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GENE NOTE

Molecular characterization of *OsRAD21-1*, a rice homologue of yeast *RAD21* essential for mitotic chromosome cohesion*

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Abstract

Rad21/Rec8 is an important component and key regulator of cohesins. A *RAD21*-like gene from rice (*Oryza sativa* L. ssp. *japonica*) has been cloned and termed *OsRAD21-1*. *OsRAD21-1* is a single-copy gene in the rice genome and is expressed in the entire plant. OsRad21-1 consists of 1055 amino acid residues and is the largest of the Rad21/Rec8 family identified to date. Based on sequence similarity comparison with other members of this family and gene expression patterns, it is concluded that OsRad21 is a rice orthologue of yeast Rad21.

Key words: Chromosome cohesin, cohesion, meiosis, mitosis, rice.

Sister chromatid cohesion is essential for accurate chromosome segregation during mitosis and meiosis. The cohesion is established in the S phase to link newly replicated sister chromatids together until metaphase-anaphase transition and is mediated by a phylogenetically conserved multiprotein complex called cohesin (for a review see Nasmyth, 2001). Rad21/Rec8 is an important component and key regulator of cohesins. In yeast, Rad21 functions in mitotic chromatid cohesion (Guacci *et al*., 1997), otherwise Rec8, a meiotic variant of Rad21, has specificity for the differential release of the chromosome arm and centromeric cohesion in meiosis I and II (Watanabe and Nurse, 1999). Rad21 homologues have been found in the metazoan *Homo sapiens* (Hoque and Ishikawa, 2001), *Mus musculus* (McKay *et al*., 1996), *Drosophila melanogaster* (Warren *et al*., 2000), *Xenopus laevis* (Losada *et al*., 1998), and *Caenorhabditis elegans* (Pasierbek *et al*., 2001), and also in the higher plant *Arabidopsis thaliana* (Dong *et al*., 2001). Rec8 homologues have been identified in mammals (Parisi *et al*., 1999), *Caenorhabditis* (Pasierbek *et al*., 2001), and *Arabidopsis* (Bai *et al*.,1999), based on their expression specificity in generative cells (Parisi *et al*., 1999; Lee *et al*., 2003) or critical roles in meiotic chromatid cohesion and disjunction (Bai *et al*., 1999; Pasierbek *et al*., 2001). These results suggest which roles of Rad21 in mitosis and Rec8 in meiosis are conserved in higher eukaryotes.

However, recent studies have indicated that the precise roles of cohesins may be different between yeasts and higher eukaryotes. Unlike yeast, in which separase mediates the simultaneous dissociation of Scc1 from the chromosome arms and centromeres at the onset of anaphase, in vertebrates, Rad21 is dissociated from the arms and centromeres in a two-step process (Waizenegger *et al*., 2000). Moreover, the *Arabidopsis* genome contains at least two *RAD21*-like genes (Dong *et al*., 2001) and *Caenorhabditis* has three *RAD21*-like genes (Pasierbek *et al*., 2001; Mito *et al*., 2003). Until now, the molecular mechanism regulating chromatid cohesion and disjunction in mitosis and meiosis has not been clearly understood in higher eukaryotes, and little is known of the molecular regulation of chromatid cohesion in higher plants. The molecular characterization of *OsRAD21-1*, a rice *RAD21*-like gene, is reported here.

Rice cultivar Zhonghua 10 (*Oryza sativa* L. ssp. *japonica*) was used in this study. Flowers in which pollen mother cells were in meiosis were collected. Leaves were collected from 3-week-old plants. Roots and buds were harvested from seeds germinated on sterile-water-soaked papers. Total RNA was isolated with a Trizol kit (GIBCO-BRL) according to the manufacturer's protocol, and was then treated with RNase-free DNaseI (TaKaRa) to remove residual genomic DNA. Performing TBLASTN searches in the DDBJ/GenBank database using the amino acid sequence of fission yeast Rad21 as the probe, a rice expressed sequence tag (EST) (AF140489) was identified which had an uncomplete ORF encoding 169 amino acids. BLASTP analyses revealed that this deduced amino acid sequence was homologous to N-termini of Rad21 proteins from yeast, *Arabidopsis*, *Xenopus*, and mammals, suggesting that the EST represented a partial sequence of a mRNA of a likely rice *RAD21* homologue, here termed *OsRAD21-1*. 5′ and 3′ ends of this transcript were isolated by RACE according to the method of Ding *et al*. (2002). The full-length cDNA of 3698 bp had an ORF of 3165 bp with a 5′ UTR of 132 bp and a 3′ UTR of 401 bp, and encoded a predicted polypeptide of 1055 amino acids (OsRad21-1) with a calculated molecular weight of 115 245 Da and a pI of 4.23.

RT-PCR experiments revealed that *OsRAD21-1* was expressed at all organs examined, with high levels in flowers and buds, middle-levels in leaves, and low-levels in roots (data not shown).

Southern blot analysis revealed that *OsRAD21-1* is a single-copy gene in the rice genome (data not shown). The gene is localized at chromosome 11 (PAC clone P0506A10) by BLAST searches in the TIGR rice genomic sequence database with OsRAD21-1 cDNA as a

* The nucleotide sequence data reported here will appear in the EMBL, GenBank and DDBJ nucleotide sequence database under the accession number AY288943.
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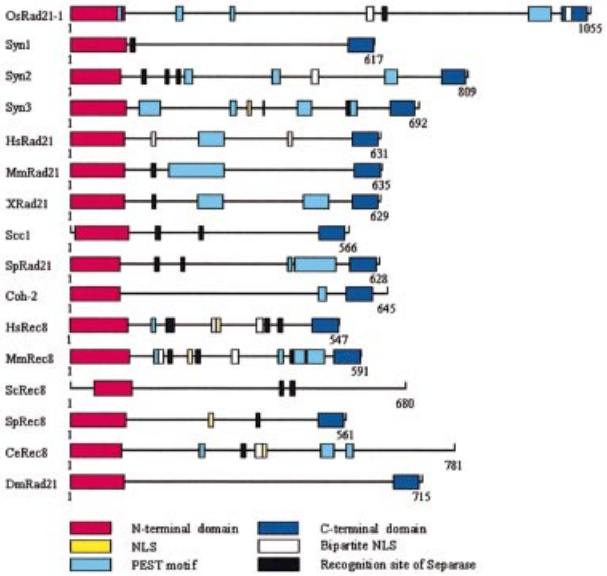
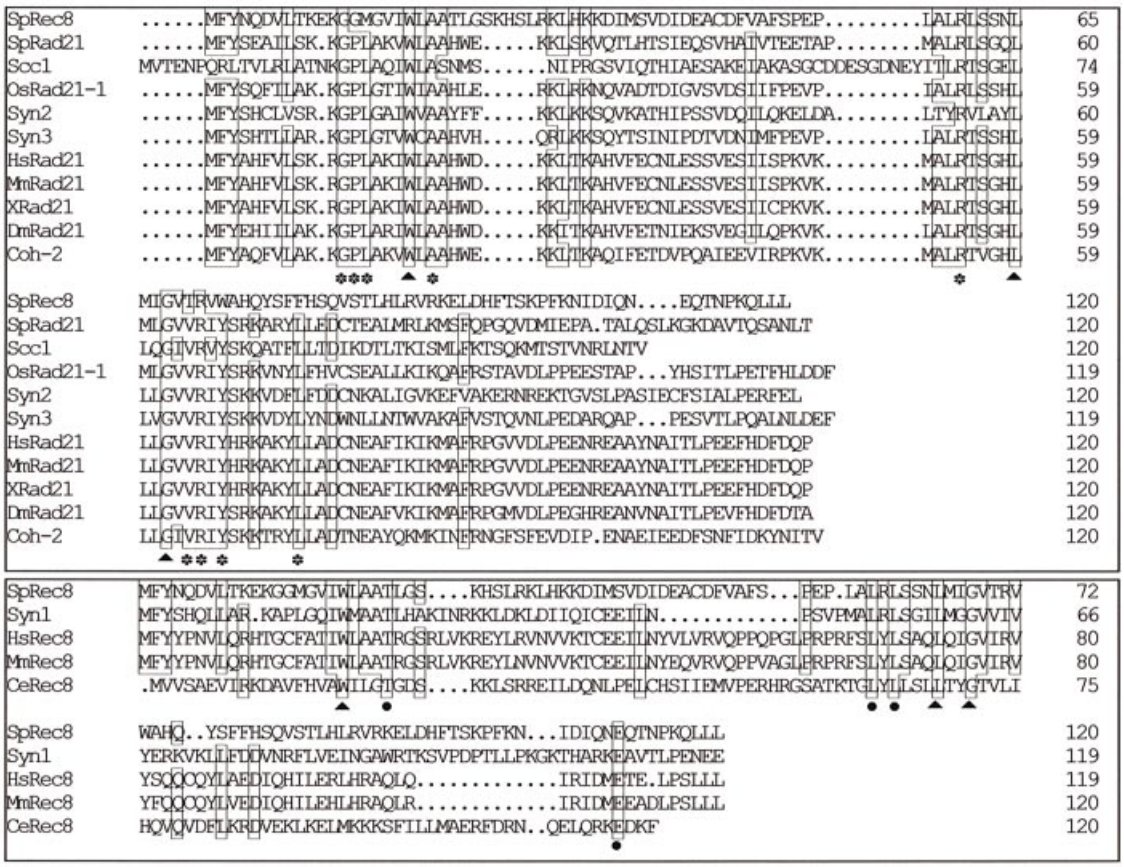
query. Comparison of this cDNA to the genomic DNA sequence revealed that a 10 kb genomic DNA sequence spanning all exons represents *OsRAD21-1*, which is composed of 14 exons and 13 introns. These introns were spliced out by canonical GT-AG sites. There is great variation in the size of exons and introns of this gene. The largest exon is 776 bp long (exon 6) with the smallest one of 34 bp (exon 11). In introns, the largest one is 2960 bp in length (intron 1), whereas the smallest one is 83 bp long (intron 7).



A



B

Predicted OsRad21-1 is a relatively hydrophilic protein and has an entire N-terminal domain (Pfam 04825) and a C-terminal domain (Pfam 04824), which are conserved in previously identified Rad21/Rec8 proteins and proposed to be involved in the interaction with Smc1–Smc3 heterodimer during cohesion establishment (Nasmyth, 2002; Gruber *et al*., 2003). In addition, This protein contains (1) two potential bipartite nuclear targeting motifs at positions 602 (KRPRLTSKSTTPKRKVQ) and 998 (RKSVAIDHLLSGKTRKE), (2) one potential cleavage site of separase at position 634 (LISTEDIRRIR), (3) multiple proposed phosphorylation sites of casein kinase II, protein kinase C, and tyrosine kinase, and (4) a PEST motif (Fig. 1A), which acts as a signal for rapid protein degradation via the ubiquitin–26S protease pathways (Rechsteiner and Rogers, 1996).

Multiple alignments of OsRad21-1 with other Rad21/Rec8 sequences revealed which similarity mainly occurred in their N- and C-terminal regions with the greatest similarity at their N-termini. The central regions of these proteins exhibited quite low similarity with great variation in length. The N-terminal domains (Pfam 04825) and the C-terminal domains (Pfam 04824) of these Rad21/Rec8 proteins isolated to date were identified further. Pfam 04825 is present in all these proteins, whereas Pfam 04824 is present in all but ScRec8 and CeRec8 (Fig. 1A). The function of ScRec8 has not yet been identified, whereas CeRec8 has been identified as being essential to *Caenorhabditis* meiotic chromosome cohesion and segregation (Pasierbek *et al*., 2001). Therefore, it seems highly possible that Pfam 04825 is a key functional domain of Rad21/Rec8 proteins in regulating the establishment of cohesion. Based on these analyses, a pairwise comparison of 240 aa N-terminal regions, containing Pfam 04825, of OsRad21-1 and other Rad21/Rec8 proteins was used to estimate OsRad21-1 as Rad21-like or Rec8-like. This comparison revealed that OsRad21-1 was closest to the Rad21 proteins from yeast and *Arabidopsis*, with 25%, 38%, 42%, 43%, and 35% similarity to Scc1, SpRad21, HsRad21, MmRad21, and Syn3, respectively, compared with 17%, 23%, 23%, 21%, and 27% to each corresponding meiotic variant, respectively.

Furthermore, conserved residues were analysed in the N-terminal domain regions of Rad21 and Rec8 proteins by optimal alignments. Some conserved residues were readily identified to be invariant in all Rad21 proteins and not in Rec8 proteins, and vice versa (Fig. 1B). OsRad21-1 and other Rad21 proteins share identical residues G, P, L, A, R, V, R, Y, and L (^{12}G, ^{13}P, ^{14}L, ^{20}A, ^{55}R, ^{65}V, ^{66}R, ^{68}Y, and ^{75}L in SpRad21) (Fig. 1A), whereas Rec8 proteins have identical residues T, L, L, L, and E (^{23}T, ^{59}L, ^{61}L, and ^{111}E in SpRec8) (Fig. 1B) except that both share identical residues W, L, and G (taking SpRec8 as reference at positions 19, 65 and 68) (Fig. 1B). All data suggest that OsRad21-1 is probably a rice orthologue of yeast Rad21.

Interestingly, besides *OsRAD21-1*, three other *RAD21*-like genes have been identified (AY 371047, AY371048, and AY371049) from rice. In order to understand the biological function of these genes in regulating mitosis and meiosis, further experiments, including chromosome localization of proteins and knock-out of these genes by RNAi, are underway.

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Fig. 1. (A) Schematic domain structure of OsRad21-1 and other proteins of the Rad21/Rec8 family. The conserved N- and C-terminal domains were determined by RPS-BLAST. Potential nuclear localization signal (NLS) and bipartite NLS were identified by PredictNLS. The potential recognition sites of separase were predicted according to this motf (XXS/E/DX**E**XX**R**XXX). PEST motifs were identified by PESTfind program. (B) Multiple alignments of the conserved N-terminal domain regions of Rad21 (upper panel) and Rec8 (lower panel) proteins from different species. Gaps introduced to maximize the alignment are marked with dots. Numbers on the right indicate the position of the amino acid residues of each polypeptide. Identical residues in both Rad21 and Rec8 proteins are highlighted with solid arrowheads, and only in Rad21 proteins or in Rec8 proteins with 'stars' or 'filled circles', respectively. These sequences are shown here as follows: OsRad21-1 (this study, *Oryza sativa*, accession number AY288943); Syn1, Syn2, and Syn3 (*A. thaliana*, accession numbers AF080619, AF281154, and AF281155); HsRad21 and HsRec8 (*H. sapiens*, accession numbers NM_006265 and NM_005132); XRad21 (*X. laevis*, accession numbers AF051786); MmRad21 and MmRec8 (*M. musculus*, accession numbers NP_033035 and AAF69524); Coh-2 and CeRec8 (*C. elegans*, accession numbers NM_062435 and CAB05309); Scc1 and ScRec8 (*S. cerevisiae*, accession numbers U23759 and NP_15332); SpRad21 and SpRec8 (*S. pombe*, accession numbers CAA19348 and AB018077); and DmRad21 (*D. melanogaster*, accession number NM_143900).

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