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The Origin Recognition Complex Links Replication, Sister Chromatid Cohesion and Transcriptional Silencing in *Saccharomyces cerevisiae*

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ABSTRACT

Mutations in genes encoding the origin recognition complex (ORC) of *Saccharomyces cerevisiae* affect initiation of DNA replication and transcriptional repression at the silent mating-type loci. To explore the function of ORC in more detail, a screen for genetic interactions was undertaken using large-scale synthetic lethal analysis. Combination of *orc2-1* and *orc5-1* alleles with the complete set of haploid deletion mutants revealed synthetic lethal/sick phenotypes with genes involved in DNA replication, chromatin structure, checkpoints, DNA repair and recombination, and other genes that were unexpected on the basis of previous studies of ORC. Many of these genetic interactions are shared with other genes that are involved in initiation of DNA replication. Strong synthetic interactions were demonstrated with null mutations in genes that contribute to sister chromatid cohesion. A genetic interaction between *orc5-1* and the cohesin mutant *scc1-73* suggested that ORC function contributes to sister chromatid cohesion. Thus, comprehensive screening for genetic interactions with a replication gene revealed a connection between initiation of DNA replication and sister chromatid cohesion. Further experiments linked sister chromatid cohesion genes to silencing at mating-type loci and telomeres.

THE origin recognition complex (ORC) plays a central role in the initiation of DNA replication in eukaryotes. ORC consist of six subunits (Orc1p–6p), all of which are required for viability. ORC, in combination with Cdc6p and a hexamer of minichromosome maintenance (MCM) proteins, forms the prereplicative complex (preRC) in the G_1 phase of the cell cycle. During S phase, preRCs activate origins by the recruitment of the Cdc45 protein and the unwinding of the DNA, presumably through the helicase activity of the MCM complex. Tight cell-cycle-mediated regulation by Cdc7p/Dbf4p and Cdc28-cyclin dependent kinase ensures that initiation at different origins is properly coordinated during the cell cycle (reviewed in Bell and Dutta 2002). Phenotypes of two temperature-sensitive mutants in *Saccharomyces cerevisiae ORC2* and *ORC5* genes, *orc2-1* and *orc5-1*, are characterized by high plasmid-loss rates and reduced firing of chromosomal origins (Bell *et al.* 1993; Foss *et al.* 1993; Fox *et al.* 1995; Liang *et al.* 1995; Loo *et al.* 1995). In addition to its replication function, ORC has a role in transcriptional silencing of the *HML* and *HMR* mating-type loci in yeast (Foss *et al.* 1993; Bell *et al.* 1995; Fox *et al.* 1995). The two roles are genetically separable (Dillin and Rine 1997). The separate silencing function of ORC, combined with ORC's association with origins during the whole cell cycle, prompted us to consider whether ORC also contributes to other processes.

The proper segregation of sister chromatids during anaphase depends on the establishment of sister chromatid cohesion during S phase and chromosome condensation before mitosis (Nasmyth 2002). An evolutionary conserved cohesin complex is required for cohesive linkage of sister chromatids (Guacci *et al.* 1997; Michaelis *et al.* 1997). The cohesin complex binds to chromosomes at distinct cohesion sites from late G_1 phase until the metaphase-anaphase transition when the Scc1p cohesin subunits are degraded (Uhlmann *et al.* 1999). Cohesin binding is enriched at centromeres where cohesion counteracts the pulling force of the mitotic spindle before the onset of anaphase. Physical and genetic evidence indicates that establishment of sister chromatid cohesion is also closely linked to DNA replication, most likely mediated by components of the replication fork (Carson and Christman 2001). *CTF7/ECO1* is an essential gene that is required in S phase for establishment of sister chromatid cohesion. Ctf7/Eco1 has been linked genetically and physically to the replication apparatus (Skibbens *et al.* 1999; Toth *et al.* 1999; Kenna and Skibbens 2003). Another link between sister chromatid cohesion and DNA replication came with the discovery of an alternative replication fork clamp loader (RFC) complex, mutations of which lead to loss of sister chro-

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matid cohesion (Mayer *et al.* 2001). The Ctf4 protein was originally identified through its binding to DNA polymerase α (Miles and Formosa 1992). Mutation of *CTF4* also leads to a cohesion defect (Hanna *et al.* 2001). Likewise, a nucleotidyl-transferase activity (polymerase σ) that is encoded by *TRF4* and *TRF5* contributes to sister chromatid cohesion (Wang *et al.* 2000). These proteins may contribute to the passage of the replication fork through a cohesion site (see Carson and Christman 2001). Finally, the *pol2-12* mutation in DNA polymerase ε also causes a cohesion defect, providing a direct connection between sister chromatid cohesion and a replicative polymerase (Edwards *et al.* 2003). Interesting links between cohesion and condensation of chromosomes to the epigenetic inheritance of transcriptional states have emerged in different organisms (reviewed in Hagstrom and Meyer 2003). For example, in *Schizosaccharomyces pombe*, the high concentration of Scc1 protein at centromeres requires stable heterochromatin formation at centromeric repeats (Bernard *et al.* 2001).

Synthetic lethal interactions have been used to establish functional relationships between genes (Guarente 1993; Hartman *et al.* 2001). In a previous synthetic lethal screen for genes that are required for viability of *orc2-1* mutants, *cdc7, cdc14*, and *orc3* mutants were isolated on the basis of the lethality of the double-mutant combinations (Hardy 1996). Strong genetic interactions between ORC and other replication genes have also been observed, but none of these studies approached genetic saturation of possible interactions (Liang *et al.* 1995; Loo *et al.* 1995; Kroll *et al.* 1996; Zou *et al.* 1997).

To provide a more comprehensive view of the processes linked to ORC and presumably therefore to origins of DNA replication, we used the synthetic genetic array (SGA) methodology to systematically evaluate double mutants between ORC genes and the deletion collection of nonessential genes. As expected, synthetic genetic interactions were seen between ORC mutants and mutations in genes involved in DNA replication. However, the combined network of interactions for ORC and other replication mutants reveals an extended link among replication initiation, sister chromatid cohesion, chromatin structure, checkpoint control, and DNA repair. Finally, new links were uncovered between the establishment of sister chromatid cohesion and transcriptional silencing.

MATERIALS AND METHODS

Synthetic genetic array analysis: To transfer the *orc5-1* allele to strains marked for SGA, the *orc5-1* allele was amplified from plasmid pJR1759 (*orc5-1* clone in pRS414) using a forward primer that is complementary to the first 25 bp of the *ORC5* open reading frame (5′-orc5-top, 5′-ATGAATGTGACCACTC CGGAAG-3′) and a reverse primer annealing 184 bp downstream from the stop codon (3′-orc5-TEF, GGGACGAGGCA AGCTAAACAGATCTCTAGTGGACTGAATTAATAACGGT). The nourseothricin-MX4 that confers resistance to the antibiotic nourseothricin (clonNAT, Werner BioAgents) was amplified from plasmid pFA6::natMX4 using primers MX5′ (5′-AGATC TGTTTAGCTTGCCTCG-3′) and MX3′-orc5 (TGTTTCGAA CGTATCCTGCCCTCTGGATACCTTCCAGGGAGATAAGA ATTCGAGCTCGTTTAAACTGGA-3′. MX3′-orc5 contains 45 bp of complementary sequence from the ORC5 stop codon. DNA (10 ng) of the two fragments was combined and amplified using primers 5′-orc5-top and MX3′-orc5. The *orc2-1* allele was amplified from plasmid pJR1675 (*orc2-1* in pRS315) and marked with natMX by the same method but using primers that were specific for *ORC2* (5′-orc2-top, 5′-ATGCTAAATGG GGAAGACTTTGT-3′; MX5′-orc2, Gggacgaggcaagctaaacagatc tGAGCTCATCAGACGTTTTTCAGT-3′; MX5′, 5′-AGATCTG TTTAGCTTGCCTCG-3′; MX3′-orc2′, CTAGCAAGCCTAGT ACTATTACAATTGTTCGTGATATGTTACATgaattcgagctcgtt taaactgga-3′). All PCR reactions used the PfuTurbo DNA polymerase kit (Stratagene, La Jolla, CA) to increase fidelity of the PCR reaction. PCR fragments were purified using the QIAEX protocol and transformed in the starting strain for synthetic genetic array analysis (Y2454: *MAT*α *mfa1*Δ*::MFA1pr-HIS3 can1*Δ *his3*Δ*1 leu2*Δ*0 ura3*Δ*0 MET15+ lys2*Δ*0*). The identity of the *orc-ts* allele was confirmed by its phenotype and correct integration was confirmed by PCR or DNA blot. SGA screening procedure with the marked *orc2-1* and *orc5-1* alleles was done essentially as described previously (Tong *et al.* 2001). Double-mutant selection was done at 23°, 26°, and 30° for the *orc5-1* strain and at 22°–23° for *orc2-1*. Potential synthetic lethal/sick interactions were confirmed by tetrad analysis at different temperatures, 23° and 30° for *orc2-1* and *orc5-1*, respectively. Positive results from the synthetic lethal screens were confirmed by tetrad dissection and growth at 23° and 30°, which are semipermissive temperatures for *orc2-1* and *orc5-1*, respectively. For the *orc5-1* screen, a *MAT***a** version of the *orc5-1::natMX4* strain was crossed with the haploid α collection strains for the confirmation of the results. A minimum of 10 tetrads were dissected from each cross. Some results from the *orc2-1* screen were also confirmed by random spore analysis as described by Tong *et al.* (2004).

Yeast strains: The genotypes of all yeast strains used are in Table 1. PCR fragments containing *ctf4*Δ*::kanMX4, ctf18*Δ*::kanMX4, dcc1*Δ*::kanMX4, trf4*Δ*::kanMX4*, and *trf5*Δ*::kanMX4* were amplified by colony PCR from the knockout collection strains. PCR products were purified using the QIAEX buffer desalting protocol and transformed into a diploid strain derived from mating of JRY4012 and JRY3009. Gene disruption in haploid segregants was confirmed by PCR and characterization of phenotypes. The *scc1-73::TRP1* allele was from strain ROY1063. Segregants from crosses with ROY1063 were tested for an intact *HMR-I* silencer by PCR.

Plasmid-loss assay: To measure plasmid-loss rates, plasmids pDK243 and pDK368-7 (Hogan and Koshland 1992) were transformed into strains JRY4012, JRY4285 (*orc5-1*), JRY7716 (*ctf4*Δ*::kanMX4*), and JRY7717 (*ctf18*Δ*::kanMX4*). Plasmid-loss assays were done as described previously (Loo *et al.* 1995), except that growth in nonselective medium was for 9 or 10 generations at 30°.

Sister chromatid cohesion assay: Tet repressor-green fluorescent protein (GFP)/Tet operator repeat constructs were used to visualize the *URA3* region on chromosome V (Michaelis *et al.* 1997). TetR-GFP was expressed from K3524/yplac128tetR-GFP (*URA3*-5′NLStetR-SuperGlowGFP-*ADH-T::LEU2::leu2-3,112*). The K2524 construct was integrated into JRY4012 to generate JRY7468. JRY7469, JRY7470, and JRY7471 were generated from JRY7468 with JRY4285 (*orc5-1*) and ROY1063 (*scc1-73*). To mark the *URA3* locus, these strains were transformed with plasmid pXH122 (p306tetO2X224 ChV-38; He *et al.* 2000) that was linearized with *Eco*RV. Cells from overnight cultures were diluted to A_{600} ~0.1 and grown for 3 hr at 31° in SC-Ura

TABLE 1

Yeast strains used in this study

Strain	Genotype	Source[a]
Y2454	*MAT*α *his3*Δ*1 leu2*Δ*0 lys2*Δ *ura3*Δ*0 can1*Δ *mfa1*Δ*::MFA1pr-HIS3*	C. Boone
JRY3009	*MAT*α *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1*	W303-1B; R. Rothstein
JRY4012	*MAT***a** *his3-11,15 leu2-3,112 lys2*Δ *trp1-1 ura3-1*	
JRY4125	W303 *MAT*α *ade2-1 orc2-1*	
JRY4250	W303 *MAT*α *ade2-1 orc5-1*	
JRY4249	W303 *MAT*α *lys2*Δ *orc5-1*	
JRY4285	W303 *MAT***a** *lys2*Δ *orc5-1*	
ROY1063	W303 *MAT*α *lys2*Δ *scc1-73::TRP1 HMR*Δ*I*	R. Kamakaka
JRY4555	W303 *MAT*α *ade2-1 orc5-1R*	
JRY5329	W303 *MAT*α *ade2-1 HMR::2EDA*	D. Shore
JRY3371	W303 *MAT***a** *ade2-1* Δ*Ahmr::TRP1*	D. Shore
JRY3372	W303 *MAT***a** *ade2-1* Δ*Ahmr::TRP1 rap1-12::LEU2*	D. Shore
JRY4441	W303 *MAT***a** *lys2*Δ *ura3*Δ*::LEU2 TEL-VII-L::TRP1::URA3*	
JRY4470	W303 *MAT***a** *ade2-1 ura3*Δ*::LEU2 TEL-VII-L::URA3::TRP1 sir2*Δ*::LEU2*	
JRY5475	*MAT*α *HMRa-e** cdc44-5::URA3(cs) ade2 his3 trp1 leu2 ura3 lys2*	
JRY6115	W303 *MAT*α *TELVII-L::URA3 TEL-V-R::ADE2*	P. Kaufman
JRY7459	Y2454 *orc5-1::natMX*	
JRY7460	Y2454 *orc2-1::natMX*	
JRY7461	*MAT***a** *his3*Δ*1 leu2*Δ*0 lys2*Δ *ura3*Δ*0 can1*Δ *mfa1*Δ*::MFA1p-HIS3 orc5-1::natMX4*	
JRY7463	W303 *MAT***a** *ade2-1 ctf4*Δ*::kanMX4*	
JRY7464	W303 *MAT***a** *ade2-1 dcc1*Δ*::kanMX4*	
JRY7465	W303 *MAT***a** *ade2-1 ctf18*Δ*::kanMX4*	
JRY7466	W303 *MAT***a** *ade2-1 trf4*Δ*::kanMX4*	
JRY7467	W303 *MAT***a** *ade2-1 trf5*Δ*::kanMX4*	
JRY7468	W303 *MAT***a** *lys2*Δ *K3524/p128tetR-GFP::LEU2::leu2-3,112*	
JRY7469	W303 *MAT***a** *lys2*Δ *K3524/p128tetR-GFP::LEU2::leu2-3,112 orc5-1*	
JRY7470	W303 *MAT***a** *lys2*Δ *K3524/p128tetR-GFP::LEU2::leu2-3,112 scc1-73::TRP1*	
JRY7471	W303 *MAT***a** *lys2*Δ *K3524/p128tetR-GFP::LEU2::leu2-3,112 scc1-73::TRP1 orc5-1*	
JRY7473	W303 *MAT***a** *ade2-1 TEL-VII-L::URA3 TEL-V-R::ADE2 ctf4*Δ*::kanMX4*	
JRY7474	W303 *MAT***a** *ade2-1 TEL-VII-L::URA3 TEL-V-R::ADE2*	
JRY7475	W303 *MAT***a** *ade2-1 TEL-VII-L::URA3 TEL-V-R::ADE2 ctf18*Δ*::kanMX4*	
JRY7476	W303 *MAT***a** *lys2*Δ *orc5-1 K3524/p128tetR-GFP::LEU2::leu2-3,112 pXH122::URA3::ura3-1*	
JRY7477	W303 *MAT***a** *lys2*Δ *orc5-1 K3524/p128tetR-GFP::LEU2::leu2-3,112 pXH122::URA3::ura3-1*	
JRY7478	W303 *MAT***a** *lys2*Δ *scc1-73::TRP1 K3524/p128tetR-GFP::LEU2::leu2-3,112 pXH122::URA3::ura3-1*	
JRY7479	W303 *MAT***a** *lys2*Δ *orc5-1 scc1-73::TRP1 K3524/p128tetR-GFP::LEU2::leu2-3,112 pXH122::URA3::ura3-1*	
JRY7480	W303 *MAT***a** *ade2-1 HMR::2EDA ctf4*Δ*::kanMX4*	
JRY7481	W303 *MAT***a** *ade2-1 HMR::2EDA*	
JRY7482	W303 *MAT***a** *ade2-1 HMR::2EDA ctf4*Δ*::kanMX4 orc5-1R*	
JRY7483	W303 *MAT***a** *ade2-1 HMR::2EDA orc5-1R*	
JRY7484	W303 *MAT***a** *ade2-1 HMR::2EDA ctf4*Δ*::kanMX4 rap1-12::LEU2*	
JRY7485	W303 *MAT*α *ade2-1 HMR::2EDA ctf4*Δ*::kanMX4*	
JRY7486	W303 *MAT***a** *ade2-1 HMR::2EDA rap1-12::LEU2*	
JRY7488	W303 *MAT***a** *ade2-1* Δ*Ahmr::TRP1 ctf4*Δ*::kanMX4*	
JRY7489	W303 *MAT***a** *ade2-1* Δ*Ahmr::TRP1 ctf4*Δ*::kanMX4 rap1-12::LEU2*	
JRY7497	W303 *MAT*α *lys2*Δ *TEL-VII-L-TRP1-URA3 ctf4*Δ*::kanMX4*	
JRY7498	W303 *MAT***a** *lys2*Δ *ura3*Δ*::LEU2 TEL-VII-L::TRP1::URA3 ctf4*Δ*::kanMX4*	
JRY7499	W303 *MAT***a** *lys2*Δ *TEL-VII-L-TRP1-URA3 ctf18*Δ*::kanMX4*	
JRY7500	W303 *MAT*α *lys2*Δ *TEL-VII-L-TRP1-URA3 ctf18*Δ*::kanMX4 ura3*Δ*::LEU2*	
JRY7501	W303 *MAT***a** *lys2*Δ *TEL-VII-L-TRP1-URA3 dcc1*Δ*::kanMX4 ura3*Δ*::LEU2*	
JRY7502	W303 *MAT*α *lys2*Δ *TEL-VII-L-TRP1-URA3 dcc1*Δ*::kanMX4 ura3*Δ*::LEU2*	
JRY7716	W303 *MAT***a** *lys2*Δ *ctf4*Δ*::kanMX4*	
JRY7717	W303 *MAT***a** *lys2*Δ *ctf4*Δ*::kanMX4*	
JRY7719	W303 *MAT*α *ade2-1 HMR::2EDA ctf18*Δ*::kanMX4*	

[a] Strains in our laboratory collection that were obtained from others are indicated.

or YPD. To monitor sister chromatid cohesion in G_2/M phase, cells were transferred to A_{600} 0.2 in YPD + 15 μg/ml nocodazole and arrested for ~2.5 hr at 31°. To control for aneuploidy, cells were arrested at A_{600} ~0.2 in YPD + 5 μg/ml α-factor for 2.5 hr at 31°. Aliquots (0.9 ml) were fixed with 100 μl 37.5% formaldehyde for 10 min at 4°. Samples were washed twice with 1 ml ice-cold phosphate-buffered saline and sonicated for 10 sec. After the second wash, the pellets were resuspended in 200 μl phosphate-buffered saline and GFP dots were directly analyzed by fluorescence microscopy. The fraction of cells with two GFP dots was compared to the total number of arrested cells. Microscopy was done using a Nikon Eclipse E600 microscope, a Nikon ×100 Plan Apo phase objective, and a Hamamatsu digital camera C4742-95. Slides were coded during scoring so that the scorer was blind to the genotype of the sample.

Silencing assays: All silencing assays were done with *ctf4Δ::kanMX4, ctf18Δ::kanMX4,* and *dcc1Δ::kanMX4* in the *W303* background. To assay silencing at *HMR*, strain JRY5329 with *HMR::2EDA* replacing *HMR*a was used (Sussel *et al.* 1993). Cultures were grown to A_{600} ~1 in liquid YPD medium and 100 μl from $1:10^4$ dilutions was plated on YPD plates. The deletion strains containing *ADE2* at the *HMR* locus were analyzed for development of red or pink color after 3 days at 30° and 3 additional days at 4°. To assay telomeric silencing for the *URA3-TRP1* reporter at telomere *VII-L*, cultures were grown to A_{600} ~0.5 in liquid YPD medium and spotted in fivefold serial dilutions on SC, SC + 0.1% fluoroorotic acid (FOA), and SC-TRP plates. Strains containing the *ADE2* reporter gene at telomere *V-L* were grown to A_{600} ~1 in YPD and plated on SC medium to obtain 100–200 colonies per plate. Photographs were taken after 3 additional days at 4°.

RESULTS

Synthetic genetic array analysis has been used to identify large networks of interacting genes (Tong *et al.* 2001). This success prompted the application of this method to genes involved in the initiation of DNA replication. Applying the synthetic genetic array methodology, we found 38 genes that show reduced growth with *orc2-1* (Figure 1A). Interactions from the synthetic lethal screens were retested by either tetrad dissection or random spore analysis, with sample data shown for *orc2-1* and *orc5-1* combined with *mrc1Δ* (Figure 1B). More interacting genes were recovered in the *orc2-1* screen than in the *orc5-1* screen (Table 2). However, all results from the *orc5-1* screen were also found in the *orc2-1* screen. This complete overlap suggests that both Orc2p and Orc5p function in only one essential process. Lower recovery of interactions with *orc5-1* was in part due to minor differences in the conditions of the screens using the two mutants and, perhaps, due to *orc2-1* being more defective than *orc5-1* at some temperatures. The results of the ORC screens overlapped extensively with those from the synthetic lethal screens with *cdc45-1* and *cdc7-1* (Figure 1C), which are described elsewhere (Tong *et al.* 2004). Considering the occurrence of false-negative results from the robotic screen, the overlap could be even more extensive. Thus, the pattern of synthetic lethal interactions clearly supported the common view that the major role of ORC is in DNA replication.

Genetic interactions with ORC: Genetic interactions were observed between *orc-ts* mutants and null alleles of genes that collectively have many different cellular roles. A subset of these synthetic interactions can be understood in light of the known function of the interacting gene. *POL32* encodes the only nonessential subunit of DNA polymerase δ, so it was not surprising that a null allele of this gene was synthetically lethal with *orc-ts* mutants. Likewise, earlier work established that replication mutants become dependent on double-strand-break repair by homologous recombination (Hartwell and Smith 1985). Hence the synthetic lethality between *orc-ts* mutants and *rad52Δ*, *rad55Δ*, and *hpr5Δ* presumably reflected an inability to rescue stalled or collapsed replication forks by recombination mechanisms. *MRC1* encodes a mediator of the DNA replication checkpoint and binds to Cdc45p as does the Tof1 protein (Katou *et al.* 2003; Osborn and Elledge 2003); hence the synthetic lethal interactions between mutations in *MRC1* and *TOF1* and *orc-ts* mutants may reflect the contribution of the replication checkpoint to cell survival when origin firing becomes limiting. On the other hand, the genetic interaction of *orc-ts* with *mrc1Δ* could be caused by an active role of Mrc1p in the replication process (Osborn and Elledge 2003). Strong interactions of *orc-ts* mutations were also found with a deletion of *CSM3* that encodes a newly identified component of the replication checkpoint (Tong *et al.* 2004). Compared with the replication checkpoint, DNA damage checkpoint mutants that exhibit reduced viability in combination with other replication mutants (see Figure 1C) were found to be only slightly sick in combination with *orc2-1* (*rad9Δ* and *rad24Δ*; Table 2) or were not picked up in the *orc-ts* screens (*chk1Δ*, *mec3Δ*, *rad17Δ*, and *ddc1Δ*).

Other double-mutant combinations with *orc-ts* mutants revealed connections that were not anticipated. For example, *FUN30* is a member of the Swi/Snf2 family, whose other members play a role in chromatin remodeling. Another possible link between replication and chromatin remodeling was revealed by the interaction with *ISW1*, an Snf2-related chromatin-remodeling factor. Factors that have a role in the deposition of nucleosomes onto newly replicated DNA were also prominent in the screens with *orc2-1*, *orc5-1*, and other replication mutants. Furthermore, different genes that have a role in histone modification were also identified, thus strengthening a link between replication and chromatin assembly or function. Growth defects in *orc-ts* strains were observed in combination with a null allele of the NAD^+-dependent histone deacetylase gene *HST3* and its paralog *HST1*. Interestingly, a synthetic sick interaction was also found when *orc5-1* and *orc2-1* were combined with the *sum1Δ* mutation. *SUM1* encodes a repressor of middle meiotic genes and requires the deacetylase activity encoded by *HST1* for its function (Xie *et al.* 1999). Several genes that have no known role in the replication process but have established roles in other cellular pro-

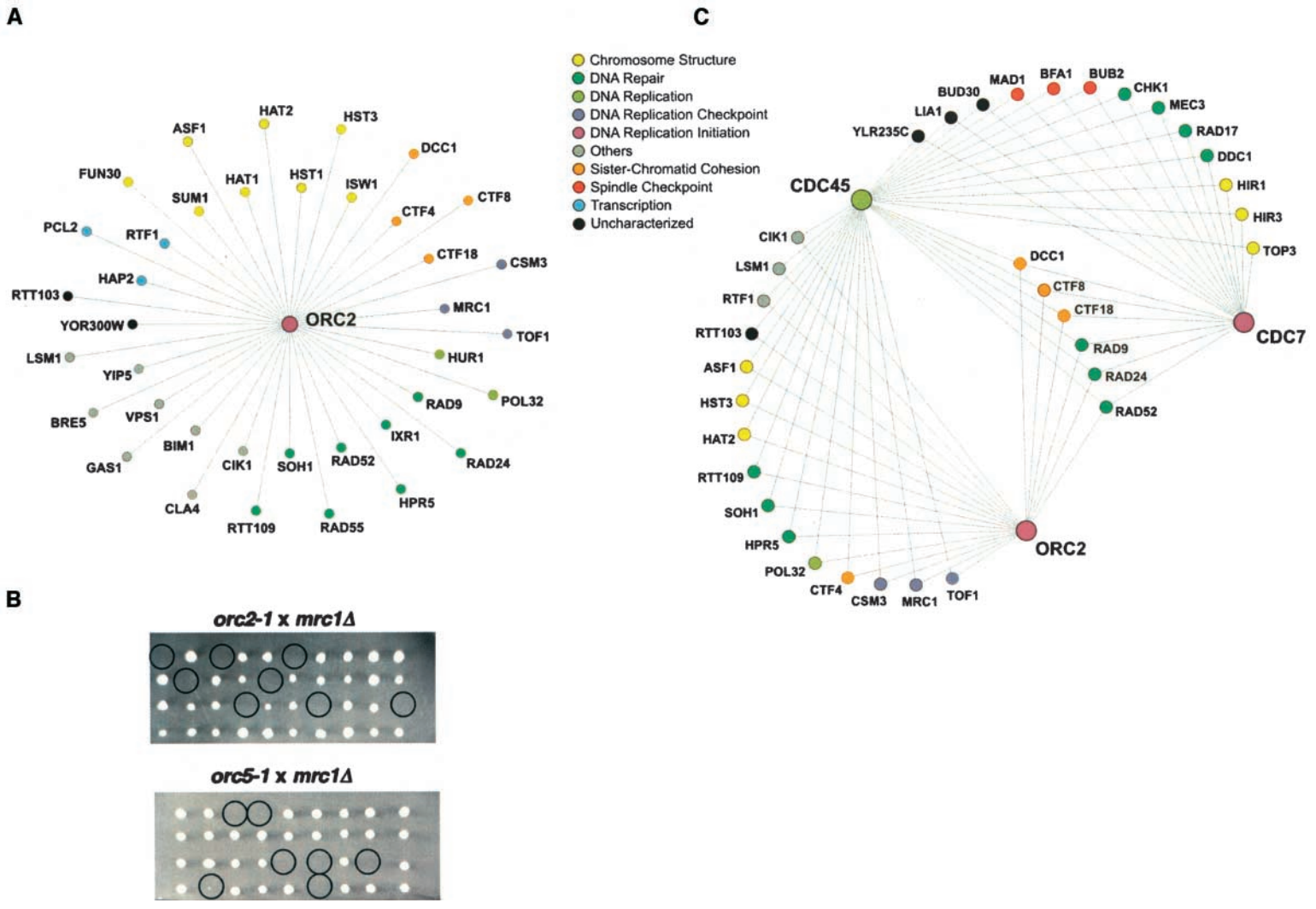

FIGURE 1.—The genetic interaction network for ORC. (A) Genes that interact with *ORC2* in SGA analysis. Genes that are required for full viability in combination with *orc2-1* cells are represented as nodes and the interactions are represented as edges connecting the nodes. Genes are colored according to a subset of their functional classification according to YPD (HODGES *et al.* 1999; COSTANZO *et al.* 2001). *TOF1* and *CSM3* are colored according to their role that was established from other SGA screens (TONG *et al.* 2004). (B) Synthetic lethality between *orc2-1* and *mrc1* and *orc5-1* and *mrc1*. Dissection of **a**/α *MRC1/mrc1*Δ*::kanMX orc2-1::natMX/ORC2* and **a**/α *MRC1/mrc1*Δ*::kanMX orc5-1::natMX/ORC5* is shown. Cells were grown at 23°. Genotyping revealed that nongrowing colonies correspond to the double mutants (circles). (C) Initiation of DNA replication network. Overlap of genetic interactions between *orc2-1, cdc45-1,* and *cdc7-1* is shown.

TABLE 2

Confirmation of *orc2-1* and *orc5-1* screens

Bait	Gene	Systematic name	RSA	Tetrad	Gene function
orc2-1		YOR300W	SS	SS	Uncharacterized
orc2-1	*ASF1*	YJL115W	SL		Chromosome structure
orc2-1	*BIM1*	YER016W	SL		Others
orc2-1	*BRE5*	YNR051C	SS		Uncharacterized
orc2-1	*CIK1*	YMR198W	SS		Others
orc2-1	*CLA4*	YNL298W	SL		Others
orc2-1	*CSM3*	YMR048W	SL		DNA replication checkpoint
orc2-1	*CTF18*	YMR078C	SL	SL[a]	Sister chromatid cohesion
orc2-1	*CTF4*	YPR135W		SL[a]	Sister chromatid cohesion
orc2-1	*CTF8*	YHR191C		SL[a]	Sister chromatid cohesion
orc2-1	*DCC1*	YCL016C	SL	SL[a]	Sister chromatid cohesion
orc2-1	*FUN30*	YAL019W	SS	SL	Chromosome structure
orc2-1	*GAS1*	YMR307W	SS	SS	Others
orc2-1	*HAP2*	YGL237C	SS		Transcription
orc2-1	*HAT1*	YPL001W	SS	SS	Chromosome structure
orc2-1	*HAT2*	YEL056W	SL	SS	Chromosome structure
orc2-1	*HPR5*	YJL092W	SS	SL[a]	DNA repair
orc2-1	*HST1*	YOL068C		SS[a]	Chromosome structure
orc2-1	*HST3*	YOR025W	SS	SS	Chromosome structure
orc2-1	*HUR1*	YGL168W	SS		Uncharacterized
orc2-1	*ISW1*	YBR245C	SS	SS	Chromosome structure
orc2-1	*IXR1*	YKL032C	SS	SL	DNA repair
orc2-1	*LSM1*	YJL124C	SS		Others
orc2-1	*MRC1*	YCL060C	SL	SL	DNA replication checkpoint
orc2-1	*PCL2*	YDL127W		SS	Transcription
orc2-1	*POL32*	YJR043C	SL	SL	DNA replication
orc2-1	*RAD24*	YER173W	SS		DNA repair
orc2-1	*RAD52*	YML032C	SS	SS	DNA repair
orc2-1	*RAD55*	YDR076W	SS		DNA repair
orc2-1	*RAD9*	YDR217C	SS		DNA repair
orc2-1	*RTF1*	YGL244W		SS	Transcription
orc2-1	*RTT103*	YDR289C	SS		Chromosome structure
orc2-1	*RTT109*	YLL002W		SL	Chromosome structure
orc2-1	*SOH1*	YGL127C	SS		DNA repair
orc2-1	*SUM1*	YDR310C	SS	SS[a]	Chromosome structure
orc2-1	*TOF1*	YNL273W	SS	SL	DNA replication checkpoint
orc2-1	*VPS1*	YKR001C	SS		Others
orc2-1	*YIP5*	YGL161C	SS	SS	Others
orc5-1	*CIK1*	YMR198W		SS	Others
orc5-1	*CSM3*	YMR048W		SL	DNA replication checkpoint
orc5-1	*CTF18*	YMR078C		SL[a,b]	Sister chromatid cohesion
orc5-1	*CTF4*	YPR135W		SL[a]	Sister chromatid cohesion
orc5-1	*CTF8*	YHR191C		SL[a]	Sister chromatid cohesion
orc5-1	*DCC1*	YCL016C		SL[a]	Sister chromatid cohesion
orc5-1	*FUN30*	YAL019W		SS[b]	Chromosome structure
orc5-1	*HAT1*	YPL001W		SS[b]	Chromosome structure
orc5-1	*HAT2*	YEL056W		SS	Chromosome structure
orc5-1	*HPR5*	YJL092W		SS[a]	Chromosome structure
orc5-1	*HST1*	YOL068C		SS[a,b]	Chromosome structure
orc5-1	*HST3*	YOR025W		SL	Chromosome structure
orc5-1	*ISW1*	YBR245C		SS[b]	Chromosome structure
orc5-1	*MRC1*	YCL061C		SL	DNA replication checkpoint
orc5-1	*RAD55*	YDR076W		SS	DNA repair
orc5-1	*RTT103*	YDR289C		SS	Chromosome structure
orc5-1	*SUM1*	YDR310C		SS[a,b]	Chromosome structure

Confirmation of synthetic lethal interactions by random spore analysis (RSA) and tetrad dissection. SL, synthetic lethal; SS, synthetic sick.

[a] These interactions were also confirmed in the *W303* background.

[b] These interactions were found only in the *orc2-1* screens, but were also confirmed for *orc5-1*.

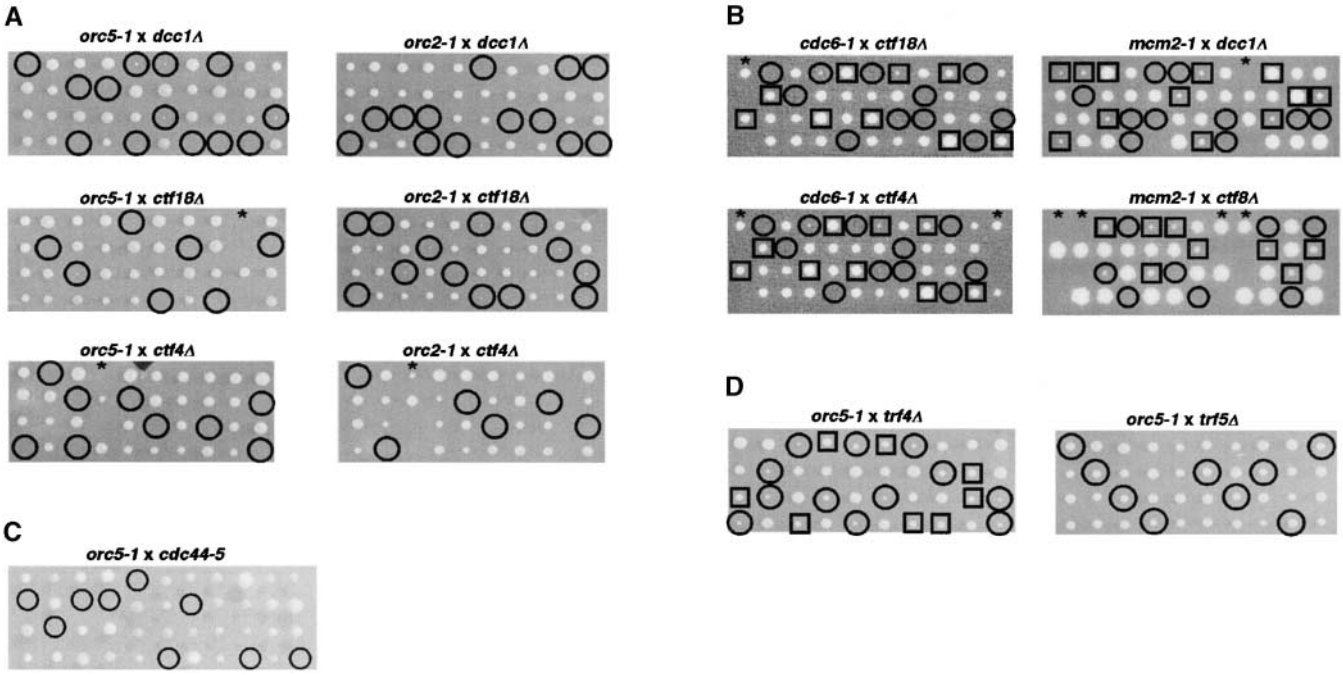

FIGURE 2.—*orc-ts* mutants and other initiation mutants genetically interacted with genes that are involved in sister chromatid cohesion. (A) Genetic interaction of *CTF18* and *DCC1* components of the alternate RFC and *CTF4* with *orc5-1* and *orc2-1*. Strains JRY7463 (*ctf4*Δ), JRY7464 (*dcc1*Δ), and JRY7465 (*ctf18*Δ) were crossed with JRY4125 (*orc2-1*) and JRY4250 (*orc5-1*) and dissected. Dissection plates were grown at 30°–31° for *orc5-1* and 23° for *orc2-1*. Double mutants (circles) were compromised for growth. (B) Genetic interactions of *cdc6-1* and *mcm2-1* with sister chromatid cohesion genes. Y2454 with *mcm2-1::natMX4* or *cdc6-1::natMX4* were crossed with strains of the deletion strain collection. *cdc6-1* crosses were grown at 25° and *mcm2-1* at 30°. Double mutants (circles) and *cdc6-1* or *mcm2-1* single mutants (squares) are indicated. (C) Synthetic lethality between *orc5-1* and *cdc44-5* mutation in the *RFC1* subunit. Cross between JRY5475 (*cdc44-5*) and JRY4249 (*orc5-1*) is shown. Tetrads were grown at 30° for 3 days. Circles denote position of expected double mutants. (D) Cross between JRY7466 (*trf4*Δ) and JRY4250 (*orc5-1*) and JRY7467 (*trf5*Δ) and JRY4250 (*orc5-1*). Double mutants (circles) and *trf4*Δ single mutants (squares) are indicated. Tetrads were grown at 31° for 3 days.

cesses [*e.g.*, *LSM1* in mRNA capping (THARUN *et al.* 2000) and *CIK1* and *BIM1* in microtubule function (PAGE and SNYDER 1992; SCHWARTZ *et al.* 1997)] also caused synthetic sickness or lethality when combined with *orc2-1* and *orc5-1*. The synthetic lethal screens also revealed interactions of *cdc45-1* and *cdc7-1* with *mad1*Δ and *bfa1*Δ, which are involved in the spindle checkpoint mechanism (see LEW and BURKE 2003). Activation of the spindle checkpoint at restrictive temperature by *orc-ts* and other replication mutations has been shown previously (GARBER and RINE 2002).

Links between ORC and sister chromatid cohesion: Among the especially strong interactors with *orc5-1* or *orc2-1* alleles were null alleles of genes that are involved in the establishment of sister chromatid cohesion during S phase. These genetic interactions included *CTF4* and three genes, *CTF8, CTF18*, and *DCC1*, that encode subunits of the alternate RFC complex that is important for sister chromatid cohesion (Figure 2A). The double-mutant segregants grew to microcolonies that consisted of large budded cells. Strong genetic interactions of *CTF* sister chromatid cohesion genes were also found with *cdc6-1* and *mcm2-1* (Figure 2B) and *cdc45-1* and *cdc7-1* (Figure 1C and data not shown). Given the strong genetic interactions of *orc-ts* alleles with components of one alternative RFC complex that replaces the canonical Rfc1 subunit (MAYER *et al.* 2001), we also analyzed the genetic interaction between ORC and the canonical Rfc1 subunit gene, *RFC1*. The combination of the *cdc44-5* mutation in *RFC1* with *orc5-1* was lethal (Figure 2C).

Previously, genetic interactions have linked *CTF4* and *CTF18* genes with components of the replication fork (HANNA *et al.* 2001). To determine whether the genetic interactions with initiation mutants revealed a role of *CTF4* and *CTF18* in the initiation of DNA replication, plasmid-loss rates were measured using plasmids that contain one (pDK243) or eight ARS1 sequences (pDK368-7) (HOGAN and KOSHLAND 1992). In *orc-ts* strains that have a defect in the initiation of DNA replication, a high plasmid-loss rate of plasmid pDK243 containing one ARS is suppressed by the addition of multiple ARSs (FOX *et al.* 1995). No suppression of plasmid loss by multiple ARSs was found in *ctf4*Δ and *ctf18*Δ strains. Plasmid-loss rates for pDK243 and pDK368-7 were 9.4 and 13.1% for *ctf4*Δ, 16.3 and 18.8% for *ctf18*Δ, 16.5 and 1.8% for *orc5-1*, and 0.3 and 0.07% for wild-type control strain. Thus, by this assay, no link was found between *CTF4* and *CTF18* and replication initiation.

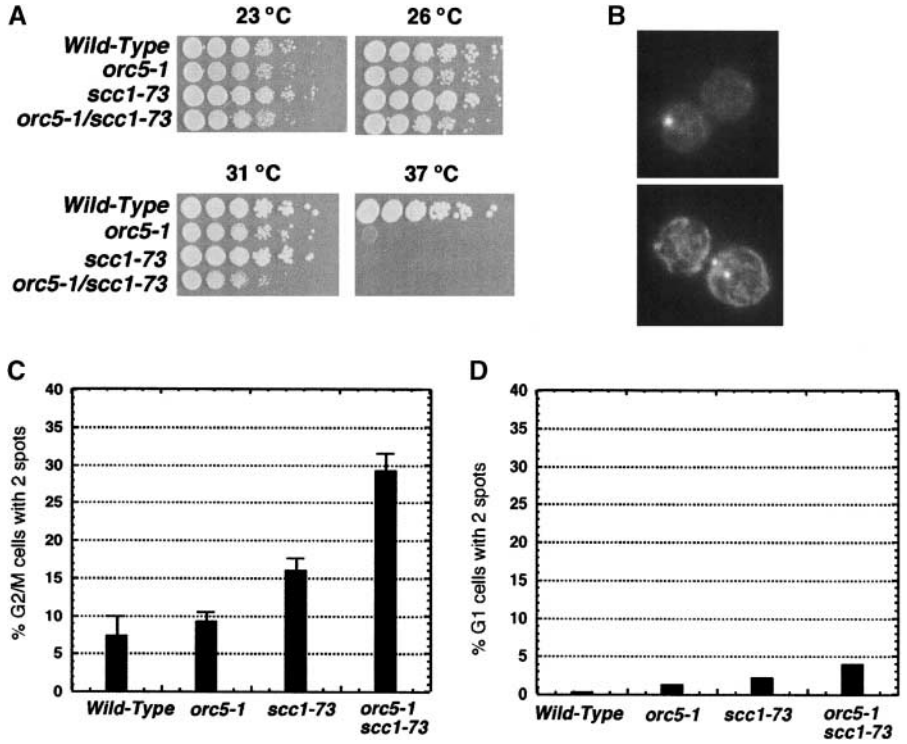

FIGURE 3.—Effect of *orc5-1* mutation on sister chromatid cohesion. (A) A genetic interaction between *orc5-1* and *scc1-73*. Strains JRY7468–JRY7471 were grown to late-log phase and spotted in 10-fold dilutions on YPD. Plates were incubated for 2 days at 23°, 30°, 31°, and 37°. (B) Visualization of chromosomal tet operator repeats by GFP in nocodazole-arrested cells. (C) *orc5-1* enhanced cohesion defect of *scc1-73* at the *URA3* locus ~30 kb from *CEN-V*. Scoring was done for cells with two GFP dots *vs.* the total number of cells scored. A total number of ~1500 cells was scored for each strain in three independent trials. (D) Chromosome missegregation in *orc5-1* and *scc1-73* cells in α-factor-arrested cells. A total of ~600–700 cells were counted.

Given the strong genetic interactions of *orc5-1* and *orc2-1* alleles with *ctf4*Δ and mutants in the CTF18-Rfc complex, the *orc5-1* allele was also tested for genetic interactions with two genes, *TRF4* and *TRF5*, that have overlapping roles in sister chromatid cohesion (WANG *et al.* 2000; EDWARDS *et al.* 2003). The *trf4*Δ *orc5-1* segregants grew only slightly less well than the *trf4*Δ single mutants (Figure 2D). No genetic interaction was evident between *orc5-1* and *trf5*Δ (Figure 2D). The weaker or nonexistent interactions between *orc-ts* mutations and *trf4*Δ or *trf5*Δ mutations may reflect a less direct role for Trf4p and Trf5p in the coupling of sister chromatid cohesion with DNA replication.

ORC function affects sister chromatid cohesion: Given that the synthetic interaction between *orc-ts* mutations and mutations in sister chromatid cohesion genes did not seem to reflect roles for the cohesion proteins in replication initiation, we explored whether ORC might contribute in some way to efficient sister chromatid cohesion. The *orc5-1* allele was combined with the *scc1-73* mutation in the core cohesin complex. The *scc1-73* mutation by itself leads to a precocious separation of sister chromatids before the onset of anaphase at its restrictive temperature (MICHAELIS *et al.* 1997). Both *orc5-1* and *scc1-73* have a restrictive temperature at 32°–33°. At 31°, both single mutants showed robust growth. However, growth of the *orc5-1 scc1-73* double mutant was compromised at this temperature, whereas at 23° and 26°, the *orc5-1 scc1-73* double mutant grows as well as the *orc5-1* single mutant (Figure 3A). Thus, the combination of *orc5-1* and *scc1-73* revealed a synthetic growth defect at the maximum permissive temperature.

To determine whether the reduced growth of the *scc1-73 orc5-1* double mutant reflected a more severe cohesion defect, strains expressing a tet repressor-GFP fusion protein and carrying tandem tet operator sequences integrated at the *URA3* locus of chromosome V were used to evaluate cohesion at a specific locus. Premature loss of sister chromatid cohesion in nocodazole-arrested cells can be detected by the appearance of cells containing two GFP dots instead of one (Figure 3B). In three different experiments, the fraction of wild-type cells arrested at G_2/M with nocodazole containing two GFP dots with tet operators at *URA3* was ~8%. In an *scc1-73* strain, an elevated loss of sister chromatid cohesion (16% cells with two spots) was evident at the semipermissive temperature of 31° (Figure 3C). The *orc5-1 scc1-73* double mutant exhibited a cohesion defect substantially greater than that of the *scc1-73* single mutant (29% of cells with two spots). Thus, reduced ORC function enhanced the cohesion defect of *scc1-73* cells. A potential source of artifacts in this analysis would be an extra copy of the chromosome containing the tet operator repeats. To determine whether the elevated level of cells with two spots resulted from a cohesion defect or from the presence of an extra chromosome, chromosomes marked at the *URA3* locus were scored in cells that were arrested in G_1 phase by α-factor. These cells are expected to contain only a single fluorescent dot, unless they have a second copy of the marked chromosome. The frequency of G_1 cells containing two marked chromosomes was low in all cases. Some chromosome missegregation was observed in *scc1-73* and *scc1-73 orc5-1* strains (<5%; Figure 3D). However, the few G_1 cells with two

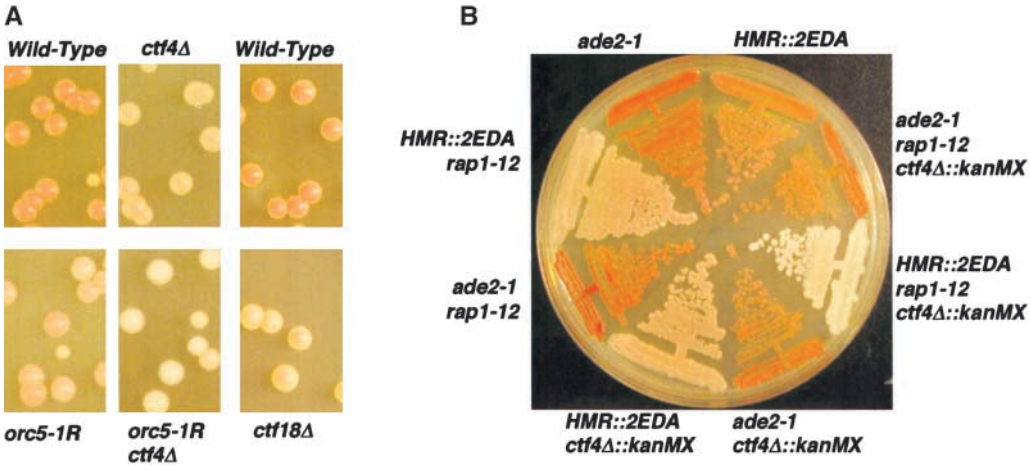

FIGURE 4.—Sister chromatid cohesion genes affected silencing of mating-type loci. (A) Effects of *ctf4*Δ, *ctf18*Δ, and *orc5-1R* on the *ADE2* gene inserted at the *HMR* locus (*HMR::EDA2*). Cultures were grown to mid-log phase A_{600} ~1 and 100 μl from $1:10^4$ dilutions was plated on YPD plates. Strains JRY7480–JRY7483 and JRY7719 contained the *HMR::2EDA* marker from JRY5329. The *orc5-1R* allele was derived from JRY4555. (B) Combined effect on silencing of *HMR::2EDA* by *ctf4*Δ and *rap1-12*. Cells were streaked on YPD at 30° for 3 days and then incubated at 4° for 3 additional days. Strains were JRY3371, JRY3372, JRY5329, JRY7484–JRY7486, JRY7488, and JRY7489.

marked chromosomes could not account for the large number of chromosomes with two spots in G_2/M phase that were therefore the consequence of a sister chromatid cohesion defect.

A subtle cohesion defect was evident in the *orc5-1* single mutant at semipermissive temperature (31°–32°) compared to wild type. This small difference was reproducible but not statistically significant. A similar result was obtained for *orc2-1* cells arrested at semipermissive temperature (not shown). Furthermore, we observed no increase in the number of separated sister chromatids in *orc5-1* single mutants at the restrictive temperature of 36° (not shown). Thus, defective sister chromatid cohesion is not a major phenotype of *orc-ts* mutations. However, the reduction in the permissive temperature and the enhanced cohesion defect in the *orc5-1 scc1-73* double mutant revealed some role of ORC in sister chromatid cohesion.

Sister chromatid cohesion genes and transcriptional silencing: Silencing functions have been established for different DNA replication genes, including *POL30, CDC44, POL2, CDC45, DPB4*, and *DPB11* (EHRENHOFER-MURRAY *et al.* 1999; ZHANG *et al.* 2000). Therefore, genes with new roles in DNA replication that were identified in our screens could, in principle, also have potential functions in silencing. Ctf4p and the alternative RFC complex are thought to help establish sister chromatid cohesion during the replication process and could connect establishment of cohesion with silencing. To study transcriptional repression in *ctf4*Δ and *ctf18*Δ strains at the *HMR* locus, strains with *ADE2* inserted into *HMR* were used (*HMR::2EDA*; Figure 4). Complete repression of the *ADE2* gene results in red colonies, whereas derepression of *ADE2* produces white colonies, and partial derepression produces pink colonies. The colony color in *ctf4*Δ colonies with the *HMR::2EDA* reporter was pink, whereas wild-type colonies were red (Figure 4A). Colonies were also predominantly pink in a *ctf18*Δ mutant strain (Figure 4A). Derepression of *ADE2* was also observed when *ctf4*Δ and *ctf18*Δ strains contained the *ADE2* gene in the opposite orientation with a weakened *HMR E* silencer (*HMR*Δ*B::ADE2*; not shown). Thus, *CTF4* and *CTF18* contributed to silencing at the *HMR* locus.

The roles of ORC in replication and silencing are genetically separable (EHRENHOFER-MURRAY *et al.* 1995; DILLIN and RINE 1997). Strains containing the *orc5-1R* allele are competent in initiation of DNA replication but compromised in silencing *HMR*. An *orc5-1R* strain that contains the *ADE2* gene at the *HMR* locus formed colonies with red and pink sectors, which indicated switching between repressed and derepressed states. This phenotype is also observed in strains lacking the *ARS* consensus sequence in the *HMR E* silencer or in strains with a mutation in the *RAP1* gene (SUSSEL *et al.* 1993). When the *ctf4*Δ mutation was combined with *orc5-1R*, the colonies were white, indicating complete derepression of *ADE2* (Figure 4A). Therefore, the effect of the *ctf4*Δ null mutation on transcriptional silencing was independent of ORC. Similarly, *ctf4*Δ led to an enhanced derepression of *HMR::2EDA* in combination with the *rap1-12* allele (Figure 4B). The pink and white colony color was caused by the growth defect in the mutants since single- and double-mutant colonies are red in strains without *ADE2*. Furthermore, silencing in an *orc5-1R ctf4*Δ double mutant at the *HML* locus was also partially defective as judged by α-factor confrontation assay. About 10% of the double-mutant cells grew into small colonies after 14 hr at 23° in the presence of α-factor, whereas all wild-type or single-mutant cells remained arrested by α-factor (not shown).

To assay silencing at telomeres in cohesion mutants, strains were used that contained either the *URA3::TRP1* reporter at telomere *VII-L* (Figure 5A) or the *ADE2* gene at telomere *V-L* (Figure 5B). When strains containing *TELVII-L::URA3::TRP1* were assayed on 0.1% 5-FOA medium, selecting against *URA3* function, *ctf4*Δ strains did not grow, whereas *ctf18*Δ and *dcc1*Δ strains formed small

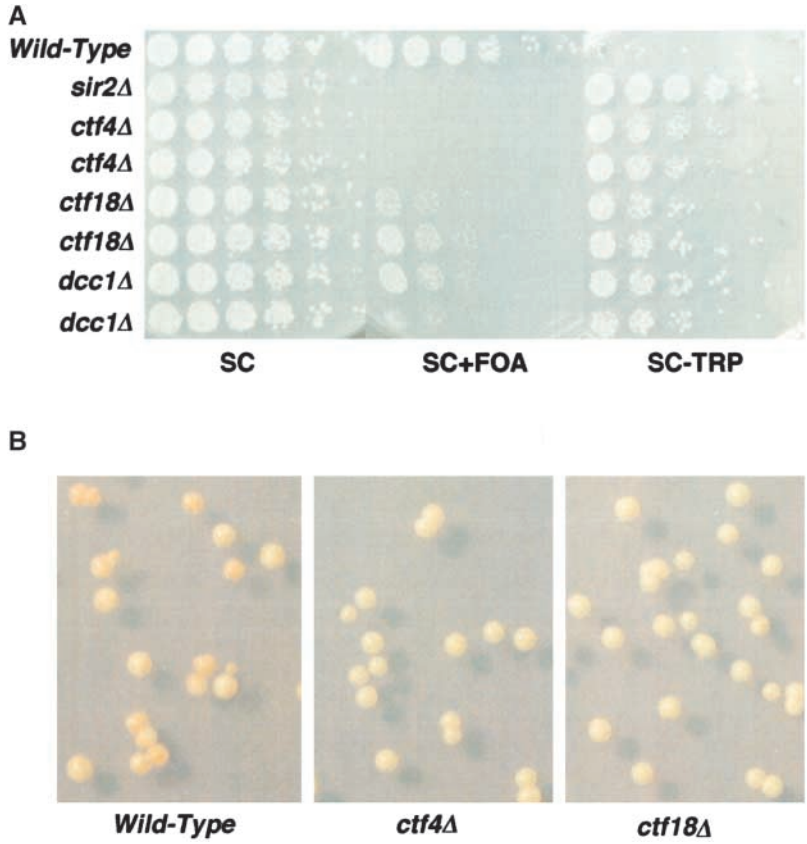

FIGURE 5.—Sister chromatid cohesion genes affect telomeric silencing. (A) Derepression of the telomeric *URA3* and *TRP1* genes. Strains JRY7497–JRY7502 were derived from crosses with JRY4441 containing the *URA3-TRP1* reporter construct at telomere *VII-R*. Control strain was *sir2*Δ (JRY4470). For the assay, cells were grown to A_{600} 0.5 in YPD medium and spotted in fivefold serial dilutions on SC containing 0.1% FOA, SC, and SC-TRP plates. (B) Derepression of the *ADE2* reporter gene at *telomere V-L*. Strains JRY7473–JRY7475 were derived from crosses of *ctf4*Δ and *ctf18*Δ strains with JRY6115. Cells were plated on SC medium from exponentially growing cultures of A_{600} ~1 in YPD. Pictures were taken after 3 days at 30° and an additional 3 days at 4°.

colonies. The wild-type control strain grew well, indicating that *URA3* was silenced at this location, whereas the silencing defective *sir2*Δ strain did not. The *TRP1* gene at telomere *VII-L* was also derepressed in *ctf4*Δ, *ctf18*Δ, and *dcc1*Δ mutants, although to a lesser extent than in the *sir2*Δ control strain. In wild-type cells, partial repression of the *ADE2* gene at telomere *V-L* leads to a phenotype of red and white sectored colonies (Figure 5B). In contrast, *ctf4*Δ and *ctf18*Δ with this reporter were white. Thus, *CTF4* and *CTF18/DCC1* contributed to transcriptional silencing at two different telomeres.

DISCUSSION

Synthetic lethal or synthetic sick interactions can occur by several mechanisms. Synthetic lethality that results from the combination of two temperature-sensitive mutations affecting the same essential process, such as DNA replication, can be understood as too little residual activity to support growth. Indeed, most previous synthetic lethal interactions with ORC mutants were of this type (LOO *et al.* 1995; HARDY 1996). The analysis here is qualitatively different from these in that our screen involved combining all null alleles of nonessential genes with temperature-sensitive alleles of *ORC* genes. This approach allowed us to ask which genes become essential when ORC function is reduced. Genes identified in this way are candidates for roles at replication origins and at the replication fork and can, in principle, uncover roles for ORC in processes that are not limited to origins of replication. Synthetic lethal or synthetic sick interactions seen with *orc-ts* mutations were, to a large extent, recapitulated with temperature-sensitive mutations in other replication genes, such as *cdc7-1* and *cdc45-1*. Thus, many of these interactions reflected gene functions that became paramount regardless of how replication was compromised. In this study, we investigated most thoroughly the links that were discovered between ORC and genes whose previously known function was in the establishment of sister chromatid cohesion. Because of ORC's role in both silencing and replication, we explored possible links among these three processes.

Temperature-sensitive *orc-ts* mutations in combination with mutations in sister chromatid cohesion genes resulted in reduced viability at temperatures permissive for the individual mutations. These interactions did not appear to reflect a defect of cohesion mutants in replication initiation. Rather the reduced viability in an *orc5-1 scc1-73* double mutant was likely caused by an enhanced cohesion defect. Synthetic lethal interactions are also observed for cohesion mutants in combination with *mcm2-1, cdc6-1, cdc7-1,* and *cdc45-1* mutations, and hence we favor the notion that the interaction reflects a dependence of robust sister chromatid cohesion on initiation of DNA replication. A link between initiation of DNA replication and sister chromatid cohesion was found in *S. pombe*. Here, a mutation in *hsk1*$^+$, which is the homolog of the *S. cerevisiae CDC7* encoded serine threonine

kinase, leads to a cohesion defect (Takeda *et al.* 2001; Bailis *et al.* 2003).

However, *orc5-1* alone showed no obvious cohesion defect compared to wild-type strain. Furthermore, the majority of chromosomal cohesin binding sites do not co-localize with origins (Blat and Kleckner 1999; Laloraya *et al.* 2000). Thus, ORC's involvement in cohesion is presumably not completed at replication origins. Cohesion between sister chromatids is established at the time of replication, presumably by processes that occur at the replication fork. At permissive temperature, the *orc2-1* mutation leads to a 30% reduction in the number of replication forks (Shimada *et al.* 2002), and presumably this loss is greater the closer to the restrictive temperature the mutant is grown. We speculate that there may be a limit to the amount of cohesion that can be established at any given fork. In this model, the reduction in the number of replication forks in the mutants leads to less cohesion, which becomes growth limiting in cells with temperature-sensitive Scc1p.

Although an enhanced cohesion defect provides an explanation for the reduced viability in the *orc5-1 scc1-73* double mutant, other mechanisms might also contribute to the strong genetic interactions of ORC with cohesion genes. *CTF4* and genes encoding components of the Ctf18-RFC are sensitive to different DNA-damaging agents (Chang *et al.* 2002). Furthermore, cohesion is important for recombinational repair (Sjogren and Nasmyth 2001). It is possible that the repair of broken replication forks could lead to a dependence of *orc-ts* mutants on cohesion genes. *CTF18* also has an overlapping role with *RAD24* in the replication checkpoint (Naiki *et al.* 2001). Moreover, replication mutants strongly interact with the replication checkpoint genes *MRC1, TOF1*, and *CSM3* (Figure 1C). Thus, proper replication checkpoint function could be important for viability of *orc-ts* and other replication mutants, and the cohesion phenotypes in the *ctf* mutants may be an indirect consequence of improper DNA replication or failure of the replication checkpoint. During the final stage of preparing this manuscript, work was in press that also demonstrated a cohesion function for other genes that were identified in our screens (Mayer *et al.* 2004; Warren *et al.* 2004). Importantly, synthetic lethal screens for genes interacting with *ctf4*Δ and *ctf8*Δ also established a role in sister chromatid cohesion for the replication checkpoint genes *MRC1, TOF1*, and *CSM3*.

Only a slight interaction was observed between *orc5-1* and *trf4*Δ and essentially none between *orc5-1* and *trf5*Δ, suggesting that the roles of Trf4p and Trf5p are mechanistically distinct from those of Ctf4p, Ctf18-RFC, and other cohesion factors. Thus, it seems likely that some cohesion factors are more intimately connected to the replication process than others. Plasmid-loss experiments suggested that Ctf4p and Ctf18p did not directly affect initiation of DNA replication. However, this does not exclude the possibility that these proteins somehow couple the initiation of DNA replication with the establishment of sister chromatid cohesion.

We demonstrated that the genes important for the establishment of sister chromatid cohesion, *CTF4*, *CTF18*, and *DCC1*, also contributed to silencing of *HMR, HML*, and telomeres. A partial defect in telomeric silencing has also been observed in an *scc1-73* strain at semipermissive temperature (P. Kaufman, personal communication). In principle, indirect effects on chromosome organization that occur in these mutants could explain such links. A mutation in a cohesin subunit removed a boundary for the spread of heterochromatin at the *HMR* locus (Donze *et al.* 1999). Spreading of heterochromatic factors into the adjacent euchromatin might dilute heterochromatin components and decreases transcriptional silencing (Meneghini *et al.* 2003). Thus, sister chromatid cohesion could be important for the proper separation and distribution of euchromatin and heterochromatin.

More direct models are supported by the temporal coincidence of the establishment of sister chromatid cohesion in S phase with chromatin assembly and the establishment of silencing (Miller and Nasmyth 1984; Shibahara and Stillman 1999; Zhang *et al.* 2000; Kirchmaier and Rine 2001; Li *et al.* 2001). Recent work suggests that cohesins have to be removed before silencing is complete (Lau *et al.* 2002), which implies an inhibitory role for cohesins in silencing. Our data present the reciprocal view that cohesion establishment contributes positively to silencing, suggesting a more complex interplay between proteins involved in the two processes. Defects in transcriptional silencing have been shown for chromatin assembly factors that are associated with the replication fork (Kaufman *et al.* 1998; Tyler *et al.* 1999; Sharp *et al.* 2001). Similarly, Ctf4p and the Ctf18-RFC complex could also affect silencing by their possible function at the replication fork. This also raises the question of whether the cohesion phenotypes in *ctf4*Δ and *cf18*Δ are a consequence of a defective organization of newly replicated chromatin. The involvement of cohesion factors in transcriptional silencing demonstrates therefore an interdependence of DNA replication, chromosome structure, and proper chromosome segregation.

DNA replication, like other DNA-dependent processes, must contend with the organization of DNA into nucleosomes and higher-order chromatin structures. Previous work established the contribution of positioned nucleosomes to replication initiation at *ARS1* (Simpson 1990; Lipford and Bell 2001). The identification of multiple genes with roles in nucleosome remodeling and histone modification in our screens suggests that specific aspects of chromatin structure influence replication. Mobilization of nucleosomes by chromatin-remodeling factors may promote origin firing or fork progression. The recovery of histone acetyltransferases, NAD^+-dependent protein deacetylase paralogs, and chromatin-remodeling factors in the screen implies a deep and potentially

complex relationship between chromatin structure and DNA replication.

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