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Characterization of the origin recognition complex (ORC) from a higher plant, rice (*Oryza sativa* L.)

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Abstract

The origin recognition complex (ORC) protein plays a critical role in DNA replication through binding to sites (origins) where replication commences. The protein is composed of six subunits (ORC1 to 6) in animals and yeasts. Our knowledge of the ORC protein in plants is, however, much less complete. We have performed cDNA cloning and characterization of ORC subunits in rice (*Oryza sativa* L. cv. Nipponbare) in order to facilitate study of plant DNA replication mechanisms. Our previous report provided a description of a gene, *ORC1* (*OsORC1*), that encodes one of the protein subunits. The present report extends this initial analysis to include the genes that encode four other rice ORC subunits, *OsORC2*, *3*, *4* and *5*. Northern hybridization analyses demonstrated the presence of abundant transcripts for all *OsORC* subunits in shoot apical meristems (SAM) and cultured cells, but not in mature leaves. Interestingly, only *OsORC5* showed high levels of expression in organs in which cell proliferation is not active, such as flag leaves, the ears and the non-tip roots. The pattern of expression of *OsORC2* also differed from other *OsORC* subunits. When cell proliferation was temporarily halted for 6–10 days by removal of sucrose from the growth medium, expression of *OsORC1*, *OsORC3*, *OsORC4* and *OsORC5* was substantially reduced. However, the level of expression of *OsORC2* remained constant. We suggest from these results that expression of *OsORC1*, *3*, *4* and *5* are correlated with cell proliferation, but the expression of *OsORC2* is not.

Keywords: DNA replication; Origin recognition complex (ORC); Cell proliferation; Plant

1. Introduction

The origin recognition complex (ORC), first described in *Saccharomyces cerevisiae* (Bell and Stillman, 1992), is a protein complex composed of six subunits showing considerable evolutionary conservation from yeasts to humans. The complex has important roles in the initiation of DNA replication, a dynamic process that occurs through the coordinated assembly and disassembly of protein complexes at replication origins (Stoeber et al., 2001; Gerbi et al., 2002). The specific sequence recognized by ORC in *S. cerevisiae* is termed the autonomous replicating sequence (ARS) (Gerbi et al., 2002); ARS elements are further classified by the functional modules known as A and B domains (Bielinsky and Gerbi, 1998, 1999). In early G1, the Cdc6 and Cdt1 proteins associate with ORC, which then interacts with the protein complex of Mcm2-7 (Tye, 1999). The MCM proteins gradually dissociate from chromatin as S phase proceeds, a

Abbreviations: ORC, Origin recognition complex; SAM, Shoot apical meristems; ARS, Autonomous replication sequence; ACS, ARS consensus sequence; MCM, Minichromosome maintenance; CDK, Cyclin-dependent kinase; Pre-RC, Pre-replication complex; RPA, Replication protein A; Os, *Oryza sativa*; Cdc, Cell division cycle; post-RC, Post-replication complex.

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behavior pattern that is consistent with their predicted DNA helicase functions. The assembly of ORC, Cdc6 and MCM proteins into the pre-replication complex (pre-RC) makes chromatin competent or 'licensed' for DNA replication (Bielinsky and Gerbi, 2001; Stoeber et al., 2001). The pre-RC can only form in G1 when cyclin-dependent kinase (CDK) activity is low, subsequent activation by CDK/cyclins convert the pre-RC to a post-RC (Liang and Stillman, 1997). Strand dissociation of dsDNA induces a conformational change in ORC before replication origins assume a post-RC chromatin state. ORC is believed to remain bound to origins throughout the cell cycle (Lei and Tye, 2001). Cdc6 is released just before or at the beginning of S phase and replaced by the Cdc45 protein, which, as the first step in establishing replication forks, recruits DNA polymerase α and RPA to the replication origin (Mimura and Takisawa, 1998; Mimura et al., 2000). Replication then proceeds bidirectionally in a semi-discontinuous manner.

Our research interest is in the mechanism of coordination of the DNA replication process. However, little is known about the mechanism. Recently, isolation and characterization of plant ORC have been reported in several plant species (Kimura et al., 2000; Witmer et al., 2003; Collinge et al., 2004; Masuda et al., 2004). In *Zea mays*, the expression patterns of *ZmORC* genes suggest they have a function related to DNA replication. *ZmORC3* may be pleiotropic, being involved in functions not requiring the whole ORC complex. Analyses of *ZmORC3–ZmORC5* indicate that they might from a core capable of binding *ZmORC1* (Witmer et al., 2003). The *AtORC2* of *Arabidopsis thaliana* is an essential gene in plant cells, as it is in fungi and animals. Post-fertilization expression of AtORC2 is essential to both embryo and endosperm development (Collinge et al., 2004). This report also showed that the *Arabidopsis* genome, unlike other eukaryotes, has two paralogues of ORC1, namely CDC6 and CDT1. In addition, the expression of the putative pre-RC genes in non-proliferating plant tissues suggests that they might have roles in processes other than DNA replication licensing (Masuda et al., 2004). *AtORC* genes are transcribed during early seed development and/or flower development (Collinge et al., 2004).

In this paper, we describe the molecular cloning and characterization of *ORC* subunits in rice, and detail their expression patterns in tissues. To date, only five *ORC* subunits, *ORC1*, *2*, *3*, *4*, and *5*, have been identified; no subunit corresponding to *ORC6* has yet been found.

2. Materials and methods

2.1. Plant material

Rice plants (*Oryza sativa* L. cv. Nipponbare) were grown in a growth cabinet under a 16 h day/8 h night cycle at 28 °C. Rice cells were grown in suspension culture as described previously (Baba et al., 1986). Suspension-cultured tobacco BY-2 cells were maintained in modified Linsmaier and Skoog medium (LSD medium) following a previously reported method (Takeda et al., 1992).

2.2. cDNA cloning of each of the ORC subunits from rice, *OsORC1, OsORC2, OsORC3, OsORC4 or OsORC5*

Genes encoding five subunits of the *O. sativa* origin recognition complex (*OsORC*) are described here (*OsORC1-5*). We have previously reported on the cDNA cloning of *OsORC1* (Kimura et al., 2000); the nucleotide sequence data of the gene have been lodged in the DDBJ/EMBL/GenBank nucleotide sequence databases (accession numberAB037135). The nucleotide sequences of the eight primers used for RT-PCR amplification of *OsORC2-4* were as follows: *OsORC2*, 5′-ATGGCACTGAGAGGCGGCCA-3′ (head region forward primer), 5′-TCAGGTCAATTCCTGAAGCA-3′ (body region forward primer); *OsORC3*, 5′-ATGGCTGCGCCGCCCGGTGA-3′ (head region forward primer), 5′-TCAAAGGCCGAATGCGATTC-3′ (body region forward primer); *OsORC4*, 5′-CTCCTTCCAACTCACATGGC-3′ (head region forward primer), 5′-TTACATGTATCTTTCACGGT-3′ (body region forward primer); *OsORC5*, 5′-ATGGATATCCTCCGGCTCCTC-3′ (head region forward primer), 5′-CTATCTTCTGTACATATACT-3′ (body region forward primer). PCR was carried out with rice cDNA produced from purified mRNA with a SuperScript First-strand system for RT-PCR kit (Invitrogen) as template. The amplified cDNA products were purified from a low-melting-point agarose gel, and cloned into the pGEM vector (Promega).

2.3. Analysis of DNA sequences

DNA sequencing was performed by the dideoxy chain termination method with a sequencing kit and DNA sequencer from Applied Biosystems. DNA sequence analysis was performed using GENTETIX MAC var. 10 (SOFTWARE DEVELOPMENT CO., LTD).

2.4. Northern hybridization

Aliquots of 20 μg of total RNA were resolved on 1.2% formaldehyde agarose gels and transferred onto nylon membranes (Hybond-N, Amersham). After pre-hybridization, the filters were probed with ^{32}P-labeled cDNAs at 42 °C for 16 h, followed by washing twice with 2 X SSC+0.1% SDS at room temperature for 15 min and three times with 0.1 X SSC+0.1% SDS at 65 °C for 20 min.

2.5. Culture of different plant materials and cell synchronization

Oc cultured cells, originally established by Baba et al. (1986) using protoplasts from rice roots (*O. sativa* L. cv. C5924), were subcultured weekly in an amino