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28. DETC lines 2-10-B6 and 5-6 and clone 30B4 were derived as described (*15*). The transformed keratinocyte line PAM2.12 was provided by A. Takashima. DETCs (10^5 per well) were cocultured with irradiated (30 gray) PAM2.12 keratinocytes (10^5 per well) in 96-well flat-bottom plates for 20 hours at 37°C, after which the extent of PAM2.12 monolayer lysis was noted. Supernatants from the cultures were tested for the ability to support the growth of the IL-2–dependent CTLL cell line (provided by K. Bottomly). CTLL cells were cultured at 5000 cells per well in 96-well round-bottom plates in the presence of medium only (CRPMI), murine recombinant IL-2 (0.01 to 40 U/ml) (Genzyme), or test culture supernatants (dilutions ranging from 1:2 to 1:50). After incubation for 24 hours at 37°C, 1 μCi of [^{3}H]thymidine was added to each well and incubation was continued for an additional 24 hours. Cells were then harvested and [^{3}H]thymidine incorporation was determined.
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32. Supported by NIH grants GM37759 and AI27855 (A.C.H.) and AI27404 (R.E.T.). We thank our colleagues for discussion; L. McVay for isolation of genomic clones; T. Taylor for FACS analysis; I. Rosewell and S. Donaldson for mouse work; and M. Bonneville, W. Born, K. Bottomly, L. Lefrancois, P. Pereira, E. Shevach, A. Takashima, and S. Tonegawa for provision of reagents. All animal experiments were performed under accredited conditions after prior approval of protocols by the local Animal Care and Use Committee.

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Roles for ORC in M Phase and S Phase

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The origin recognition complex (ORC), a six-subunit protein, functions as the replication initiator in the yeast *Saccharomyces cerevisiae*. Initiation depends on the assembly of the prereplication complex in late M phase and activation in S phase. One subunit of ORC, Orc5p, was required at G_1/S and in early M phase. Asynchronous cells with a temperature-sensitive *orc5-1* allele arrested in early M phase. In contrast, cells that were first synchronized in M phase, shifted to the restrictive temperature, and then released from the block arrested at the G_1/S boundary. The G_1/S arrest phenotype could not be suppressed by introducing wild-type Orc5p during G_1. Although all *orc2* and *orc5* mutations were recessive in the conventional sense, this dominant phenotype was shared with other *orc5* alleles and an *orc2* allele. The dominant inhibition to cell-cycle progression exhibited by the *orc* mutants was restricted to the nucleus, suggesting that chromosomes with mutant ORC complexes were capable of sending a signal that blocked initiation on chromosomes containing functional origins.

In *Saccharomyces cerevisiae*, replication initiates from specific DNA sequences called autonomous replication sequences (ARSs), many of which have proven to be chromosomal origins of replication. A six-subunit protein complex, the ORC, binds to ARSs in an ATP-dependent manner and is required for initiation (*1–3*). Homologs of ORC subunits have been identified in other eukaryotes including humans, suggesting that the mechanism by which ORC initiates replication is highly conserved (*4*). Additionally, these homologs are essential for in vitro replication of *Xenopus* egg extracts (*5–7*) and for amplification of the chorion gene cluster of *Drosophila* (*8*).

Eukaryotes can initiate replication at a given origin only once per cell cycle, and there are hundreds of origins whose activation is coordinated. Some origins initiate early and others initiate late in S phase. ORC remains bound to origins throughout the cell cycle (*9*), so initiation of replication, at least in *S. cerevisiae*, is not regulated by simply controlling the binding of ORC to origins.

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Origin initiation is regulated by a two-step mechanism. The first step, referred to historically as origin licensing, occurs in M phase, and the second step, origin activation, occurs in S phase (*10*). Once a licensed origin has been activated during S phase, it is incapable of initiating again until it is licensed in the next M phase. The factors that make up a licensed origin are not diffusible, because otherwise these factors could diffuse from a late origin that had not initiated to a recently initiated early origin, allowing its reinitiation.

ORC has properties consistent with its being the target of factors that control initiation. From S phase to late M phase, the in vivo footprint at an origin is similar to the footprint created in vitro by purified ORC, suggesting that ORC is the only factor bound during this time. During late M phase, when origins are licensed, the footprint is extended, reflecting the assembly of a prereplication complex (pre-RC) (*9*). Genetic and molecular data suggest that the pre-RC contains ORC, Cdc6p, and the MCM family of proteins (*11–13*). Mutation of the MCM genes causes defects in minichromosome propagation (*14*), and MCM homologs in *Xenopus* are components of licensing factor (*6*, *7*, *15*). The *Xenopus* MCM proteins bind chromatin in an ORC-dependent manner, suggesting that ORC interactions with MCM proteins make an origin competent to initiate replication (*6*).

This study revealed that at least one subunit of the ORC complex, Orc5p, was required for at least two steps in the cell cycle, G_1/S and early M phase. The execution point for the G_1/S function occurred before Start, probably in M phase. Moreover, the level of ORC5 function required for entry into M phase was higher than the level of function required for entry into S phase.

Strains with either *orc2-1* or *orc5-1* recessive mutations are compromised for replication initiation, but differ in their arrest point in the cell cycle upon shift to the nonpermissive temperature. Haploid cells with a defective Orc2 protein (Orc2p) arrest with 1C DNA content (*16*). In contrast, *orc5-1* mutant cells arrest with an apparent 2C DNA content, suggesting that these cells are in G_2 or M phase of the cell cycle (*12*) (Fig. 1) or arrested in late S phase, with most of the genome replicated. A late S phase arrest would suggest that the *orc5-1* allele was defective at a small subset of origins of replication, perhaps the late-initiating ones. Arrest after S phase would argue that the function of Orc5p was not confined to S phase. To distinguish between these possibilities, we examined the arrest phenotype of *orc5-1* mutant cells.

We performed pulsed-field gel electrophoresis (PFGE) on the chromosomal DNA of arrested *orc5-1* cells (*17*). Chromosomal DNA of wild-type (WT) cells arrested in S phase by treatment with hydroxyurea does not enter the gel matrix (*18*). In contrast, the fully replicated chromosomal DNA of cells arrested either in G_1 by α-factor or in M phase by nocodazole entered the gel and migrated in a characteristic manner. By this assay, *orc5-1* cells arrested with a 2C DNA content and contained fully replicated chromosomal DNA similar to that of *orc5-1* cells grown at 23°C and to that of WT cells grown at either 23° or 37°C (Fig. 2).

The *RAD9* checkpoint pathway is activated in response to small amounts of unreplicated and damaged DNA. For example,

both DNA ligase (*CDC9*) and DNA polymerase α (*CDC2*) and γ (*CDC17*) mutant cells arrest in G_2, and this arrest depends on *RAD9* (*19*). Double mutants containing the *rad9Δ* and *cdc9*, *cdc2*, or *cdc17* mutations lose viability rapidly at the nonpermissive temperature and form microcolonies of dead cells. To test if the *orc5-1* arrest required the *RAD9* checkpoint, we analyzed the viability of an *orc5-1 rad9Δ* double mutant (*20*). There was no enhanced lethality in the *orc5-1 rad9Δ* double mutant (*21*). Moreover, after 4 days at 37°C, the double mutants remained as single, large budded cells, indicating that the arrest of *orc5-1* cells was independent of *RAD9* (*22*). We also tested whether other checkpoint genes involved in monitoring DNA metabolism were required for the *orc5-1* arrest. Combining the *mec1-1*, *mec2-1*, or *rad17Δ* mutations with *orc5-1* also did not alter the viability of *orc5-1* strains (*21*). Thus, *orc5-1* cells appeared to arrest after S phase with fully replicated chromosomes.

Activity of the Cdc28 protein peaks at Start and rapidly declines upon entry into S phase, then starts to appear again during S phase, finally peaking shortly before entry into anaphase. Cells arrested before M phase have low Cdc28p kinase activity, whereas cells arrested in M phase before anaphase have high activity (*23*). After 6 hours, *orc5-1* cells had Cdc28p activity equivalent to that of cells arrested in early M phase with nocodazole at the permissive temperature (Fig. 3) (*24*). These mutant cells arrested with a single nucleus positioned at the bud neck and a short spindle that spanned the nucleus (*22*). Because *orc5-1* cells arrest at the restrictive temperature with fully replicated chromosomes, a high Cdc28p kinase activity, and short nuclear spindles less than half the length of anaphase spindles (*22*), they appear to be arrested in early M phase.

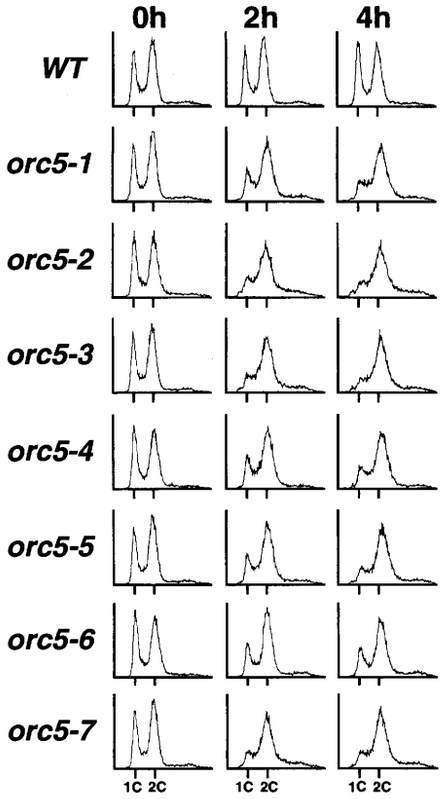


Fig. 1. Arrest of *orc5* ts strains with a 2C DNA content. Cells grown at 37°C for 0, 4, or 6 hours were harvested and subjected to FACScan analysis to assess cellular DNA content. All 25 alleles were tested; 7 representative alleles are shown.

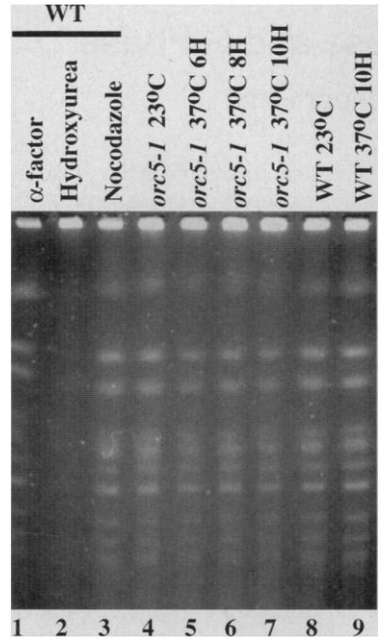


Fig. 2. PFGE analysis of *orc5-1* cells. The chromosomes of cells in S phase do not enter the gel matrix during PFGE (hydroxyurea-treated cells, lane 2). The chromosomes of cells arrested in G_1 (α-factor–treated cells, lane 1) or M phase (nocodazole-treated cells, lane 3) enter the gel matrix. The chromosomes of *orc5-1* cells (JRY4253) grown at 37°C for up to 10 hours migrated identically to those of WT cells (JRY3009) (*35*).

The M phase arrest phenotype of *orc5-1* was unexpected given the central role of ORC in the initiation of replication. We therefore tested whether this arrest phenotype was unique to the *orc5-1* allele by analyzing the arrest of 20 unique temperature-sensitive (ts) alleles (*25*). At the nonpermissive temperature, all alleles caused the cells to arrest with a single, large bud after 4 hours at 37°C (*22*) with a 2C DNA content, with some alleles showing a stronger arrest than others (Fig. 1) (*26*). Additionally, these alleles were distinct from the original *orc5-1* allele because they showed no silencing defect (*22*). Thus, the M phase arrest phenotype of *orc5-1* represented a loss of *ORC5* function in this region of the cell cycle and was not allele specific.

We examined whether Orc5p might have a second essential role in the cell cycle. If Orc5p function were required only in early M phase, then *orc5-1* mutant cells would arrest only in M phase regardless of how *ORC5* function was inactivated. We synchronized *orc5-1* cells in M phase with nocodazole at the permissive temperature, and then shifted the cells to the restrictive temperature while maintaining the M phase block. The cells were then released from the block and monitored for cell cycle progression (*27*). Single unbudded cells containing 1C DNA appeared 40 min after release from the nocodazole block, indicating that Orc5p function was not required for exit from mitosis. Cells were able to pass Start, as

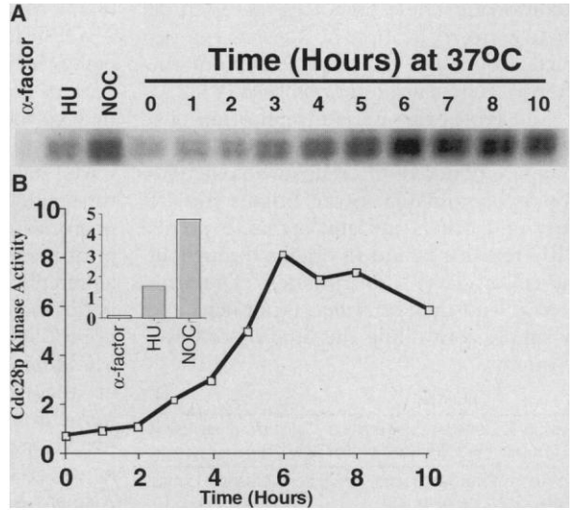


Fig. 3. Cdc28p kinase activity in *orc5-1* cells. (**A**) *orc5-1* cells (JRY4253) were shifted to the nonpermissive temperature and samples, harvested at the indicated times, were prepared for assay of Cdc28p kinase (*24*). Control cells (JRY4253) were arrested in G_1 (α-factor), S (HU), or M phase (Nocodazole) at the permissive temperature. (**B**) Quantitation of the gel in (A). Relative Cdc28 kinase activity was determined by Imagequant Software. (**Inset**) The control lanes from (A) are represented as a bar graph.

indicated by the emergence of buds. However, the cells arrested as large budded cells with 1C DNA content (Fig. 4). Thus, Orc5p function was required to begin S phase.

To test whether these cells arrested completely before S phase or whether they entered S phase, we analyzed the chromosomal DNA of cells from the 200-min time point of Fig. 4 by PFGE. The chromosomal DNA from these cells was indistinguishable from that of WT cells arrested in G_1 (*22*). Therefore, cells released from M phase with inactivated Orc5p traversed Start but arrested before the onset of S phase, consistent with a second requirement for Orc5p in the cell cycle, at the G_1/S boundary.

Because *orc5-1* is recessive, one would expect that addition of WT Orc5p to *orc5-1* cells arrested at the G_1/S boundary would restore replication if the pre-RC can assemble at any point before the beginning of S phase. However, if there is a critical point for forming the pre-RC before S phase, then injection of WT Orc5p might not rescue the replication block. We tested these models by using mating itself, which occurs at Start in G_1, to introduce Orc5p protein genetically into an *orc5-1* mutant (Fig. 5A). The *orc5-1* cells were arrested in M phase with nocodazole and shifted to the restrictive temperature. The cells were then released from the M phase block and mixed with asynchronous WT cells of the opposite mating type at the restrictive temperature. After 4 hours the mixture was plated onto medium that selected for diploids at the restrictive temperature. A parallel experiment was also performed in which all incubations were at 23°C, including the M phase arrest (*28*). Both the *MAT* α *orc5-1* and *ORC5* strains could form diploids at 23°C with the *MAT***a** WT strains, indicating that nocodazole arrest did not affect mating. In contrast, at 37°C, the *orc5-1* strain failed to form diploid colonies when mated with the WT strain, whereas the *ORC5* strain did (Fig. 5B). We isolated 11 zygotes during the mating reaction by micromanipulation and followed their progression by microscopy at 37°C. All 11 zygotes derived from the *orc5-1* cross formed a single, large bud that did not divide, even after 4 days at 37°C on rich medium, which could support the growth of either haploid parent strain (*22*). This

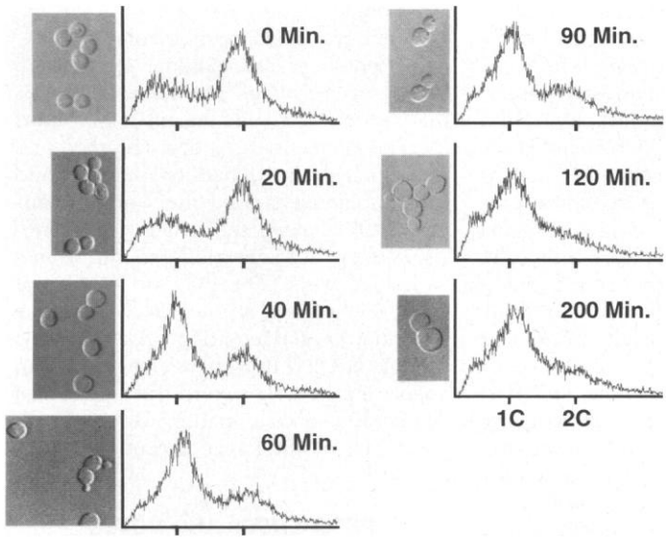
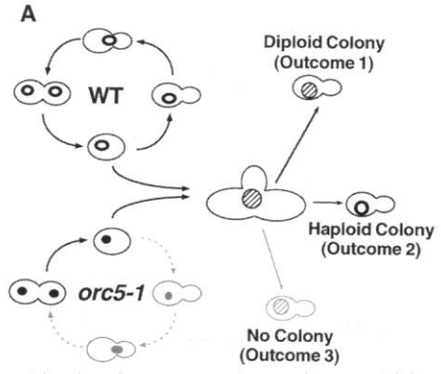


Fig. 4. Arrest of *orc5-1* cells with 1C DNA content. *orc5-1* cells (JRY4253) were arrested in M phase at 23°C, then shifted to 37°C for 2 hours while maintaining the M phase block. Cells were released from the block into prewarmed 37°C medium, and samples were harvested at the time points indicated. Cytology of the arrested cells is shown to the left of the corresponding FACScan profile.



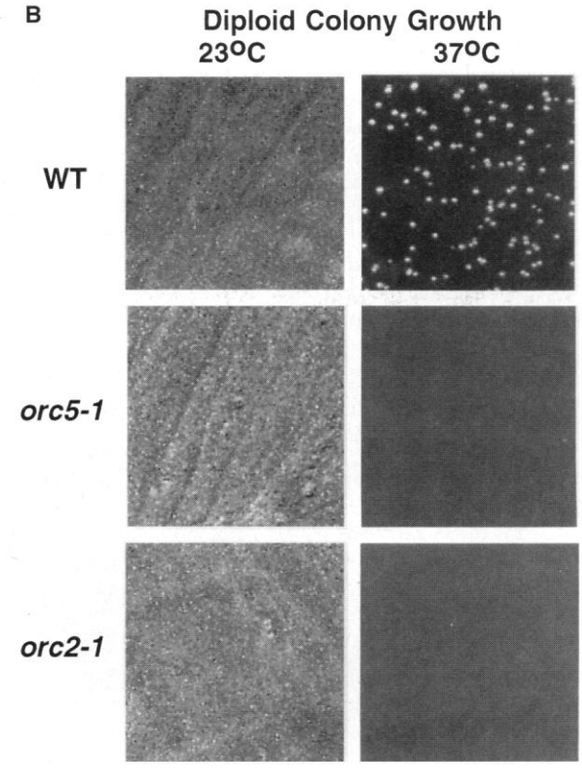


Fig. 5. Failure of addition of WT ORC by mating to rescue the G_1 arrest of *orc* cells. (**A**) Diagram of the experimental procedure and possible results. *orc5-1* cells were arrested in M phase at 23°C, shifted to 37°C for 2 hours, and then released from the M phase block at 37°C with a mixture of WT cells of the opposite mating type. Control cultures were incubated at 23°C throughout all stages. Solid nucleus represents an *orc5-1* nucleus that was inactivated at the restrictive temperature in M phase; clear nucleus represents a WT *ORC5* nucleus; striped nucleus represents a nucleus containing chromosomes from an *orc5-1* and an *ORC5* nucleus. The growth of diploid colonies would indicate rescue of the replication defect by WT Orc5p added in G_1 (Outcome 1). The growth of haploid colonies containing only the chromosomes contributed by the *ORC5* nucleus would suggest no rescue by Orc5p and cis-dominant inhibition of the chromosomes contributed by the *orc5-1* parent (Outcome 2). Absence of colonies would suggest trans-dominant inhibition by the chromosomes from the *orc5-1* strain on the replication or inheritance of the chromosomes from the *ORC5* parent (Outcome 3). (**B**) Nocodazole-arrested *orc5-1* (JRY5493) or *orc2-1* (JRY4503) cells incubated at 23°C, but not at 37°C, were able to form diploid colonies when mated to a WT strain (JRY2334 and JRY4012, respectively). Additionally, WT cells (JRY3009xJRY4012) formed diploids at 23°C at a much higher frequency than at 37°C, due to the temperature sensitivity of karyogamy (*30*).

dominant arrest phenotype was not allele-specific because either *orc5-2* or *orc5-3* mutant cells behaved identically to *orc5-1* cells (*22*). Thus, cells lacking Orc5p from M to G_1, when fused with a WT cell, inhibited cell cycle progression of the resulting heterozygous diploid cell in a dominant manner.

We repeated the mating experiment with a strain carrying the *orc2-1* allele. The *orc2-1* strain also formed diploids when mated with the WT strain at 23°C, but not at 37°C (Fig. 5B). Taken together, these results indicated that inactivation of ORC in M phase could not be rescued by addition of WT ORC in G_1. We also repeated the mating experiments with either a *cdc6-1* or a *mcm5* (*cdc46-1*) mutant strain. Either strain could form viable diploids when mated with WT cells at 23° and at 37°C (*22*). Therefore, this phenotype was ORC specific, as other *cdc* mutants involved in replication initiation were rescued in G_1 by mating with a WT strain.

We examined whether the dominant inhibitory effect of ORC mutations resulted from a defect restricted to the nucleus, such as inactivated origins of replication, or whether it was a property distributed throughout the cell. The mating experiments were repeated as before, this time with a strain containing the *kar1-1* mutation, which blocks nuclear fusion (*29*). A cell that mates with a *kar1-1* mutant cell forms a zygote with two unfused nuclei, one from the WT strain and the other from the *kar1-1* strain. The first bud from the zygote typically contains only one haploid nucleus, resulting in a haploid colony containing the cytoplasmic material from both strains but the nuclear contribution from only one parent (*30*). If the dominant effect of *orc5-1* were restricted to the nucleus containing the inactivated ORC, then haploid buds with the WT nucleus would be propagated (*31*).

Indeed, haploid colonies having the *ORC5 kar1-1* nucleus were produced from a cross with the *orc5-1* strain at the restrictive temperature with approximately equal frequency to that in crosses with the WT strain (Fig. 6) (*28*). Thus, the dominant effect on cell-cycle progression of an *orc5-1* mutant resulted from a property restricted to the nucleus.

Because ORC is the yeast replicator, it was not unexpected that one termination point of *orc5* mutants was at the G_1/S transition. Our data suggest that the execution point for the G_1/S function is between the nocodazole block in M phase and the Start point in G1. *orc5-1* mutants can recover from arrest at the restrictive temperature in mitosis but not in G_1 or S phase (*12*). Moreover, introduction of WT ORC in G_1 failed to rescue the cell cycle defect of *orc5-1* cells released from M phase at the nonpermissive temperature. This execution point matches closely the time at which the pre-RC is assembled. In contrast, functional Cdc6p and Mcm5p proteins, which are also part of the pre-RC, could be introduced as late as G_1 to support the next cell cycle (*22*). Similarly, Cdc6p can promote entry into S phase when expression is activated after Start but before activation of cyclin-B/Cdc28p (*32*).

The G_1/S arrest of *orc5* and *orc2* mutants could not be rescued by introducing WT Orc5p or Orc2p in G_1 in a mating reaction. Although we have no direct measure of where in the cell cycle these cells arrested, the simplest model is that the arrest occurred at G_1/S, as in the *orc5-1* parent. However, we cannot rule out the possibility that these cells are arrested in early M phase. It is unlikely that the arrested cell cycle reflects insufficient amounts of ORC to complete a cell cycle, for two reasons. First, overexpression of *ORC5* in the WT nucleus during the mating reaction failed to suppress the lethality (*22*). Second, diploid cells with a single *ORC5* gene divide with normal rates, indicating that Orc5p is present in excess. The inactive Orc5-1p, and, by inference, origins with inactivated ORC complexes, may send a signal that blocks initiation at origins with functional ORC complexes. This signal appears to be restricted to the nucleus because the dominant inhibition caused by *orc5-1* did not block the *ORC5* haploid nucleus from the *kar1-1* cell from forming a fully functional daughter cell when nuclear fusion was blocked.

The G_1/S phase arrest of *orc5-1* cells was detectable only if the cells were first arrested in M phase and then shifted to the nonpermissive temperature for 2 hours before release from the mitotic block. Thus, the G_1/S function of Orc5p required a lower level of activity than its M phase function.

There are several possible roles for ORC in M phase of chromosome segregation. Proper segregation of chromosomes requires that sister chromatids remain paired until the end of metaphase, that chromosomes are attached to a functional spindle, and that chromosomes condense. The positioning of ORC along the length of chromosomes allows it to contribute to mitosis in a variety of ways. The ATPase activity of ORC could have a role in chromosome cohesion or condensation. Alternatively, the role of ORC in M phase may reflect an unanticipated early step in the assembly of the pre-RC in combination with a checkpoint that monitors the execution of this early step.

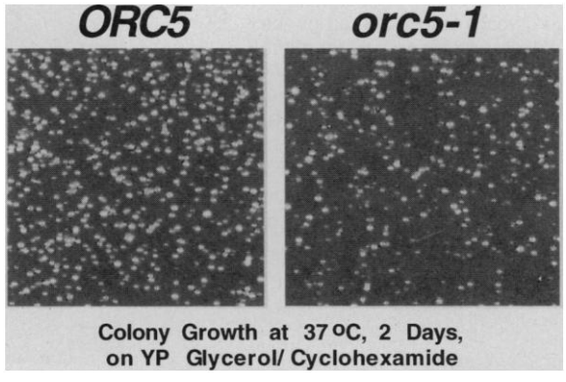


Fig. 6. Requirement for nuclear fusion for *orc5-1* cells to inhibit cell cycle progression. At 37°C, *orc5-1 KAR1* (JRY4249) and WT *KAR1* (JRY3009) cells formed approximately the same number of viable colonies containing only the nucleus of the *kar1-1* strain (JRY5449). Growth of cells on glycerol and cyclohexamide-containing medium required the presence of functional mitochondria, from the *KAR1* parent, and the recessive *cyh*^{r} nuclear gene, from the *kar1-1* parent (*31*).

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17. Chromosomal DNAs were prepared with a Bio-Rad CHEF Genomic DNA Plug Kit. Cells grown to early log phase were arrested either in G_1 with 24 μM α-factor, in S phase with 0.2 M hydroxyurea, or in M phase with nocodazole (10 μg/ml) for 3 hours at 23°C. The *orc5-1* and WT cells were grown to early log phase at 23°C and then either shifted to 37°C or kept at 23°C for up to 10 hours. DNA plugs consisted of 2.5×10^8 cells/ml. Gels were run on a BRL CHEF apparatus in the absence of ethidium bromide at 175 V for 15 hours with a 70-s switch interval

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20. Cells were grown overnight in rich medium at 23°C to early log phase. Cells were diluted to an absorbance at 600 (A_{600}) of 0.1 and incubated at 37°C. Samples were tested every hour for 9 hours. Cell viability was calculated as the number of viable colonies formed after plating at 23°C at each time point divided by the number of colonies that formed after plating at 23°C at the 0 hour time point.
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24. Cdc28p was isolated and assayed essentially as described (*33*). The *orc5-1* cultures were grown to A_{600} = 0.1 and either arrested with α-factor, hydroxyurea, or nocodazole for 3 hours at 23°C or shifted to 37°C. Cells (1×10^8) were harvested and lysed for each hourly time point.
25. The *orc5* ts alleles were created by in vitro mutagenesis of the cloned *ORC5* gene by the polymerase chain reaction (PCR). PCR-mutagenized DNA pools were cotransformed into yeast cells with a gapped plasmid missing most of the *ORC5* reading frame such that in vivo recombination would restore plasmid-borne copies of *ORC5*. In one screen, an *orc5-1* strain (JRY4253) was transformed and the transformants were screened for their inability to complement the ts defect of *orc5-1* (*34*). Putative new ts alleles were then recovered and transformed into an *orc5* Δ strain. *orc5-2* through *orc5-12* were identified in this manner. The remaining alleles were identified by directly transforming the *orc5*Δ strain (JRY4154) with the mutagenic PCR reaction and the gapped plasmid in a plasmid-shuffle protocol. Transformants were screened for their ability to complement *orc5*Δ at 23°C, but not at 37°C.
26. Cells were prepared and analyzed by flow cytometry as described (*12*), with the exception that 10 ml of cells were harvested at A_{600} = 0.05 for each time point. All strains were made ρ°, hence lacking mitochondrial DNA, to avoid interference of mitochondrial DNA.
27. WT or *orc5-1* cells were grown and diluted to A_{600} = 0.05 in rich medium. Diluted cultures were arrested by the addition of nocodazole (10 μg/ml) for 3 hours at 23°C. Cells were harvested and resuspended in either 23° or 37°C rich medium containing nocodazole (10 μg/ml) and incubated for an additional 2 hours at either 23° or 37°C. For DNA content analysis, the cultures were washed two times with prewarmed medium and resuspended at A_{600} = 0.05 at either 37° or 23°C. For PFGE, cells were similarly released from the M phase block and cells were harvested after 200 min at either 37° or 23°C.
28. WT and mutant cells were arrested in M phase (*27*). Cells (3×10^6) were released from the M phase block by filtration onto a 0.45-μm filter and washed two times with 37°C YPD. WT cells (3×10^6) of the opposite mating type were filtered onto the same filter and placed onto a YPD plate and incubated at 37°C for 4 hours to allow mating. The filter was washed with minimal medium and the cells were plated onto solid selective medium and incubated at 37°C for up to 4 days. To select for cytoductants during the *kar1-1* mutant matings, cells were plated onto YP plates containing 2% glycerol and cyclohexamide (3 mg/liter) to select for rho$^+$ cytoductants that retained the chromosomes of the *kar1-1* (*cyh*^{R}) parent but not the chromosomes of the Kar$^+$ parent (*cyh*^{S}).
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Recognition of Stress-Induced MHC Molecules by Intestinal Epithelial γδ T Cells

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T cells with variable region $V_\delta1$ γδ T cell receptors (TCRs) are distributed throughout the human intestinal epithelium and may function as sentinels that respond to self antigens. The expression of a major histocompatibility complex (MHC) class I–related molecule, MICA, matches this localization. MICA and the closely related MICB were recognized by intestinal epithelial T cells expressing diverse $V_\delta1$ γδ TCRs. These interactions involved the α1α2 domains of MICA and MICB but were independent of antigen processing. With intestinal epithelial cell lines, the expression and recognition of MICA and MICB could be stress-induced. Thus, these molecules may broadly regulate protective responses by the $V_\delta1$ γδ T cells in the epithelium of the intestinal tract.

T cells expressing γδ TCRs recognize antigens without restriction by polymorphic MHC class I or class II molecules and their associated peptide ligands (*1–3*). Of two main subsets in humans, $V_\gamma2/V_\delta2$ T cells predominate in the circulation and respond to bacterial infections by recognizing soluble nonpeptide antigens (*4*). The other subset defined by expression of $V_\delta1$, however, is of unknown function and no antigens have been identified. These T cells represent 70 to 90% of the γδ T cells in the intestinal epithelium (*5*). Because they are oligoclonal and uniformly distributed, they are believed to recognize self antigens that may be stress-induced (*6, 7*).

The localization of the intestinal intraepithelial $V_\delta1$ γδ T cells is matched by the restricted expression of MICA, a divergent MHC class I–related molecule of unknown function (*8*). Its characteristics include the lack of association with β_2-microglobulin (β_2M), stable expression without conventional class I peptide ligands, and the absence of a CD8 binding site (*8, 9*). Notably, the 5′-end flanking regions of the genes for MICA and a closely related molecule, MICB, include putative heat shock elements similar to those of *hsp70* genes, and the encoded mRNAs are increased in heat shock–stressed epithelial cells (*8*).

To explore a functional relation, we established T cell lines from lymphocytes extracted from intestinal epithelial tumors (*10*). Other adequate sources of human intestinal epithelium are generally unavailable. Freshly isolated tumor cells gave positive stainings with monoclonal antibodies (mAbs) 2C10 and 6D4, which are specific for MICA and for MICA and MICB, respectively (*8, 11, 12*). $V_\delta1$ γδ T cells isolated by cell sorting were grown as two lines, δ1A and δ1B, which were cultured in the presence of cytokines and irradiated C1R cells transfected with cDNA for MICA or MICB, respectively (*10, 13*). After expansion, the T cell lines were tested for phenotype and function. They were homogeneously positive for $V_\delta1$ γδ TCRs, $CD4^-$, and $CD8^-$ (Fig. 1A). As is characteristic of intestinal intraepithelial T cells, they expressed the $\alpha_E\ \beta_7$ integrin (*12, 14*). In chromium release assays with C1R- MICA or C1R-MICB cells as targets, the δ1A and δ1B T cells were cytotoxic against both of these transfectants, but not against untransfected C1R cells (Fig. 1B) (*15*). Two $CD8^+$ αβ T cell lines grown and tested under identical

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