 84.4 79.4 PSPHSP1 D29709 Heat shock protein 80* 293 65.3 97 78.4 PSPHSP2 D29710 Heat shock protein 80* 293 75.1 95 84.2 THEHSP80 D29711 Higtonebility group protein* 163 72.4 50 68.0 ZMHMGPMR D29712 Histone H2* 204 81.4 65 97.0 SPHISH3 D29714 Hydroxyproline-rich glycoprotein 133 100 40 </td <td>D29700</td> <td>Chloroplast genome (psbB)</td> <td>174</td> <td>99.4</td> <td>55</td> <td>98.2</td> <td>CHOSXX</td>	D29700	Chloroplast genome (psbB)	174	99.4	55	98.2	CHOSXX
D23702 Cytochrome b 5* 150 67.3 51 71.0 BNACYTB5A D29703 Elongation factor 1-x* 264 95.9 86 100 S116489 D29704 G-protein J-aubunit-like polypeptide 141 60.3 25 80.00 CRECFLP D29705 GLutamate 1-semialdehyde-aninotransferase 122 75.2 43 90.7 BLYCSA D29706 Glutamate 1-semialdehyde-aninotransferase 122 84.4 34 79.4 ZMGLYR D29709 Heat shock protein 70* 293 65.1 95.0 84.2 THEHSP90 D29710 Heat shock protein 90* 293 75.1 95 84.2 THEHSP90 D29711 High mobility group protein* 163 72.4 50 68.0 ZMHMGPMR D29712 Histone H3* 204 81.4 65 97.0 SPHISH34 D29711 Hydroxyproline-rich glycoprotein 133 100 40 100 865624 D29715 Initiation facto	D29701	Cyclophilin*	269	81.4	89	79.8	MZECYP
D29703Elongation factor $1-a^*$ 26493.986100S116489D29704G-protein β -subunit-like polypeptide14160.32580.0CREGPLPD29705GAmRNA (cloneF)14070.04777.0PEAGAMRFD29706Glutamate 1-semialdehyde-aminotransferase12975.24390.7BLYCSAD29707Glycine-rich protein*12284.43479.4ZMGLYRD29708Heat shock protein 70*29369.39778.4PSPHSP1D29709Heat shock protein 82*30066.079.0TOBHSP82D29710Heat shock protein 90*29375.19584.2THEHSP90D29711Histone H3*20481.46597.0SPHISH34D29712Histone H3*20481.46597.0SPHISH34D29713Histone H3*20481.46597.0SPHISH34D29714Hydroxyproline-rich glycoprotein13310040100S85024D29715Initiation factor (eIF-4A)*29398.07100RICEIF4AD5570Initiation factor (eIF-4A)*20485.1RICANT2D29715NADPH HC-toxin reductase22680.57178.0MZETOXRD17443NdOH2H HC-toxin reductase23660.520WHTPSB0D29715Ortyzacystatin20466.662.0WHTPSB0D29717O	D29702	Cytochrome b–5*	150	67.3	51	71.0	BNACYTB5A
D29704G-protein β -subunit-like polypeptide14160.32580.0CREGPLPD29705GAmRNA (clonef)14070.04777.0PEAGAMRFD29706Glutamate 1-semialdehyde-aminotransferase12975.24390.7BLYGSAD29707Glycine-rich protein*12284.43474.4ZMGLYRD29708Heat shock protein 70*29369.39778.4PSPHSP1D29709Heat shock protein 82*30363.06679.0TOBHSP82D29711High mobility group protein*16372.45066.0ZMHMGPMRD29712Histone H2B*19587.76590.8TAHISTH2BD29713Histone H3*20481.46597.0SPHISH3D29714Hydroxyproline-rich glycoprotein13310040100S85024D29715Initiation factor (eIF-4A)*29398.07100RICEIF4AD50570Initiation factor (eIF-4A)*21064.86980.0SOVNOD26RD29715NADPH HC-toxin reductase22680.57178.0MZETOXRD1744Nodulin 2621064.86980.0SOVNOD26RD29717Oryzacystatin20410068100RICOCSD29718PLB0724865.5 $-$ LUPPLB07MD50599Protein kinase-Alfalfa (MSK-3)25366.08257.3AMMSK3A	D29703	Elongation factor $1-\alpha^*$	264	93.9	86	100	S116489
D29705 GAmRNA (cloneF) 140 70.0 47 77.0 PEAGAMRF D29706 Gutamate 1-semialdehyde-aminotransferase 129 75.2 43 90.7 BLYCSA D29707 Glycine-rich protein* 122 84.4 34 79.4 ZMGLYR D29708 Heat shock protein 70* 293 66.3 97 78.4 PSPHSP1 D29709 Heat shock protein 82* 330 63.0 66 79.0 TOBHSP82 D29710 Heat shock protein 90* 293 75.1 95 84.2 THEIHSP90 D29711 Histone H2B* 195 87.7 65 90.8 TAHISTH2B D29713 Histone H3* 204 81.4 65 97.0 SPHISH34 D29714 Hydroxyproline-rich glycoprotein 133 100 40 100 85024 D29715 Initiation factor (eIF-4A)* 293 98.0 7 100 RICEIFAA D50570 Initiation factor (eIF-4C) 163	D29704	G-protein β -subunit-like polypeptide	141	60.3	25	80.0	CREGPLP
D29706 Glutamate 1-semialdehyde-aminotransferase 129 75.2 43 90.7 BLYGSA D29707 Glycine-rich protein* 122 84.4 34 79.4 ZMGLYR D29708 Heat shock protein 70* 293 69.3 97 78.4 PSPHSP1 D29709 Heat shock protein 82* 330 63.0 66 79.0 TOBHSP82 D29710 Heat shock protein 90* 293 75.1 95 84.2 THEHSP90 D29711 Higt mobility group protein* 163 72.4 50 68.0 ZMIHMOPMR D29712 Histone H3* 204 81.4 65 97.0 SPHISH34 D29714 Hydroxyproline-rich glycoprotein 133 100 40 100 S85024 D29715 Initiation factor (eIF 4A)* 293 98.0 7 100 RICEIF4A D30507 Initiation factor (eIF 4A)* 293 98.0 7 100 RICEIF4A D29715 Inititation factor (eIF 4A)*	D29705	GAmRNA (cloneF)	140	70.0	47	77.0	PEAGAMRF
D29707 Glycine-rich protein* 122 84.4 34 79.4 ZMGLYR D29708 Heat shock protein 70* 293 69.3 97 78.4 PSPHSP1 D29709 Heat shock protein 82* 330 63.0 66 79.0 TOBHSP82 D29711 Higt mobility group protein* 163 72.4 50 68.0 ZMHMGPMR D29712 Histone H3* 204 81.4 65 90.8 TAHISTH2B D29713 Histone H3* 204 81.4 65 97.0 SPHISH34 D29714 Indition factor (eIF-4A)* 293 98.0 7 100 RICEIF4A D50570 Initiation factor (eIF-4C) 163 65.6 46 63.0 2066333A D29714 NADPH HC toxin reductase 226 80.5 71 78.0 MZETOXR D17443 Nodulin 26 210 64.8 69 80.0 S0YNOD26R D29717 Oryzacystatin 004 66 100	D29706	Glutamate 1-semialdehyde-aminotransferase	129	75.2	43	90.7	BLYGSA
D29708 Heat shock protein 70* 293 69.3 97 78.4 PSPHSP1 D29709 Heat shock protein 82* 330 63.0 66 79.0 TOBHSP82 D29710 Heat shock protein 90* 293 75.1 95 84.2 THEHSP90 D29711 Histone H2B* 195 87.7 65 90.8 TAHISTH2B D29712 Histone H3* 204 81.4 65 97.0 SPHISH34 D29713 Histone H3* 204 81.4 65 97.0 SPHISH34 D29714 Hydroxyproline-rich glycoprotein 133 100 40 100 885024 D29715 Initiation factor (eIF-4A)* 293 98.0 7 100 RICEFAA D50570 Initiation factor (eIF-4C) 163 65.6 46 63.0 206633A D29716 NADPH HC-toxin reductase 226 80.5 71 78.0 MZETOXR D29717 Oryzacystatin 204 100 68 <td>D29707</td> <td>Glycine-rich protein*</td> <td>122</td> <td>84.4</td> <td>34</td> <td>79.4</td> <td>ZMGLYR</td>	D29707	Glycine-rich protein*	122	84.4	34	79.4	ZMGLYR
D29709 Heat shock protein 82* 330 63.0 66 79.0 TOBHSP82 D29710 Heat shock protein 90* 293 75.1 95 84.2 THEHSP90 D29711 High mobility group protein* 163 72.4 50 66.0 ZMHMGPMR D29712 Histone H2B* 195 87.7 65 90.8 TAHISTH2B D29713 Histone H3* 204 81.4 65 97.0 SPHISH34 D29714 Hydroxyproline-rich glycoprotein 133 100 40 100 S85024 D29715 Initiation factor (eIF-4A)* 293 98.0 7 100 RICEIF4A D50570 Initiation factor (eIF-4C) 163 65.6 46 63.0 2006333A D29715 NADPH HC-toxin reductase 226 80.5 71 78.0 MZETOXR D17443 Nodulin 26 204 100 68 100 RICOCS D29717 Oryzacystatin 204 100 68	D29708	Heat shock protein 70*	293	69.3	97	78.4	PSPHSP1
D29710Heat shock protein 90*29375.19584.2THEHSP90D29711High mobility group protein*16372.45068.0ZMHMGPMRD29712Histone H2B*19587.76590.8TAHISTH2BD29713Histone H3*20481.46597.0SPHISH34D29714Hydroxyproline-rich glycoprotein13310040100S85024D29715Initiation factor (eIF-4A)*29398.07100RICEIF4AD50570Initiation factor (eIF-4C)16365.64663.02006333AD29716NADPH HC-toxin reductase22680.57178.0MZETOXRD17443Nodulin 2621064.86980.0SOYNOD26RD29739Osc631585.1RICANT2D2110933kDa oxygen evolving protein8076.32662.0WHTPSB0D2113Polypeptide chain-binding protein43187.214183MZEB70AD50572Polyubiquitin10186.1CSPORUBID29718pPLB0724868.5LUPLB07MD50569Protein kinase-Alfalfa (MSK-3)25366.08257.3AMMSK3AD29719Ras-related GTP binding protein*26497.07100RICSS20D29720Ribosomal protein L2614262.04459.1HUMRPL7YD29721 <t< td=""><td>D29709</td><td>Heat shock protein 82*</td><td>330</td><td>63.0</td><td>66</td><td>79.0</td><td>TOBHSP82</td></t<>	D29709	Heat shock protein 82*	330	63.0	66	79.0	TOBHSP82
D29711High mobility group protein*16372.45068.0ZMHMGPMRD29712Histone H2B*19587.76590.8TAHISTH2BD29713Histone H3*20481.46597.0SPHISH34D29714Hydroxyproline-rich glycoprotein13310040100S85024D29715Initiation factor (eIF \cdot A)*29398.07100RICEIF4AD50570Initiation factor (eIF \cdot C)16365.64663.02006333AD29715NADPH HC-toxin reductase22680.57178.0MZETOXRD17443Nodulin 2621064.86980.0SOYNOD26RD29717Oryzacystatin20410068100RICOCSD29739Osc631585.1RICANT2D2110933kDa oxygen evolving protein8076.32662.0WHTPSB0D21113Polypeptide chain-binding protein43187.214183MZEB70AD29718pPLB0724868.5LUPLB07MD50569Protein kinase-Alfalfa (MSK-3)25366.08257.3AMMSK3AD29719Ras-related GTP binding protein*26497.07100RICSS20D21114Ribosomal protein L213320461.86364.0CRERIBPL31D29720Ribosomal protein L3320461.86364.0CRERIBPL31 <t< td=""><td>D29710</td><td>Heat shock protein 90*</td><td>293</td><td>75.1</td><td>95</td><td>84.2</td><td>THEHSP90</td></t<>	D29710	Heat shock protein 90*	293	75.1	95	84.2	THEHSP90
D29712Histone H2B*19587.76590.8TAHISTH2BD29713Histone H3*20481.46597.0SPHISH34D29714Hydroxyproline-rich glycoprotein13310040100S85024D29715Initiation factor (eIF-4A)*29398.07100RICEIF4AD50570Initiation factor (eIF-4C)16365.64663.02006333AD29716NADPH HC-toxin reductase22680.57178.0MZETOXRD17443Nodulin 2621064.86980.0SOYNOD26RD29717Oryzacystatin20410068100RICOCSD29719Osc631585.1RICANT2D2110933kDa oxygen evolving protein43187.214183MZEB70AD50572Polyubiquitin10186.1OSPORUBID29718pPLB0724868.5LUPPLB07MD50569Protein kinase-Alfalfa (MSK-3)25366.08257.3AMMSK3AD29720Ribosomal protein A233458.79363.0YSPRPA2D29721Ribosomal protein L1713972.74678.0HUMRPL2KXD29722Ribosomal protein L3820069.56274.0ENRIPRL38D29724Ribosomal protein L3820069.56274.0ENRIPRL38D29725Ribosomal protein L39161	D29711	High mobility group protein*	163	72.4	50	68.0	ZMHMGPMR
D29713Histone H3*204 $\$1.4$ 65 97.0SPHISH34D29714Hydroxyproline-rich glycoprotein13310040100S85024D29715Initiation factor (eIF-4A)*29398.07100RICEIF4AD50570Initiation factor (eIF-4C)16365.64663.02006333AD29716NADPH HC-toxin reductase22680.57178.0MZETOXRD17443Nodulin 2621064.86980.0SOYNOD26RD29717Oryzacystatin20410068100RICOCSD29739Osc631585.1RICANT2D2110933kDa oxygen evolving protein8076.32662.0WHTPSB0D21113Polypeptide chain-binding protein43187.214183MZEB70AD50572Polyubiquitin10186.1OSPORUBID29718pPLB0724868.5LUPPLB07MD50569Protein kinase-Alfalfa (MSK-3)25366.08257.3AMMSK3AD29720Ribosomal protein A233458.79363.0YSPRPA2D29721Ribosomal protein L713972.74678.0HUMRPL7YD29721Ribosomal protein L3721765.07270.8MMRP37AD29722Ribosomal protein L3820069.56274.0ENRIPRL38D29722Ribosomal prote	D29712	Histone H2B*	195	87.7	65	90.8	TAHISTH2B
D29714Hydroxyproline-rich glycoprotein13310040100S85024D29715Initiation factor (eIF-4A)*29398.07100RICEIF4AD50570Initiation factor (eIF-4C)16365.64663.02006333AD29716NADPH HC-toxin reductase22680.57178.0MZETOXRD17443Nodulin 2621064.86980.0SOYNOD26RD29717Oryzacystatin20410068100RICOCSD29739Osc631585.1RICANT2D2110933kDa oxygen evolving protein8076.32662.0WHTPSB0D21113Polypeptide chain-binding protein43187.214183MZEB70AD50572Polyubiquitin10186.1OSPORUBID29718pPLB0724868.5LUPPLB07MD50569Protein kinase-Alfalfa (MSK-3)25366.08257.3AMMSK3AD29710Ribosomal protein A233458.79363.0YSPRPA2D29720Ribosomal protein L713972.74678.0HUMRPL7YD29721Ribosomal protein L3120461.86364.0CRERIBPL31D29722Ribosomal protein L3820069.56274.0ENRIPRL38D29724Ribosomal protein L3820069.56274.0ENRIPRL38D29725Ribos	D29713	Histone H3*	204	81.4	65	97.0	SPHISH34
D29715 Initiation factor (eIF-4A)* 293 98.0 7 100 RICEIF4A D50570 Initiation factor (eIF-4C) 163 65.6 46 63.0 2006333A D29716 NADPH HC-toxin reductase 226 80.5 71 78.0 MZETOXR D17443 Nodulin 26 210 64.8 69 80.0 SOYNOD26R D29717 Oryzacystatin 204 100 68 100 RICCOS D29719 Osc6 315 85.1 - - RICANT2 D21113 Polypeptide chain-binding protein 80 76.3 26 62.0 WHTPSB0 D21113 Polypeptide chain-binding protein 431 87.2 141 83 MZEB70A D50572 Polyubiquitin 101 86.1 - - SOPRUBI D29718 pPLB07 248 68.5 - - LUPPLB07M D50569 Protein kinase-Alfalfa (MSK-3) 253 66.0 82 57	D29714	Hydroxyproline-rich glycoprotein	133	100	40	100	S85024
D50570 Initiation factor (eIF-4C) 163 65.6 46 63.0 2006333A D29716 NADPH HC-toxin reductase 226 80.5 71 78.0 MZETOXR D17443 Nodulin 26 210 64.8 69 80.0 SOYNOD26R D29717 Oryzacystatin 204 100 68 100 RICCCS D29739 Osc6 315 85.1 - - RICANT2 D21109 33kDa oxygen evolving protein 80 76.3 26 62.0 WHTPSB0 D29718 Polypeptide chain-binding protein 431 87.2 141 83 MZEB70A D50572 Polyubiquitin 101 86.1 - - OSPORUBI D29718 pPLB07 248 68.5 - - LUPPL807M D50569 Protein kinase-Alfalfa (MSK-3) 253 66.0 82 57.3 AMMSK3A D29719 Ras-related GTP binding protein* 264 97.0 7	D29715	Initiation factor (eIF-4A)*	293	98.0	7	100	RICEIF4A
D29716 NADPH HC-toxin reductase 226 80.5 71 78.0 MZETOXR D17443 Nodulin 26 210 64.8 69 80.0 SOYNOD26R D29717 Oryzacystatin 204 100 68 100 RICOCS D29739 Osc6 315 85.1 - - RICANT2 D21109 33kDa oxygen evolving protein 80 76.3 26 62.0 WHTPSB0 D21113 Polypeptide chain-binding protein 431 87.2 141 83 MZEB70A D50572 Polyubiquitin 101 86.1 - - UPPLB07 D50569 Protein kinase-Alfalfa (MSK-3) 253 66.0 82 57.3 AMMSK3A D29719 Ras-related GTP binding protein* 264 97.0 7 100 RICSS230 D29720 Ribosomal protein L7 139 72.7 46 78.0 HUMRPL7Y D29721 Ribosomal protein L31 204 61.8 63	D50570	Initiation factor (eIF-4C)	163	65.6	46	63.0	2006333A
D17443 Nodulin 26 210 64.8 69 80.0 SOYNOD26R D29717 Oryzacystatin 204 100 68 100 RICOCS D29739 Osc6 315 85.1 - - RICANT2 D21109 33kDa oxygen evolving protein 80 76.3 26 62.0 WHTPSB0 D21113 Polypeptide chain-binding protein 431 87.2 141 83 MZEB70A D50572 Polyubiquitin 101 86.1 - - OSPORUBI D29718 pPLB07 248 68.5 - - LUPPLB07M D50569 Protein kinase-Alfalfa (MSK-3) 253 66.0 82 57.3 AMMSK3A D29719 Ras-related GTP binding protein* 264 97.0 7 100 RICSS230 D29720 Ribosomal protein L7 139 72.7 46 78.0 HUMRPL7Y D29721 Ribosomal protein L37a 217 65.0 72 70.8 <td>D29716</td> <td>NADPH HC-toxin reductase</td> <td>226</td> <td>80.5</td> <td>71</td> <td>78.0</td> <td>MZETOXR</td>	D29716	NADPH HC-toxin reductase	226	80.5	71	78.0	MZETOXR
D29717 Oryzacystatin 204 100 68 100 RICOCS D29739 Osc6 315 85.1 - - RICANT2 D21109 33kDa oxygen evolving protein 80 76.3 26 62.0 WHTPSB0 D21113 Polypeptide chain-binding protein 431 87.2 141 83 MZEB70A D50572 Polyubiquitin 101 86.1 - - OSPORUBI D29718 pPLB07 248 68.5 - - LUPPLB07M D50569 Protein kinase-Alfalfa (MSK-3) 253 66.0 82 57.3 AMMSK3A D29719 Ras-related GTP binding protein* 264 97.0 7 100 RICSS230 D21114 Ribosmal protein L7 139 72.7 46 78.0 HUMRPL7Y D29720 Ribosmal protein L31 204 61.8 63 64.0 CRERIBPL31 D29721 Ribosmal protein L37a 217 65.0 72 <td< td=""><td>D17443</td><td>Nodulin 26</td><td>210</td><td>64.8</td><td>69</td><td>80.0</td><td>SOYNOD26R</td></td<>	D17443	Nodulin 26	210	64.8	69	80.0	SOYNOD26R
D29739 Osc6 315 85.1 RICANT2 D21109 33kDa oxygen evolving protein 80 76.3 26 62.0 WHTPSB0 D21113 Polypeptide chain-binding protein 431 87.2 141 83 MZEB70A D50572 Polyubiquitin 101 86.1 - - OSPORUBI D29718 pPLB07 248 68.5 - - LUPPLB07M D50569 Protein kinase-Alfalfa (MSK-3) 253 66.0 82 57.3 AMMSK3A D29719 Ras-related GTP binding protein* 264 97.0 7 100 RICSS230 D21114 Ribosomal protein A2 334 58.7 93 63.0 YSPRPA2 D29720 Ribosomal protein L7 139 72.7 46 78.0 HUMRPL7Y D29721 Ribosomal protein L26 142 62.0 44 59.1 HUMRPL26X D29722 Ribosomal protein L31 204 61.8 63 64.0 </td <td>D29717</td> <td>Oryzacystatin</td> <td>204</td> <td>100</td> <td>68</td> <td>100</td> <td>RICOCS</td>	D29717	Oryzacystatin	204	100	68	100	RICOCS
D21109 33kDa oxygen evolving protein 80 76.3 26 62.0 WHTPSB0 D21113 Polypeptide chain-binding protein 431 87.2 141 83 MZEB70A D50572 Polyubiquitin 101 86.1 - - OSPORUBI D29718 pPLB07 248 68.5 - - LUPPLB07M D50569 Protein kinase-Alfalfa (MSK-3) 253 66.0 82 57.3 AMMSK3A D29719 Ras-related GTP binding protein* 264 97.0 7 100 RICSS230 D21114 Ribosomal protein A2 334 58.7 93 63.0 YSPRPA2 D29720 Ribosomal protein L7 139 72.7 46 78.0 HUMRPL7Y D29721 Ribosomal protein L19* 86 89.5 20 90.0 RICSS504 D50571 Ribosomal protein L37a 217 65.0 72 70.8 MMRPL26X D29722 Ribosomal protein L37a 217 65.0 <td>D29739</td> <td>Osc6</td> <td>315</td> <td>85.1</td> <td>_</td> <td>_</td> <td>RICANT2</td>	D29739	Osc6	315	85.1	_	_	RICANT2
D21113 Polypeptide chain-binding protein 431 87.2 141 83 MZEB70A D50572 Polyubiquitin 101 86.1 - - OSPORUBI D29718 pPLB07 248 68.5 - - LUPPLB07M D50569 Protein kinase-Alfalfa (MSK-3) 253 66.0 82 57.3 AMMSK3A D29719 Ras-related GTP binding protein* 264 97.0 7 100 RICSS230 D21114 Ribosomal protein A2 334 58.7 93 63.0 YSPRA2 D29720 Ribosomal protein L7 139 72.7 46 78.0 HUMRPL7Y D29721 Ribosomal protein L19* 86 89.5 20 90.0 RICSS504 D50571 Ribosomal protein L31 204 61.8 63 64.0 CRERIBPL31 D29722 Ribosomal protein L37a 217 65.0 72 70.8 MMRP37A D29725 Ribosomal protein L38 200 69.5 62 74.0 ENRIPRL38 D29724 Ribosomal protein L41*	D21109	33kDa oxygen evolving protein	80	76.3	26	62.0	WHTPSB0
D50572 Polyubiquitin 101 86.1 OSPORUBI D29718 pPLB07 248 68.5 LUPPLB07M D50569 Protein kinase-Alfalfa (MSK-3) 253 66.0 82 57.3 AMMSK3A D29719 Ras-related GTP binding protein* 264 97.0 7 100 RICSS230 D21114 Ribosomal protein A2 334 58.7 93 63.0 YSPRPA2 D29720 Ribosomal protein L7 139 72.7 46 78.0 HUMRPL7Y D29721 Ribosomal protein L19* 86 89.5 20 90.0 RICSS504 D50571 Ribosomal protein L26 142 62.0 44 59.1 HUMRPL26X D29722 Ribosomal protein L31 204 61.8 63 64.0 CRERIBPL31 D29723 Ribosomal protein L37a 217 65.0 72 70.8 MMRP37A D29725 Ribosomal protein L38 200 69.5	D21113	Polypeptide chain-binding protein	431	87.2	141	83	MZEB70A
D29718 pPLB07 248 68.5 LUPPLB07M D50569 Protein kinase-Alfalfa (MSK-3) 253 66.0 82 57.3 AMMSK3A D29719 Ras-related GTP binding protein* 264 97.0 7 100 RICSS230 D21114 Ribosomal protein A2 334 58.7 93 63.0 YSPRPA2 D29720 Ribosomal protein L7 139 72.7 46 78.0 HUMRPL7Y D29721 Ribosomal protein L19* 86 89.5 20 90.0 RICSS504 D50571 Ribosomal protein L26 142 62.0 44 59.1 HUMRPL26X D29722 Ribosomal protein L31 204 61.8 63 64.0 CRERIBPL31 D29723 Ribosomal protein L37a 217 65.0 72 70.8 MMRP37A D29724 Ribosomal protein L38 200 69.5 62 74.0 ENRIPRL38 D29725 Ribosomal protein L41* 78 92.3 26 <td>D50572</td> <td>Polyubiquitin</td> <td>101</td> <td>86.1</td> <td>_</td> <td>_</td> <td>OSPORUBI</td>	D50572	Polyubiquitin	101	86.1	_	_	OSPORUBI
D50569 Protein kinase-Alfalfa (MSK-3) 253 66.0 82 57.3 AMMSK3A D29719 Ras-related GTP binding protein* 264 97.0 7 100 RICSS230 D21114 Ribosomal protein A2 334 58.7 93 63.0 YSPRPA2 D29720 Ribosomal protein L7 139 72.7 46 78.0 HUMRPL7Y D29721 Ribosomal protein L19* 86 89.5 20 90.0 RICSS504 D50571 Ribosomal protein L26 142 62.0 44 59.1 HUMRPL26X D29722 Ribosomal protein L31 204 61.8 63 64.0 CRERIBPL31 D29723 Ribosomal protein L37a 217 65.0 72 70.8 MMRP37A D29724 Ribosomal protein L38 200 69.5 62 74.0 ENRIPRL38 D29725 Ribosomal protein L41* 78 92.3 26 92.3 GHRP60S D29740 Ribosomal protein S5 121	D29718	pPLB07	248	68.5	_		LUPPLB07M
D29719Ras-related GTP binding protein*26497.07100RICSS230D21114Ribosomal protein A233458.79363.0YSPRPA2D29720Ribosomal protein L713972.74678.0HUMRPL7YD29721Ribosomal protein L19*8689.52090.0RICSS504D50571Ribosomal protein L2614262.04459.1HUMRPL26XD29722Ribosomal protein L3120461.86364.0CRERIBPL31D29723Ribosomal protein L37a21765.07270.8MMRP37AD29724Ribosomal protein L3820069.56274.0ENRIPRL38D29725Ribosomal protein L3916163.44969.4HUMRIBPROGD50573Ribosomal protein L41*7892.32692.3GHRP60SD29726Ribosomal protein rp21c15472.74362.8DRORP21CD29726Ribosomal protein S512184.33835.0RNRPS5D29727Ribosomal protein S1123992.979100ZMRPS11CD29728Ribosomal protein S12*12562.42860.7TTRPS12	D50569	Protein kinase-Alfalfa (MSK-3)	253	66.0	82	57.3	AMMSK3A
D21114Ribosomal protein A233458.79363.0YSPRPA2D29720Ribosomal protein L713972.74678.0HUMRPL7YD29721Ribosomal protein L19*8689.52090.0RICSS504D50571Ribosomal protein L2614262.04459.1HUMRPL26XD29722Ribosomal protein L3120461.86364.0CRERIBPL31D29723Ribosomal protein L37a21765.07270.8MMRP37AD29724Ribosomal protein L3820069.56274.0ENRIPRL38D29725Ribosomal protein L3916163.44969.4HUMRIBPROGD50573Ribosomal protein L41*7892.32692.3GHRP60SD29726Ribosomal protein S512184.33835.0RNRPS5D29727Ribosomal protein S1123992.979100ZMRPS11CD29728Ribosomal protein S12*12562.42860.7TTRPS12	D29719	Ras-related GTP binding protein*	264	97.0	7	100	RICSS230
D29720Ribosomal protein L713972.74678.0HUMRPL7YD29721Ribosomal protein L19*8689.52090.0RICSS504D50571Ribosomal protein L2614262.04459.1HUMRPL26XD29722Ribosomal protein L3120461.86364.0CRERIBPL31D29723Ribosomal protein L37a21765.07270.8MMRP37AD29724Ribosomal protein L3820069.56274.0ENRIPRL38D29725Ribosomal protein L3916163.44969.4HUMRIBPROGD50573Ribosomal protein L41*7892.32692.3GHRP60SD29726Ribosomal protein S512184.33835.0RNRPS5D29727Ribosomal protein S1123992.979100ZMRPS11CD29728Ribosomal protein S12*12562.42860.7TTRPS12	D21114	Ribosomal protein A2	334	58.7	93	63.0	YSPRPA2
D29721 Ribosomal protein L19* 86 89.5 20 90.0 RICSS504 D50571 Ribosomal protein L26 142 62.0 44 59.1 HUMRPL26X D29722 Ribosomal protein L31 204 61.8 63 64.0 CRERIBPL31 D29723 Ribosomal protein L37a 217 65.0 72 70.8 MMRP37A D29724 Ribosomal protein L38 200 69.5 62 74.0 ENRIPRL38 D29725 Ribosomal protein L39 161 63.4 49 69.4 HUMRIBPROG D50573 Ribosomal protein L41* 78 92.3 26 92.3 GHRP60S D29726 Ribosomal protein rp21c 154 72.7 43 62.8 DRORP21C D29726 Ribosomal protein S5 121 84.3 38 35.0 RNRPS5 D29727 Ribosomal protein S11 239 92.9 79 100 ZMRPS11C D29728 Ribosomal protein S12* 125 62.4 28 60.7 TTRPS12	D29720	Ribosomal protein L7	139	72.7	46	78.0	HUMRPL7Y
D50571Ribosomal protein L2614262.04459.1HUMRPL26XD29722Ribosomal protein L3120461.86364.0CRERIBPL31D29723Ribosomal protein L37a21765.07270.8MMRP37AD29724Ribosomal protein L3820069.56274.0ENRIPRL38D29725Ribosomal protein L3916163.44969.4HUMRIBPROGD50573Ribosomal protein L41*7892.32692.3GHRP60SD29726Ribosomal protein S512184.33835.0RNRPS5D29727Ribosomal protein S1123992.979100ZMRPS11CD29728Ribosomal protein S12*12562.42860.7TTRPS12	D29721	Ribosomal protein L19*	86	89.5	20	90.0	RICSS504
D29722 Ribosomal protein L31 204 61.8 63 64.0 CRERIBPL31 D29723 Ribosomal protein L37a 217 65.0 72 70.8 MMRP37A D29724 Ribosomal protein L38 200 69.5 62 74.0 ENRIPRL38 D29725 Ribosomal protein L39 161 63.4 49 69.4 HUMRIBPROG D50573 Ribosomal protein L41* 78 92.3 26 92.3 GHRP60S D29726 Ribosomal protein rp21c 154 72.7 43 62.8 DRORP21C D29726 Ribosomal protein S5 121 84.3 38 35.0 RNRPS5 D29727 Ribosomal protein S11 239 92.9 79 100 ZMRPS11C D29728 Ribosomal protein S12* 125 62.4 28 60.7 TTRPS12	D50571	Ribosomal protein L26	142	62.0	44	59.1	HUMRPL26X
D29723 Ribosomal protein L37a 217 65.0 72 70.8 MMRP37A D29724 Ribosomal protein L38 200 69.5 62 74.0 ENRIPRL38 D29725 Ribosomal protein L39 161 63.4 49 69.4 HUMRIBPROG D50573 Ribosomal protein L41* 78 92.3 26 92.3 GHRP60S D29726 Ribosomal protein rp21c 154 72.7 43 62.8 DRORP21C D29726 Ribosomal protein S5 121 84.3 38 35.0 RNRPS5 D29727 Ribosomal protein S11 239 92.9 79 100 ZMRPS11C D29728 Ribosomal protein S12* 125 62.4 28 60.7 TTRPS12	D29722	Ribosomal protein L31	204	61.8	63	64.0	CRERIBPL31
D29724 Ribosomal protein L38 200 69.5 62 74.0 ENRIPRL38 D29725 Ribosomal protein L39 161 63.4 49 69.4 HUMRIBPROG D50573 Ribosomal protein L41* 78 92.3 26 92.3 GHRP60S D29726 Ribosomal protein rp21c 154 72.7 43 62.8 DRORP21C D29726 Ribosomal protein S5 121 84.3 38 35.0 RNRPS5 D29727 Ribosomal protein S11 239 92.9 79 100 ZMRPS11C D29728 Ribosomal protein S12* 125 62.4 28 60.7 TTRPS12	D29723	Ribosomal protein L37a	217	65.0	72	70.8	MMRP37A
D29725 Ribosomal protein L39 161 63.4 49 69.4 HUMRIBPROG D50573 Ribosomal protein L41* 78 92.3 26 92.3 GHRP60S D29740 Ribosomal protein rp21c 154 72.7 43 62.8 DRORP21C D29726 Ribosomal protein S5 121 84.3 38 35.0 RNRPS5 D29727 Ribosomal protein S11 239 92.9 79 100 ZMRPS11C D29728 Ribosomal protein S12* 125 62.4 28 60.7 TTRPS12	D29724	Ribosomal protein L38	200	69.5	62	74.0	ENRIPRL38
D50573 Ribosomal protein L41* 78 92.3 26 92.3 GHRP60S D29740 Ribosomal protein rp21c 154 72.7 43 62.8 DRORP21C D29726 Ribosomal protein S5 121 84.3 38 35.0 RNRPS5 D29727 Ribosomal protein S11 239 92.9 79 100 ZMRPS11C D29728 Ribosomal protein S12* 125 62.4 28 60.7 TTRPS12	D29725	Ribosomal protein L39	161	63.4	49	69.4	HUMRIBPROG
D29740 Ribosomal protein rp21c 154 72.7 43 62.8 DRORP21C D29726 Ribosomal protein S5 121 84.3 38 35.0 RNRPS5 D29727 Ribosomal protein S11 239 92.9 79 100 ZMRPS11C D29728 Ribosomal protein S12* 125 62.4 28 60.7 TTRPS12	D50573	Ribosomal protein L41*	78	92.3	26	92.3	GHRP60S
D29726 Ribosomal protein S5 121 84.3 38 35.0 RNRPS5 D29727 Ribosomal protein S11 239 92.9 79 100 ZMRPS11C D29728 Ribosomal protein S12* 125 62.4 28 60.7 TTRPS12	D29740	Ribosomal protein rp21c	154	72.7	43	62.8	DRORP21C
D29727 Ribosomal protein S11 239 92.9 79 100 ZMRPS11C D29728 Ribosomal protein S12* 125 62.4 28 60.7 TTRPS12	D29726	Ribosomal protein S5	121	84.3	38	35-0	RNRPS5
D29728 Ribosomal protein S12* 125 62.4 28 60.7 TTRPS12	D29727	Ribosomal protein S11	239	92.9	79	100	ZMRPS11C
	D29728	Ribosomal protein S12*	125	62.4	28	60.7	TTRPS12

Accession No.	Gene	Length compared (bp)	% matched (bp)	Length compared (aa)	% matched (aa)	Source of comparison
D29729	Ribosomal protein S15*	121	76.9	33	93.9	CHKRIGA
D29730	Ribosomal protein S19	286	66.4	85	66.0	HUMS19RP
D21117	Signal recognition particle 7SRNA	208	72.6	_	<u></u>	WHTSRP7S2
D29731	smD small nuclear ribonucleoprotein	130	73.1		_	MUSSMDAUTA
D29732	Sucrose synthase (RSs1)*	358	98.3	54	94.4	OSSUPHSY
D29733	Sucrose synthase (RSs2)*	137	95.6	33	94.0	RICRSS2
D29734	Superoxide dismutase*	330	99.1	109	99.1	RICSODAOA
D25534	Tonoplast intrinsic protein	188	71.8	60	86.7	ATHGTIP
D17766	Triosephosphate isomerase*	223	96.0	62	97.0	RICRIC
D29735	α−tubulin*	143	87.4	49	93.9	PRUATUB
D50574	Ubiquitin-conjugating enzyme*	163	75.5	46	97.8	ATHUBCB-1

Accession numbers registered in DDBJ, EMBL NCBI and GenBank databases are indicated. Homologies are given as percent identity (% matched) and length compared in base pairs (bp) for nucleotide matches and in amino acid residues (aa) for peptide matches. Sources of comparison were based on the recorded sequences in the GenBank database. Genes with asterisks had been identified in other cDNA libraries previously (Uchimiya *et al.* 1992, Umeda *et al.* 1994).

expected functions of the identified genes were distributed in a broad spectrum. Putative genes encoding ribosomal proteins accounted for most of the identified genes as in previous studies [11, 13–15]. In plants, ribosomes are classified into three types, namely chloroplastic, mitochondrial and cytosolic. Though many chloroplastic ribosomal protein genes which are homologous to those of bacteria have been identified [18], there are relatively few reports about isolation of cytosolic ones. In this study, we isolated 17 cytosolic ribosomal protein genes. For instance, genes encoding cytosolic ribosomal proteins L7, L26, L31, L39, rp21c, S5 and S19 (**Table 1**) are not well understood in higher plants.

We isolated several genes associated with signal transduction pathways: ADP-ribosylation factor, Calmodulin, Casein kinase I- γ , Casein kinase II- α , G-protein β -subunit-like polypeptide, protein kinase isolated from Alfalfa, and Ras-related GTP-binding protein (**Table 1**). We identified a cDNA clone similar to the gene (Osc6) which is specifically expressed in rice anther tissue [19]. We also isolated a homologue to CARSR12 which is a senescence-related gene strictly regulated by ethylene, and specific to floral organs, primarily to petals [20].

Concerning the redundancy of cDNA clones of rice anther, about 80% of the clones appeared once. We did not see much redundancy in other clones. Nevertheless, cDNA encoding sucrose synthase appeared six times, and those encoding glycine-rich protein were detected three times, suggesting their important role in the early stage of anther development. Glycine-rich protein works as a cell-wall component. Since pollen wall is rapidly synthesized immediately after meiosis, proteins such as glycine-rich protein are expected to be transcribed and translated actively in tapetal cells and be secreted to microspores.

To analyze gene expression pattern during anther development, we conducted RNA blot analysis using cDNA clones identified in this study. We have chosen two cDNAs encoding sucrose synthase isozymes which were repeatedly identified from the anther library, and several cDNAs encoding ribosomal proteins. cDNAs were amplified by PCR method from plasmid clones, and labeled with $[\alpha^{-32}P]$ dCTP (110 TBg/mmol, ICN) using a random primer DNA labeling kit (TAKARA). Total RNA was isolated from anthers at the uninucleate microspore stage, at the binucleate pollen stage $(2.1 \sim 2.2 \text{ mm in length})$ and at the trinucleate pollen stage (ready to dehiscence), and also from shoots and roots of 1-week-old seedlings by the phenol-SDS method [21]. RNAs (3 μ g/slot) were blotted on the nylon membrane (Hybond-N+: Amersham) using a BIO-DOT SF (BioRad). Membranes were prehybridized at 65°C for 1 h in hybridization solution containing 1% SDS, 1 M NaCl, 10% dextran sulfate and 0.1 mg/ml heat-denatured salmon sperm DNA. They were then hybridized with cDNA probes for 15 h. After hybridization, they were washed in 2 imesSSC for 10 min. at 65°C with shaking, and then twice in $2 \times SSC$ containing 1% SDS for 30 min. at 65°C. Membranes were dried and exposed to X-ray films (Fuji RX-50). Quantities of slot blots were measured using a Imaging Plate Scanner BAS2000 (Fuji Film Co.).

As expected from the redundancy in the cDNA library, sucrose synthase gene was highly expressed in anthers compared with shoots and roots (**Fig. 1a**). We repeated the same experiments with different clones, and the result was reproduced. Sucrose synthase catalyzes the reversible reaction, namely, sucrose + UDP \leftrightarrow UDP-glucose + fructose. Its main physiological



Fig. 1 Expression analysis of various genes during anther development.
(a) Sucrose synthase gene and (b) ribosomal protein gene for (1) S12, (2) S19, (3) S22, (4) L3, (5) L7a, (6) L31, (7) L37a, (8) L38 and (9) P0. Results of RNA blot analysis were quantitated using a Imaging Plate Scanner BAS2000 (Fuji Film). Amounts of transcript in anther at uninucleate microspore stage were taken as 100%. 1: anthers at uninucleate microspore stage. 2: anthers at binucleate microspore stage. 3: anthers at trinucleate microspore stage. S: shoots. R: roots.

role is to cleave sucrose, providing UDP-glucose for the metabolism such as starch, cellulose and hemicellulose synthesis, and respiration [22, 23]. During microspore development, the accompanying cell enlargement, cell division, starch accumulation and formation of intine, the innermost wall of the microspore containing cellulose and hemicellulose [24], may require a constant supply of UDP-glucose. Throughout such metabolic pathway, sucrose synthase as well as glycine-rich protein may take an important role in microspore development.

In addition to six clones encoding ribosomal protein

(S12, S19, L31, L37a, L38, P0) from the anther library, we used three cDNA clones (S22, L3, L7a) previously isolated in our laboratory [13]. As shown in **Fig. 1b**, several clones (S19, L3, L7a, L37a, L38 and P0) showed relatively lower transcript levels throughout anther development than those observed in shoots and roots of rice seedlings. With respect to the transcript levels in anthers at different developmental stages, coordinated decline of transcript accumulation from uninucleate stage to bi- and/or tri-nucleate stage was noted with the cDNA probes such as S22, L3, L7a, L31, L37a, L38 and P0, suggesting the ribosomal proteins may be

involved in active translation in young anthers. In order to supply some 60 ribosomal proteins at equimolar concentration, it is known that both transcriptional and post-transcriptional regulation is important [25]. Although there is little information on the structure and regulation of cytoplasmic ribosomal protein in plants, reported instances suggest that their gene expression is regulated in a coordinated manner. For example, two ribosomal protein genes encoding S19 and L7 showed coordinated expression patterns in parallel to cell growth in potato [26]. Namely, they are strongly expressed in the stolon tip during the early stage of tuberization, and transcript levels declined as the tuber increased in size. It is also demonstrated here that ribosomal protein gene expression seems to be coordinately regulated during anther development.

The expression analysis described here could be applicable to other genes. This enables us to make a transcriptional map of genes associated with anther development. Further work may be needed to explore the temporal and spatial gene expression events in rice anther.

Acknowledgement

This research was supported in part by a grant from the Rockefeller Foundation.

References

- [1] Mascarenhas, J.P., 1975. Bot. Rev., 41: 259-314.
- [2] McCormick, S.M., 1991. Trends Genet. 7: 298– 303.
- [3] Mascarenhas, J.P., 1992. Int. Rev. Cytol., 140: 3– 18.
- [4] Kidou, S., Umeda, M., Uchimiya, H., 1995. Plant Tissue Cult. Lett., 12: 1-7.
- [5] Adams, M.D., Kelley, J.M., Gocayne, J.D., Dubnick, M., Polymeropoulos, M.H., Xiao, H., Merril, C.R., Wu, A., Olde, B., Moreno, R.F., Kerlavage, A.R., McCombie, W.R., Venter, J.C., 1991. Science, 252: 1651-1656.
- [6] Okubo, K., Hori, N., Matoba, R., Niiyama, T., Fukushima, A., Kojima, Y., Matsubara, K., 1992.
 Nature Genet., 2: 173-179.
- McCombie, W.R., Adams, M.D., Kelley, J.M., FitzGerald, M.G., Utterback, T.R., Khan, M., Dubnick, M., Kerlavage, A.R., Venter, J.C., Fields, C., 1992. Nature Genet., 1: 124-131.
- [8] Waterston, R., Martin, C., Craxton, M., Huynh, C., Coulson, A., Hillier, L., Durbin, R., Green, P., Shownkeen, R., Halloran, N., Metzstein, M., Hawkins, T., Wilson, R., Berks, M., Du, Z., Thomas, K., Thierry-Mieg, J., Sulston, J., 1992. Nature Genet., 1: 114-123.
- [9] Höög, C., 1991. Nucl. Acids Res., 19: 6123-6127.

- [10] Keith, C.S., Hoang, D.O., Barret, B.M., Feigelmam, B., Nelson, M.C., Thai, H., Baysdorfer, C., 1993. Plant Physiol., 101: 329-332.
- [11] Höfte, H., Desprez, T., Amselem, J., Chiapello, H., Caboche, M., Moisan, A., Jourjon, M.-F., Charpenteau, J.-L., Berthomieu, P., Guerrier, D., Giraudat, J., Quigley, F., Thomas, F., Yu, D.-Y., Mache, R., Raynal, M., Cooke, R., Grellet, F., Delseny, M., Parmentier, Y., de Marcillac, G., Gigot, C., Fleck, J., Philipps, G., Axelos, M., Bardet, C., Tremousaygue, D., Lescure, B., 1993. Plant J., 4: 1051-1061.
- [12] Park, Y.S., Kwak, J.M., Kwon, O.Y., Kim, Y.S., Lee, D.S., Cho, M.J., Lee, H.H., Nam, H.G., 1993. Plant Physiol., 103: 359-370.
- [13] Uchimiya, H., Kidou, S., Shimazaki, T., Aotsuka, S., Takamatsu, S., Nishi, R., Hashimoto, H., Matsubayasi, Y., Kidou, N., Umeda, M., Kato, A., 1992. Plant J., 2: 1005-1009.
- [14] Sasaki, T., Song, J., Koga-Ban, Y., Matsui, E., Fang, F., Higo, H., Nagasaki, H., Hori, M., Miya, M., Murayama-Kayano, E., Takiguchi, T., Takasuga, A., Niki, T., Isimaru, K., Ikeda, H., Yamamoto, Y., Mukai, Y., Ohta, I., Miyadera, N., Havukkala, I., Minobe, Y., 1994. Plant J., 6: 615-624.
- [15] Umeda, M., Hara, C., Matsubayashi, Y., Li, H.-H., Liu, Q., Tadokoro, F., Aotsuka, S., Uchimiya, H., 1994. Plant Mol. Biol., 25: 469-478.
- [16] Umeda, M., Uchimiya, H., 1994. Plant Physiol., 106: 1015-1022.
- [17] Smith, L.M., Sanders, J.Z., Kaiser, R.J., Hughes,
 P., Dodd, C., Connell, C.R., Heiner, C., Kent, S.B.
 H., Hood, L.E., 1986. Nature, 321: 674-679.
- [18] Mache, R., 1990. Plant Sci., 72: 1-12.
- [19] Tsuchiya, T., Toriyama, K., Nasrallah, M.E.,
 Ejiri, S., 1992. Plant Mol. Biol., 20: 1189-1193.
- [20] Lawton, K.A., Raghothama, K.G., Goldsbrough, P.B., Woodson, W.R., 1990. Plant Physiol., 93: 1370-1375.
- [21] Palmiter, R.D., 1974. Biochemistry, 13: 3606-3615.
- [22] Chourey, P.S., Taliercio, E.W., Kane, E.J., 1991.Plant Physiol., 96: 485-490.
- [23] Huber, S.C., Akazawa, T., 1986. Plant Physiol., 81: 1008-1013.
- [24] Heslop-Harrison, J., 1971. In "Pollen: Developmenet and Physiology" (ed by Heslop-Harrison, J.), p. 75-98, Butterworths, London.
- [25] Mager, W.H., 1988. Biochim. Biophys. Acta, 949: 1–15.
- [26] Taylor, M.A., Arif, S.A.M., Pearce, S.R., Davies, H.V., Kumar, A., George, L.A., 1992. Plant Physiol., 100: 1171-1176.