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A Higher Plant Has Three Different Types of RPA Heterotrimeric Complex

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Replication protein A (RPA) is a protein complex composed of three subunits known as RPA70, RPA32, and RPA14. Generally, only one version of each of the three RPA genes is present in animals and yeast (with the exception of the human RPA32 ortholog). In rice (*Oryza sativa* L.), however, two paralogs of RPA70 have been reported. We screened the rice genome for RPA subunit genes, and identified three OsRPA70 (OsRPA70a, OsRPA70b and OsRPA70c), three OsRPA32 (OsRPA32-1, OsRPA32-2 and OsRPA32-3), and one OsRPA14. Through two-hybrid assays and pull down analyses, we showed that OsRPA70a interacted preferentially with OsRPA32-2, OsRPA70b with OsRPA32-1, and OsRPA70c with OsRPA32-3. OsRPA14 interacted with all OsRPA32 paralogs. Thus, rice has three types of RPA complex: OsRPA70a-OsRPA32-2-OsRPA14 (type A), OsRPA70b-OsRPA32-1-OsRPA14 (type B), and OsRPA70c-OsRPA32-3-OsRPA14 (type C). Subcellular localization analysis suggested that the type-A RPA complex is required for chloroplast DNA metabolism, whereas types B and C function in nuclear DNA metabolism.

Key words: DNA repair, DNA replication, rice, RPA subunit.

Abbreviations: DBD, single-stranded DNA binding domain; Hs, *Homo sapiens*; PCNA, proliferating cell nuclear antigen; RPA, replication protein A; Xs, *Xenopus laevis*.

Replication protein A (RPA) is a heterotrimeric complex composed of 70, 32, and 14 kDa subunits (referred to as RPA70, RPA32, and RPA14, respectively) that is involved in various steps of DNA metabolism (*1*, *2*). RPA was first identified as a factor necessary for SV40 replication *in vitro* (*3*, *4*), and subsequently was characterized in mammals and yeast (*5*, *6*).

RPA accumulates along stretches of ssDNA generated on DNA replication and repair (*5*, *7–9*) by means of its 6 ssDNA-binding domains (DBDs). In the RPA70 subunit, there are four DBDs (DBD-A, -B, -C and -F), two of which (DBD-A and DBD-B) exhibit the greatest ssDNA-binding activity, and mediate DNA identification and docking (*10*, *11*). DBD-C and the RPA32-located DBD-D exhibit weaker ssDNA-binding activities (*12*, *13*). DBD-F interacts with DNA as well as with various proteins (5), and is important for the unwinding of double-stranded DNA (*14–16*). Little is known about the function of DBD-E on RPA14. The DNA sequences that become occluded following binding of DBD-A and DBD-B are only 8–11 nt in size, but much longer sequences of about 30 nt are required for high affinity binding by DBD-A, DBD-B, DBD-C, and DBD-D (*17–20*).

In almost all animal species examined so far and yeast, there is only one gene each for the 70, 32, and 14 kDa subunits of RPA. On the contrary, humans carry two homologs of RPA32, *i.e.*, HsRPA2 and HsRPA4 (*21*). HsRPA2, but not HsRPA4, interacts with uracil-DNA glycosylase and XPA, which are involved in the recognition of damaged DNA (*22*). Contrary to that of the *HsRPA2* gene, the expression level of *HsRPA4* is constant during the cell cycle (*23*), suggesting that HsRPA2 and HsRPA4 have different functions.

In contrast to humans, animals, and yeast, little is known about RPA in plants. In tobacco, RPA enhanced the activity of DNA polymerase (*24*). In rice, the gene for RPA32 could be induced by UV irradiation (*25*). A rice RPA70 was cloned and found to be induced by gibberellins, a class of phytohormones (*26*). Subsequently, two isoforms called OsRPA70a and OsRPA70b were identified in this species, OsRPA70b closely resembling the gibberellin-inducible homolog previously described (*27*). Transcripts of *OsRPA70a* were expressed preferentially in proliferating tissues such as root tips, young leaves, ears, and flag leaves, but also more weakly in mature leaves. *OsRPA70b* expression was more strictly restricted to proliferating tissues (*27*). T-DNA and RNAi mutant analyses were performed using the *Arabidopsis* homologs of *OsRPA70a* and *OsRPA70b* (*AtRPA70a* and *AtRPA70b*). Mutations in *AtRPA70a* were lethal. On the other hand, *AtRPA70b* mutants were morphologically normal but hypersensitive to mutagens such as UV-B and MMS, suggesting that RPA70a and RPA70b have different roles in the cell and that the latter functions in DNA repair. In two hybrid, pull down, and coexpression analyses, OsRPA70b was found to interact with OsRPA32 more selectively than OsRPA70a (*28*). Thus, at least two different types of RPA heterotrimer are present in seed plants and there may be more, as not

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Table 1. **Comparison of the predicted amino acid sequences of OsRPA70a, OsRPA70b, and OsRPA70c.**

	OsRPA70a	*OsRPA70b*	*OsRPA70c*
DBD-A, -B			
OsRPA70a	–	40.3	57.3
OsRPA70b	40.3	–	42.9
OsRPA70c	57.3	42.9	–
DBD-C, -F			
OsRPA70a	–	22.9	40.7
OsRPA70b	22.9	–	28.1
OsRPA70c	40.7	28.1	–

Numbers indicate the percentage identity in the DBD-A and -B domains or DBD-C and -F domains.

Table 2. **Comparison of the predicted amino acid sequences of OsRPA32-1, OsRPA32-2, and OsRPA32-3.**

	OsRPA32-1	*OsRPA32-2*	*OsRPA32-3*
DBD-D			
OsRPA32-1	–	49.6	41.9
OsRPA32-2	49.6	–	40.8
OsRPA32-3	41.9	40.8	–
Total			
OsRPA32-1	–	38.8	23.4
OsRPA32-2	38.8	–	20.5
OsRPA32-3	23.5	20.5	–

Numbers indicate the percentage identity in the DBD-D domains or the total sequence.

yet identified isoforms of the 32 and 14 kDa subunits may exist that interact specifically with OsRPA70a (*28*).

In this study, we screened the rice genome to identify new potential elements of RPA complexes and found not only three paralogs of *OsRPA70* but also three versions of *OsRPA32*. However, there was only one gene for RPA14.

MATERIALS AND METHODS

Plant Material—Rice plants (*Oryza sativa* L. cv. Nipponbare) were grown in a growth cabinet with a 16 h light/8 h dark cycle at 28°C. Cells were grown in suspension culture as described previously (*29*).

Cloning of OsRPA70c, OsRPA32-2, and OsRPA32-3—The rice database was searched using the BLAST algorithm. The cDNAs of three newly identified homologs of known RPA genes, named *OsRPA70c*, *OsRPA32-2*, and *OsRPA32-3*, were prepared from rice mRNA using an RNeasy Plant Mini kit (Qiagen), and was amplified with a SuperScript One-Step RT-PCR System (Invitrogen).

The Nucleotide sequence data for *OsRPA70a*, *OsRPA70b*, *OsRPA70c*, *OsRPA32-1*, *OsRPA32-2*, *OsRPA32-3*, and *OsRPA14* have been deposited in the DDBJ/EMBL/GenBank nucleotide sequence databases under accession numbers AB042415, AB111916, AK073598, AB037145, AK073723, AK102353, and AB111915, respectively.

Yeast Two-Hybrid and β-Galactosidase Analyses—The yeast two-hybrid assay was performed with a MATCHMAKER Two-Hybrid System 3 (Clontech). Yeast transformants were grown overnight at 30°C, centrifuged and then resuspended in buffer (10 mM HEPES, 50 mM NaCl, 5 mM L-aspartic acid, 150 μM BSA, 0.05% Tween 20, pH 7.3). The OD_{600} was determined for each sample. CPRG (2.23 mM) was added and when the medium color had changed from yellow to red, the reaction was stopped. The OD_{578} of the supernatant were determined. β-Galactosidase unit (*U*) was calculated as

$$U = 1000 \times \mathrm{OD}_{578}/(5 \times t \times \mathrm{OD}_{600})$$

where *t* represents the time of reaction (min), OD_{600} the cell density at the start of the experiment, and OD_{578} the absorbance with chlorophenol red. All measurements were performed in triplicate (*30, 31*).

Pull Down Analysis—The *OsRPA70a*, *OsRPA70b*, *OsRPA70c*, *OsRPA32-1*, *OsRPA32-2*, and *OsRPA32-3* coding regions were cloned into the pET21a or pET28a expression vector (Novagen). Protein expression was performed by transforming the six constructs obtained into the Rosseta (DE3) line of *E. coli* (Novagen). The bacteria were grown in 300 ml LB medium to an OD of 0.8 and IPTG was added to a final concentration of 1 mM. Cells were harvested after 3 h by centrifugation at 3,000 × *g* for 10 min. Cell pellets were resuspended in 10 ml ice-cold binding buffer (20 mM Tris-HCl, pH 7.9, 0.5 M NaCl, 5 mM imidazole, 0.1% NP-40) and then sonicated with 15 bursts of 10 s each, before cell lysates were centrifuged at 39,000 × *g* for 30 min. Cell pellets were soluble in the presence of 6 M urea. Soluble fractions were loaded onto columns containing 2 ml His-Bind resin (Novagen). The columns were washed with 30 ml binding buffer including 6 M urea and then with 30 ml washing buffer (20 mM Tris-HCl, pH 7.9, 0.5 M NaCl, 20 mM imidazole, 0.1% NP-40, 6 M urea). The bound proteins were eluted with 6 ml elution buffer (20 mM Tris-HCl, pH 7.9, 0.5 M NaCl, 500 mM imidazole, 6 M urea), and then dialyzed against buffer A (50 mM NaH_2PO_4, pH 8.0, 50 mM NaCl, 5 mM 2-mercaptoethanol, 15% glycerol, 0.1% NP-40) containing 3 M urea for 1.5 h, buffer A containing 1.5 M urea for 1.5 h, 0.5 M urea for 1.5 h, and finally 0 M urea for 12 h.

Following cell harvesting, sonification, and centrifugation, the three histidine fusion proteins of OsRPA32-1, OsRPA32-2, and OsRPA32-3 were soluble. The soluble protein fractions were loaded onto columns filled with 2 ml His-Bind resin (Novagen). The column was washed with 30 ml binding buffer followed by 30 ml washing buffer (20 mM Tris-HCl, pH 7.9, 0.5 M NaCl, 20 mM imidazole, 0.1% NP-40). The bound proteins were eluted with 6 ml elution buffer (20 mM Tris-HCl, pH 7.9, 0.5 M NaCl, 500 mM imidazole), and then dialyzed against buffer A.

Five milligrams dialyzed proteins was loaded onto a 0.5 ml Ni^+-NTA Sepharose column (Qiagen), and then washed with buffer B (50 mM NaH_2PO_4, pH 8.0, 5 mM 2-mercaptoethanol, 15% glycerol, 0.1% NP-40, 40 mM NaCl, 50 mM imidazole). Then, cultured rice cells were loaded onto the column, followed by washing with buffer B, buffer C (50 mM NaH_2PO_4, pH 8.0, 5 mM 2-mercaptoethanol, 15% glycerol, 0.1% NP-40, 400 mM NaCl, 40 mM imidazole), and finally buffer D (50 mM NaH_2PO_4, pH 8.0, 5 mM 2-mercaptoethanol, 15% glycerol, 0.1% NP-40, 1 M NaCl, 40 mM imidazole). The bound proteins were eluted with 3 ml elution buffer (50 mM NaH_2PO_4, pH 8.0, 5 mM 2-mercaptoethanol, 15% glycerol, 0.1% NP-40, 500 mM NaCl, 400 mM imidazole). The eluted proteins were analyzed by Western blotting.

In Vivo *Immunoprecipitation*—Polyclonal antibodies against OsRPA70a, OsRPA70b, OsRPA70c, and OsRPA14

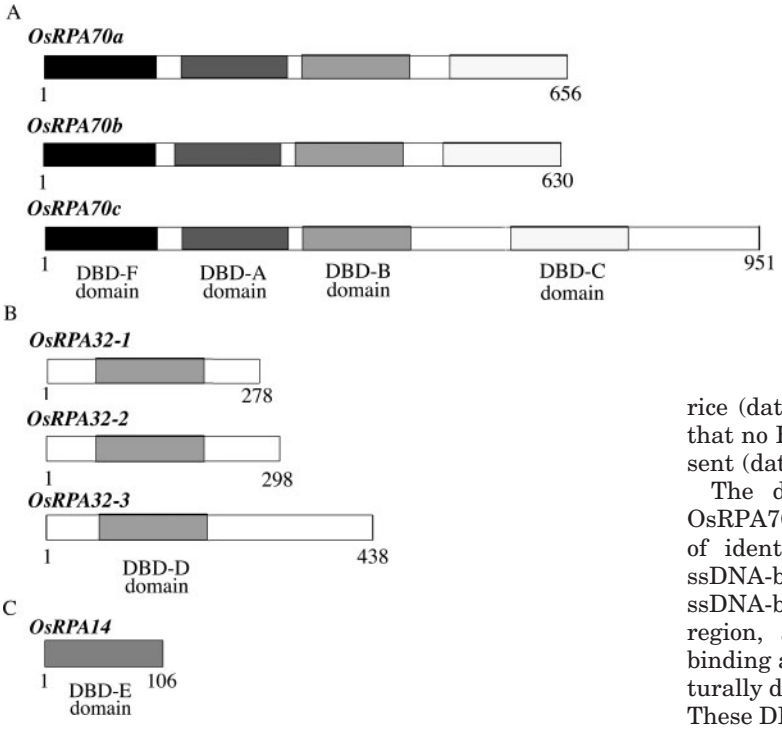

Fig. 1. **Three types of RPA exist in rice.** A: Domain structures of OsRPA70. The black and gray boxes indicate the DBD (DNA Binding Domain)-A, DBD-B, DBD-C, and DBD-F. B: Domain structures of OsRPA32. The gray boxes indicate the DBD-D domains. C: Domain structure of OsRPA14. The gray box indicates the DBD-E domain.

were coupled to Protein G using a Seize X immunoprecipitation kit (PIRECE). The beads were incubated with a crude cell extract on ice for 2 h, and then washed three times with PBS. The bound proteins were eluted with 0.1 M glycine (pH 2.5).

Cellular Localization—Nucleus and chloroplast fractions were isolated from 10-day-old rice seedlings using a Nucleus Isolation Kit (SIGMA) and a Chloroplast Isolation Kit (SIGMA), respectively, according to the manufacturer's protocols.

RESULTS

Molecular Cloning of Rice Genes Encoding RPA Complex Proteins—Our search of the rice genome for homologs of RPA70 yielded a novel cDNA sequence that contained all conserved domains, in addition to previously known *OsRPA70a* and *OsRPA70b*. We termed it *OsRPA70c* (Fig. 1A). Moreover, three different cDNAs homologous with *RPA32* were identified, and termed *OsRPA32-1* (corresponding to known *OsRPA32* described before), *OsRPA32-2*, and *OsRPA32-3* (Fig. 1B). Only one version of *OsRPA14* was found (Fig. 1C).

We isolated cDNAs of the novel rice RPA genes (*OsRPA70c*, *OsRPA32-2*, and *OsRPA32-3*); cloning of the previously discovered *OsRPA70a*, *OsRPA70b*, *OsRPA32* (*OsRPA32-1*), and *OsRPA14* was described elsewhere (*27*, *28*). RT-PCR was performed to amplify the full-length cDNAs of the new paralogs as described under Materials and Methods. We then prepared recombinant RPA proteins and raised antibodies against all seven subunits. All RPA antibodies only recognized the corresponding subunit without crossreactivity, as shown on subtractive affinity purification. Northern blotting analyses indicated that we had detected all *RPA*s transcribed in rice (data not shown), while Western blotting confirmed that no RPA proteins other than those detected were present (data not shown).

The deduced amino acid sequences of OsRPA70a, OsRPA70b, and OsRPA70c showed relatively low levels of identity (about 30%). In mammalian RPA70, two ssDNA-binding domains (DBD-A and DBD-B with high ssDNA-binding activity) are located within the central region, and a third one (DBD-C with weak ssDNA-binding activity) in the C-terminal region. A fourth, structurally defined DBD (DBD-F) is located at the N terminus. These DBD domains were conserved in all three OsRPA70 isoforms (Fig. 1D), with the highest level of similarity between the isoforms in DBD-A and DBD-B.

The situation was similar for *OsRPA32-1*, *OsRPA32-2*, and *OsRPA32-3*, which also showed relatively low levels of identity (Fig. 1E). An ssDNA-binding domain (DBD-D) is located in the central region of mammalian RPA32 (Fig. 1B).

Interactions between OsRPAs—To examine the interactions between the OsRPA subunits, two-hybrid assays (β-galactosidase assay) were performed. As shown in Fig. 2A, OsRPA70a interacted more selectively with OsRPA32-2 than OsRPA70b or OsRPA70c (Fig. 2A). On the other hand, OsRPA32-1 preferentially interacted with OsRPA70b, and OsRPA32-3 with OsRPA70c (Fig. 2A). All OsRPA32 paralogs showed strong interaction with OsRPA14 (Fig. 2A). The signal levels were generally lower for OsRPA32-3 than for OsRPA32-1 and -2, which could be due to either the genuinely lower reactivity of this paralog or to a lower expression level in the yeast system.

Pull down analyses were carried out using His-tagged OsRPA32-1, OsRPA32-2, and OsRPA32-3. A purified protein was loaded onto a Ni^{+}-NTA column, followed by loading of a crude extract of cultured rice cells. The column was washed with 1 M NaCl to remove proteins that weakly interacted with the his-tagged protein in the column, before strongly interacting ligands were eluted. The eluate was then examined for the presence of OsRPA70s by Western blot analysis, using antibodies specific for OsRPA70a, -b, and -c. The results corroborated our conclusion based on the results of the yeast two-hybrid experiments: OsRPA70a interacted preferentially with OsRPA32-2, OsRPA70b with OsRPA32-1, and OsRPA70c with OsRPA32-3 (Fig. 2B). Analogous experiments with his-tagged OsRPA70s coupled to the Ni-NTA yielded identical results (Fig. 2C).

In vivo immunoprecipitation using anti-OsRPA70a, anti-OsRPA70b, anti-OsRPA70c, and anti-OsRPA14

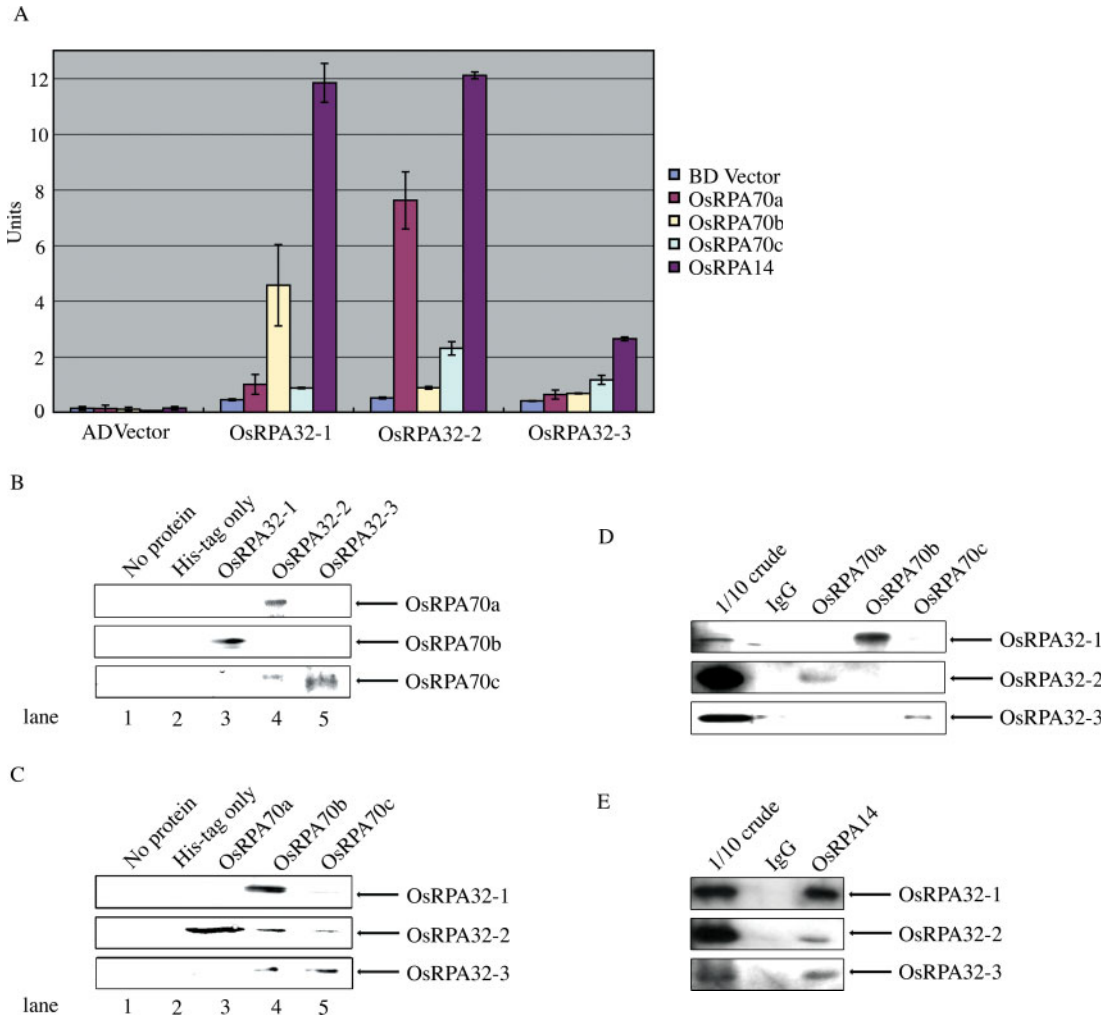

Fig. 2. **Interaction analysis.** A: Pairs of plasmids consisting of pGBKT7-OsRPA32-1, pGBKT7-OsRPA32-2, or pGBKT7-OsRPA32-2 plus either pGADT7-OsRPA70a, pGADT7-OsRPA70b, pGADT7-OsRPA70c, or pGADT7-OsRPA14 were cotransfected into yeast cell line AH109, and three independent transformants were isolated for each plasmid pair. Liquid cultures of the transformants were prepared and examined for β-galactosidase activity. Data shown are means (±SE, n = 3) of enzyme activity in units. B: His-tagged OsRPA32-1, OsRPA32-2, and OsRPA32-3 were immobilized in Ni^{+}-NTA columns, and crude extracts of cultured rice cells were added. OsRPA70s that had bound to the OsRPA32s were identified in the eluate by Western blotting, using antibodies specific for OsRPA70a, OsRPA70b, or OsRPA70c (as indicated on the right). Lane1, no protein control. lane 2, pET vector only. lane 3, OsRPA32-1. lane 4, OsRPA32-2. lane 5, OsRPA32-3. C: Experiment analogous to in (B), but with his-tagged OsRPA70a, OsRPA70b, and OsRPA70c in the colums and western blot detection of interacting OsPRA32s. D: *In vivo* interactions between OsRPA70a, OsRPA70b, OsRPA70c, OsRPA32-1, OsRPA32-2, and OsRPA32-3. Crude extracts of cultured rice cells were immunoprecipitated with polyclonal antibodies against OsRPA32s, and then the immunoblots were probed with polyclonal antibodies against OsRPA70s. E: *In vivo* interactions between OsRPA32-1, OsRPA32-2, OsRPA32-3, and OsRPA14. Crude extracts of cultured rice cells were immunoprecipitated with polyclonal antibodies against OsRPA32s; the immunoblots were probed with OsRPA14 polyclonal antibodies.

antibodies further confirmed that preferred binding occurred between OsRPA70a and OsRPA32-2, OsRPA70b and OsRPA32-1, and OsRPA70c and OsRPA32-3 (Fig. 2D); OsRPA14 interacted with all OsRPA32s (Fig. 2E). These data implied specific interactions between OsRPA70s and OsRPA32s *in vivo*. Thus, three different RPA complexes are formed in rice: OsRPA70a-OsRPA32-2-OsRPA14 (Type A), OsRPA70b-OsRPA32-1-OsRPA14 (Type B), and OsRPA70c-OsRPA32-3-OsRPA14 (Type C). This conclusion probably holds for higher plants in general, because *Arabidopsis* possesses homologs of all three *OsRPA70*s (*28*).

Subcellular Localization of Type A, B, and C RPA Complexes—The subcellular localization of the type-A, -B, and -C RPA complexes was examined in isolated nucleus and chloroplast fractions by Western blotting. DS9, a chloroplast-localized homolog of bacterial FtsH (*32*), and OsPCNA, a nuclear factor involved in DNA replication and repair (*33*), served as controls. Interestingly, signals of all three OsRPA32s were deteced in both the nucleus and chloroplast fractions (Fig. 3). OsRPA70a only appeared in the chloroplast fraction, whereas both OsRPA70b and OsRPA70c were restricted to nuclei (Fig. 3). In human and yeast cells, RPA32 occurs in the nucleus and cytoplasm, while RPA70 is only present in the nucleus. OsRPA32-1, OsRPA32-2, and OsRPA32-3 were present in both fractions (Fig. 3), maybe because OsRPA32s and OsRPA14 exist in their free forms or as a heterodimer

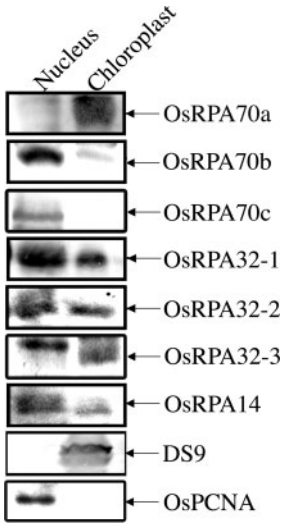

Fig. 3. **Subcellular localization of RPA.** Western blotting analysis of nucleus and chloroplast fractions of 10-day-old rice seedlings probed with anti-OsRPA70a, anti-OsRPA70b, anti-OsRPA70c, anti-OsRPA32-1, anti-OsRPA32-2, anti-OsRPA32-3, and anti-OsRPA14 antibodies was performed; anti-DS9 and anti-OsPCNA antibodies were used as chloroplast- and nucleus-specific controls, respectively.

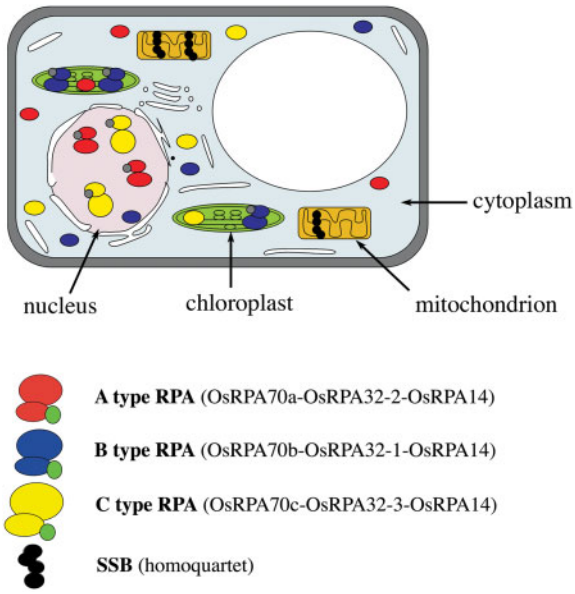

Fig. 4. **Hypothetic model of the cellular function of A-, B-, and C-type RPA complexes.**

complex (OsRPA32s-OsRPA14). These data suggested that the type-A RPA complex functions in chloroplast DNA synthesis, and that the type-B and -C complexes act in the nucleus.

DISCUSSION

In almost all animals examined so far and yeast, there is just one version of many genes involved in DNA replication and repair, whereas higher plants sometimes carry several paralogs, as in the cases of PCNA (*34*) and FEN-1 (*35*). We here described three versions of OsRPA70 in the rice genome, but the sequence identity between them was low except for in the conserved ssDNA-binding domains (DBD-A, DBD-B, DBD-C, and DBD-F). Similarly, there were three RPA32s exhibiting particularly high sequence identity in the ssDNA-binding domain (DBD-D). Generally, DBD-A, DBD-B, DBD-C, DBD-D, DBD-E, and DBD-F showed high sequence homology with their counterparts in animals and yeast. Thus, the proteins decribed must be rice homologs of the 70, 32, and 14 kDa RPA subunits, respectively, described for other taxa. We were able to demonstrate that rice possesses three types of RPA complex (A, OsRPA70a-OsRPA32-2-OsRPA14; B, OsRPA70b-OsRPA32-1-OsRPA14; and C, OsRPA70c-OsRPA32-3-OsRPA14), which have only the smallest subunit in common.

DBD-A and DBD-B of all three OsRPA70s showed a higher degree of homology with their human and yeast counterparts than DBD-C and DBD-F. Noteworthily, DBD-A and DBD-B are the most important elements for DNA binding by RPA (*10*, *11*). OsRPA70a was localized in the chloroplast, but OsRPA70b and OsRPA70c were detected in the nucleus. As all RPAs lacked nucleus- and chloroplast-specific signal sequences, the mechanism of their localization to these organelles is unclear. Noteworthily, the RPA homologue SSB is localized in mitochrondria in human cells (*36*, *37*). Similarly, SSB, but not RPA, may be localized in mitochrondria in plants. In *Arabidopsis*, mutations in the homologs of OsRPA70a and OsRPA70b were either lethal (*AtRPA70a*), or conferred hypersensitivity to UV and MMS (*AtRPA70b*) (*28*). Therefore, the type-A RPA complex could be essential for DNA replication, transcription, and repair in the chloroplast, while type B may have a role in nuclear DNA repair (Fig. 4). Currently, we are characterizing *AtRPA70c*-deletion mutants; preliminary data suggest that generally they are lethal (data not shown). Thus the type-C RPA complex appears to be essential for the nuclear DNA metabolism (Fig. 4). The existence of three different RPA complexes in higher plants may be an adaptation to the severe mutagenic UV irradiation and photo-oxidative stress that these sessile, photosynthetic organisms cannot avoid.

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REFERENCES

1. Kornberg, A. and Baker, T. (1992) *DNA Replication*, Freeman, New York
2. Hubscher, U., Mega, G., and Podust, V.N. (1996) In *DNA Replication in Eukaryotic Cells* (DePamphilis, M.L., ed.) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
3. Fairman, M.P. and Stillman, B. (1988) Cellular factors required for multiple stages of SV40 DNA replication in vitro. *EMBO J.* **7**, 1211–1218
4. Wold, M.S. and Kelly, T. (1988) Purification and characterization of replication protein A, a cellular protein required for in vitro replication of simian virus 40 DNA. *Proc. Natl. Acad. Sci. USA* **85**, 2523–2527
5. Wold, M.S. (1997) Replication protein A: a heterotrimeric, single-stranded DNA-binding protein required for eukaryotic DNA metabolism. *Annu. Rev. Biochem.* **66**, 61–92

6. Iftode, C., Daniely, Y., and Borowiec, J.A. (1999) Replication protein A (RPA): the eukaryotic SSB. *Crit. Rev. Biochem. Mol. Biol.* **34**, 141–180
7. Raderschall, E., Golub, E.I., and Haaf, T. (1999) Nuclear foci of mammalian recombination proteins are located at single-stranded DNA regions formed after DNA damage. *Proc. Natl. Acad. Sci. USA* **96**, 1921–1926
8. Oakley, G.G., Loberg, L.I., Yao, J., Risinger, M.A., Yunker, R.L., Zernik-Kobak, M., Khanna, K.K., Lavin, M.F., Carty, M.P., and Dixon, K. (2001) UV-induced hyperphosphorylation of replication protein a depends on DNA replication and expression of ATM protein. *Mol. Biol. Cell* **12**, 1199–1213
9. Wang, H., Guan, J., Perrault, A.R., Wang, Y., and Iliakis, G. (2001) Replication protein A2 phosphorylation after DNA damage by the coordinated action of ataxia telangiectasia-mutated and DNA-dependent protein kinase. *Cancer Res.* **61**, 8554–8563
10. Gomes, X.V. and Wold, M.S. (1995) Structural analysis of human replication protein A. Mapping functional domains of the 70-kDa subunit. *J. Biol. Chem.* **270**, 4534–4543
11. Gomes, X.V. and Wold, M.S. (1996) Functional domains of the 70-kilodalton subunit of human replication protein A. *Biochemistry* **35**, 10558–10568
12. Brill, S.J. and Bastin-Shanower, S. (1998) Identification and characterization of the fourth single-stranded-DNA binding domain of replication protein A. *Mol. Cell Biol.* **18**, 7225–7234
13. Bochkarev, A., Bochkareva, E., Frappier, L., and Edwards, A.M. (1999) The crystal structure of the complex of replication protein A subunits RPA32 and RPA14 reveals a mechanism for single-stranded DNA binding. *EMBO J.* **18**, 4498–4504
14. Braun, K.A., Lao, Y., He, Z., Ingles, C.J., and Wold, M.S. (1997) Role of protein-protein interactions in the function of replication protein A (RPA): RPA modulates the activity of DNA polymerase alpha by multiple mechanisms. *Biochemistry* **36**, 8443–8454
15. Lao, Y., Lee, C.G., and Wold, M.S. (1999) Replication protein A interactions with DNA. 2. Characterization of double-stranded DNA-binding/helix-destabilization activities and the role of the zinc-finger domain in DNA interactions. *Biochemistry* **38**, 3974–3984
16. Binz, S.K., Lao, Y., Lowry, D.F., and Wold, M.S. (2003) The phosphorylation domain of the 32-kDa subunit of replication protein A (RPA) modulates RPA-DNA interactions. Evidence for an intersubunit interaction. *J. Biol. Chem.* **278**, 35584–35591
17. Blackwell, L.J. and Borowiec, J.A. (1994) Human replication protein A binds single-stranded DNA in two distinct complexes. *Mol. Cell Biol.* **14**, 3993–4001
18. Kim, C. and Wold, M.S. (1995) Recombinant human replication protein A binds to polynucleotides with low cooperativity. *Biochemistry* **34**, 2058–2064
19. Blackwell, L.J., Borowiec, J.A., and Masrangelo, I.A. (1996) Single-stranded-DNA binding alters human replication protein A structure and facilitates interaction with DNA-dependent protein kinase. *Mol. Cell Biol.* **16**, 4798–4807
20. Arunkumar, A.I., Stauffer, M.E., Bochkareva, E., Bochkarev, A., and Chazin, W.J. (2003) Independent and coordinated functions of replication protein A tandem high affinity single-stranded DNA binding domains. *J. Biol. Chem.* **278**, 41077–41082
21. Keshav, K.F., Chen, C., and Dutta, A. (1995) Rpa4, a homolog of the 34-kilodalton subunit of the replication protein A complex. *Mol. Cell Biol.* **15**, 3119–3128
22. Nagelhus, T.A., Haug, T., Singh, K.K., Keshav, K.F., Skorpen, F., Otterlei, M., Bharati, S., Lindmo, T., Benichou, S., Benarous, R., and Krokan, H.E. (1997) A sequence in the N-terminal region of human uracil-DNA glycosylase with homology to XPA interacts with the C-terminal part of the 34-kDa subunit of replication protein A. *J. Biol. Chem.* **272**, 6561–6566
23. Bouziane, M., Miao, F., Bates, S.E., Somsouk, L., Sang, B.C., Denissenko, M., and O'Connor, T.R. (2000) Promoter structure and cell cycle dependent expression of the human methylpurine-DNA glycosylase gene. *Mutat. Res.* **461**, 15–29
24. Garcia-Maya, M.M. and Buck, K.W. (1997) Isolation and characterization of replication protein A (RP-A) from tobacco cells. *FEBS Lett.* **413**, 181–184
25. Marwedel, T., Ishibashi, T., Lorbiecke, R., Jacob, S., Sakaguchi, K., and Sauter, M. (2003) Plant-specific regulation of replication protein A2 (OsRPA2) from rice during the cell cycle and in response to ultraviolet light exposure. *Planta* **217**, 457–465
26. van der Knaap, E., Jagoueix, S., and Kende, H. (1997) Expression of an ortholog of replication protein A1 (RPA1) is induced by gibberellin in deepwater rice. *Proc. Natl. Acad. Sci. U S A* **94**, 9979–9983
27. Ishibashi, T., Kimura, S., Furukawa, T., Hatanaka, M., Hashimoto, J., and Sakaguchi, K. (2001) Two types of replication protein A 70 kDa subunit in rice, Oryza sativa: molecular cloning, characterization, and cellular & tissue distribution. *Gene* **272**, 335–343
28. Ishibashi, T., Koga, A., Yamamoto, T., Uchiyama, Y., Mori, Y., Hashimoto, J., Kimura, S., and Sakaguchi, K. (2005) Two types of replication protein A in seed plants. *FEBS J.* **272**, 3270–3281
29. Baba, A., Hasezawa, S., and Syono, K. (1986) Cultivation of rice protoplasts and their transformation mediated by Agrobacterium spheroplast. *Plant Cell Physiol.* **27**, 463–471
30. Miller, J.H. (1972) *Experiments in Molecular Genetics*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
31. Miller, J.H. (1992) In *A Short Course in Bacterial Genetics*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
32. Seo, S., Okamoto, M., Iwai, T., Iwano, M., Fukui, K., Isogai, A., Nakajima, N., and Ohashi, Y. (2000) Reduced levels of chloroplast FtsH protein in tobacco mosaic virus-infected tobacco leaves accelerate the hypersensitive reaction. *Plant Cell* **12**, 917–932
33. Kimura, S., Suzuki, T., Yanagawa, Y., Yamamoto, T., Nakagawa, H., Tanaka, I., Hashimoto, J., and Sakaguchi, K. (2001) Characterization of plant proliferating cell nuclear antigen (PCNA) and flap endonuclease-1 (FEN-1), and their distribution in mitotic and meiotic cell cycles. *Plant J.* **28**, 643–653
34. Hata, S., Tsukamoto, T., Osumi, T., Hashimoto, J., and Suzuka, I. (1992) Analysis of carrot genes for proliferating cell nuclear antigen homologs with the aid of the polymerase chain reaction. *Biochem. Biophys. Res. Commun.* **184**, 576–581
35. Kimura, S., Furukawa, T., Kasai, N., Mori, Y., Kitamoto, H.K., Sugawara, F., Hashimoto, J., and Sakaguchi, K. (2003) Functional characterization of two flap endonuclease-1 homologues in rice. *Gene* **314**, 63–71
36. Tiranti, V., Rocchi, M., DiDonato, S., and Zeviani, M. (1993) Cloning of human and rat cDNAs encoding the mitochondrial single-stranded DNA-binding protein (SSB). *Gene* **126**, 219–225
37. Curth, U., Urbanke, C., Greipel, J., Gerberding, H., Tiranti, V., and Zeviani, M. (1994) Single-stranded-DNA-binding proteins from human mitochondria and *Escherichia coli* have analogous physicochemical properties. *Eur. J. Biochem.* **221**, 435–443