Two Jobs for the Origin Replication Complex

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In bacteria and viruses, a key event in cell division—DNA replication—begins at the origin of replication and is triggered by the interaction of specific DNA sequences and initiation proteins (*1*). New information suggests that DNA replication is likely to be triggered similarly in a simple eukaryote, yeast. The DNA sequences at the chromosomal origins of replication have been identified in the yeast *Saccharomyces cerevisiae* (*2*). These sequences are a subset of a larger group of sequences called autonomously replicating sequence (*ARS*) elements and are identified by their ability to promote extrachromosomal replication of plasmids in yeast cells (*2*). A protein complex specifically binds to an essential, conserved domain of these *ARS* elements (*3*). In this issue of *Science*, three papers (*4–6*), together with a fourth recent paper in *Nature* (*7*), provide the first evidence that this complex, the origin recognition complex (ORC), indeed mediates replication initiation in vivo. A remarkable aspect of these findings is that ORC proteins also participate in yeast transcriptional "silencing," a form of repression of transcription seen at telomeres and at the two mating type loci of *S. cerevisiae*.

Chromosomal origins (as well as other *ARS* elements not active as chromosomal origins) contain two domains, A and B (*2*). Domain A contains an *ARS* consensus sequence (ACS), a degenerate 11-nucleotide sequence [5'-(A/T)TTTA(T/C)(A/G)TTT(A/T)-3'] that is essential for chromosomal origin function (*8*, *9*). Domain B, located 3' to the T-rich strand of the ACS, is highly variable, but contains several elements (B1, B2, and B3). Individually, these elements are not essential, but they independently contribute to *ARS* function (*10*). Thus, the essential ACS was the prime candidate for a recognition site for proteins that initiate replication.

The ORC contains proteins of 120, 72, 62, 56, 53, and 50 kilodaltons and has properties similar to those of prokaryotic replication initiators. Specific binding of ORC to DNA requires adenosine triphosphate and the ACS. Mutations in the ACS that interfere with binding also interfere with *ARS* function. Moreover, a pattern of alternating protected and susceptible deoxyribonuclease I cleavage sites 3' to the ACS suggests that DNA might be wrapped around a protein core, a property of both the *Escherichia coli* and bacteriophage λ initiator proteins (*3*). A similar pattern of deoxyribonuclease protection is seen at *ARS1* in gently lysed cells, further suggesting that ORC interacts with the ACS in vivo (*11*).

Mutations in the gene encoding the 72-kilodalton subunit of ORC, *ORC2*, were isolated by two groups while they were screening with an ACS-containing DNA sequence element that participates in the transcriptional repression ("silencing") of the silent mating type locus *HMR* (*12*). This repression requires cis-acting elements, called silencers, that flank The mating type loci *HML* and *HMR*, as well as trans-acting gene products (*12*). Foss, Rine, and colleagues screened for trans-acting mutations that derepressed an *HMR* locus (under the control of a silencer that depended on an ACS for function) at the permissive temperature and were lethal at high temperature (*4*). Some of these mutations identified a gene that functioned through the ACS in the silencer. The gene encoded the purified 72-kilodalton subunit of ORC (*5*), *ORC2*. Micklem and co-workers identified the same gene in a screen for trans-acting mutations that abolished the ability of an *HMR* silencer to repress transcription at a heterologous promoter controlling a *lacZ* reporter gene (*7*). The mutations that derepressed transcription (in a gene called *RRR1*) acted through the ACS of the silencer. *RRR1* turned out to be identical to *ORC2*.

Li and Herskowitz (*6*) identified *ORC6* more directly, with a screen for proteins that interact with the ACS. The strategy was derived from the powerful two-hybrid screen, which detects protein-protein interactions (*13*). A reporter gene was constructed with several copies of the ACS in its promoter. Hybrid expression libraries, consisting of a transcriptional activation domain fused to random protein segments, were screened for proteins capable of activating transcription of the reporter gene. The resulting *ORC6* gene encodes peptides found in the 50-kilodalton subunit of ORC (*5*).

The predicted amino acid sequences of the proteins encoded by *ORC2* and *ORC6* offer little insight into their functions. Nevertheless, the properties of *orc2* and *orc6* mutants and the genetic interactions of *ORC6* with other DNA replication mutants support the idea that ORC is required for the initiation of DNA replication. First, both *ORC2* and *ORC6* are essential genes

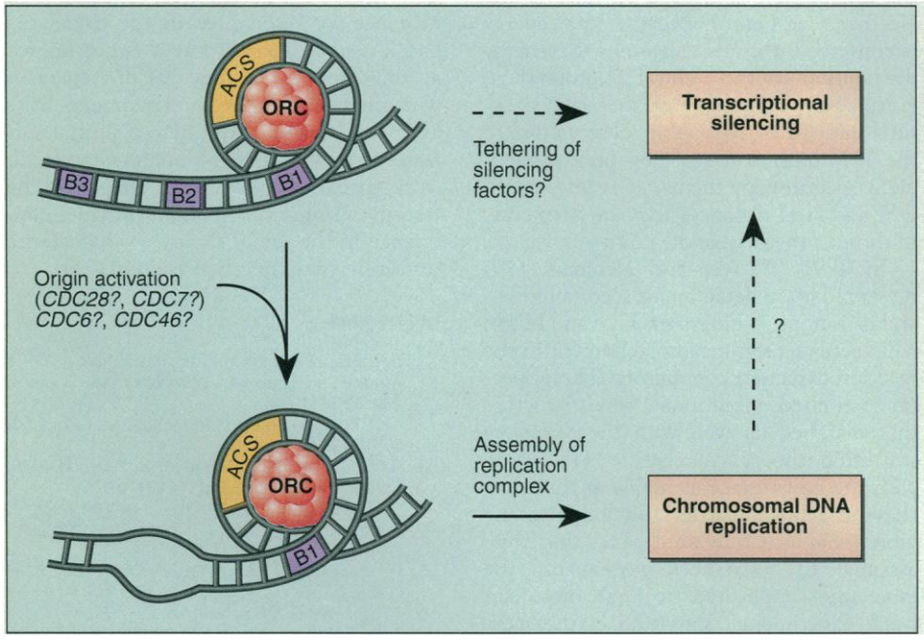

ILLUSTRATION: KATHARINE SUTLIFF

Roles for ORC in DNA replication and transcriptional silencing. ORC is likely to bind replication origins throughout the cell cycle. Origins are activated during S phase by activation of ORC by other factors. Domain B of *ARS* elements is easily unwound, suggesting that the DNA replication machinery may assemble on single-stranded DNA in domain B (*2*). Interaction of ORC with the transcriptional silencer *ARS* elements is required directly or indirectly for silencing.

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(*4*, *6*, *7*). The phenotype of the temperature-sensitive *orc2-1* mutant and *orc6* null mutants, a large budded cell with a single undivided nucleus, resembles other well-characterized mutants defective in DNA replication (*4*, *6*). Second, the reduced stability of plasmids in *orc2* mutants is consistent with a role for *ORC2* in DNA replication (*4*, *7*). Third, overexpression of *ORC6* in two cell-cycle mutants (*cdc6* and *cdc46*), which are thought to be faulty in the initiation of DNA replication, enhanced their temperature-sensitive defects (*6*). The functions of the *CDC6* and *CDC46* genes are unknown, but they act at a time in the cell cycle consistent with their functioning in an early step of DNA replication and show the loss-of-plasmid phenotypes characteristic of DNA replication mutants. Indeed, the *cdc6* loss-of-plasmid phenotype can be suppressed by the addition of *ARS* elements to the plasmid (*14*). Finally, *orc2-1* mutants in S phase complete replication and cell division and accumulate with a haploid (1C) DNA content, suggesting that ORC is required for the initiation of DNA synthesis (*5*).

ORC2 was identified in screens for mutations that relieve transcriptional silencing (*4*, *7*). By definition then, ORC can negatively regulate gene expression, in addition to its participation in DNA replication. The mechanism may be indirect (see figure): Initiation of replication at the silencer *ARS* elements could be essential for establishing the repressed state. Indeed, several observations suggest that DNA replication may have a role in silencing: The establishment of the repressed state requires passage through S phase (*12*). Further, a mutation in an *HMR* silencer that blocks its origin function also abolishes silencing (*9*), and mutations in *CDC7*, a gene required for the initiation of replication, suppress silencing defects (*12*). An event during replication may allow the establishment of the alternative chromatin structure of silent loci.

An alternative model is that ORC directly acts by protein-protein interactions to tether silencing factors to the appropriate sites in the chromosome (see figure). In this case, ORC itself or a protein complex different from ORC, but sharing one or more subunits with ORC, could be required for silencing. The silencing-defective allele of *ORC5* described by Foss and co-workers (*4*) supports the view that several ORC subunits participate in silencing. This direct model is supported by the observation that the *HML* silencers have no detectable chromosomal origin function (*15*). In addition, targeting a protein required for silencing to *HMR* by the addition of a DNA binding domain can bypass the requirement for the normal silencer element (*16*).

In an interesting parallel to its negative effects on transcription, ORC may also negatively regulate replication by preventing inappropriate initiation of DNA replication. ORC is likely to be bound to the ACS throughout the cell cycle where it could exert a repressive effect (*11*). In mutant *orc2-1* cells in which ORC is not functional, aberrant, delayed DNA synthesis can occur after prolonged exposure to the nonpermissive temperature (*5*). This phenotype is shared with the *cdc46* mutants (*14*); in fact, *CDC46* is known to interact with *ORC6* (*6*). The abundant potential protein phosphorylation sites in the ORC2 and ORC6 proteins, which include consensus sites for cyclin-dependent kinase (*4*, *6*), suggest that these kinases could regulate ORC function during the appropriate part of the cell cycle. Both the kinase encoded by the *CDC7* gene (*2*) and activation of the *CDC28* kinase by the B-type cyclins (encoded by *CLB5* and *CLB6*) have been implicated in the initiation of S phase (*17*).

The possibility that ORC may have a function in transcriptional silencing independent of its role in DNA replication adds it to a growing list of eukaryotic proteins that function in both transcription and replication. These proteins include ARS binding factor–1, which functions as a replication enhancer and in transcriptional activation and silencing (*2*); MCM1, which has a clear role in the transcriptional regulation of genes under mating type control and is implicated in DNA replication by the plasmid maintenance defect of *mcm1* mutants (*2*); and replication factor–A, a eukaryotic single-stranded DNA binding protein that may repress transcription (*18*). A clear challenge for the future is to understand how these proteins mediate their various functions.

We also do not yet know how ORC works during DNA replication. The sequences of the other four ORC subunits may reveal homologies that suggest their function. The establishment of an in vitro replication system dependent on yeast origins of replication would allow the detailed analysis of the function of ORC and other proteins in this process. The SV40 in vitro replication system depends on large amounts of its initiator protein, T antigen. Perhaps ORC will prove to be the key to yeast replication in vitro.

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