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18 June 1993; accepted 19 October 1993

Isolation of *ORC6*, a Component of the Yeast Origin Recognition Complex by a One-Hybrid System

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Here a method is described to identify genes encoding proteins that recognize a specific DNA sequence. A bank of random protein segments tagged with a transcriptional activation domain is screened for proteins that can activate a reporter gene containing the sequence in its promoter. This strategy was used to identify an essential protein that interacts in vivo with the yeast origin of DNA replication. Matches between its predicted amino acid sequence and peptide sequence obtained from the 50-kilodalton subunit of the yeast origin recognition complex (ORC) established that the gene isolated here, *ORC6*, encodes this subunit. These observations provide evidence that ORC recognizes yeast replication origins in vivo.

The replication of DNA in eukaryotic cells is tightly controlled and coordinated with other events in the cell division cycle. This control is thought to be exerted primarily at the initiation of DNA replication. Replication initiation depends on the completion of earlier events in the cell cycle that commit the cell to a new round of cell division, and reinitiation is prevented until later events are completed, particularly mitosis.

Eukaryotic chromosomal replication initiates at multiple sites in the genome and proceeds bidirectionally. The position of these sites is believed to be specified by DNA elements called origins of replication. Much of our knowledge about the initiation of bidirectional replication comes from prokaryotic and viral systems, most notably the replication systems of *Escherichia coli*, phage λ, and SV40. In these systems, replication origins have been identified, and in vitro systems are available to dissect the initiation reaction (*1*). Studies on the initiation of eukaryotic DNA replication, however, have been hampered by difficulty in the identification of origin sequences. Putative origins have been isolated in a number of eukaryotic systems (*2*), but proof of origin function has remained elusive, and the definition of these elements at the nucleotide level has proven frustrating.

Only in the yeast *Saccharomyces cerevisiae* have eukaryotic origin sequences been clearly identified [reviewed in (*3*)]. Yeast origins were first detected as DNA elements that allow plasmids to be maintained autonomously in yeast cells and were called autonomous replicating sequences (*ARS*s). *ARS*s act as replication origins on plasmids and, in many cases, behave as origins in their native chromosomal location [reviewed in (*4*)]. *ARS*s have a bipartite structure. Domain A is primarily composed of a degenerate 11-bp *ARS* consensus sequence (ACS), 5′-(T/A)-TTTA(T/C)(A/G)TTT(T/A)-3′ found in virtually all *ARS*s (*5*). Domain B, which is approximately 100 bp in size and positioned 3′ to the T-rich strand of domain A, exhibits little sequence similarity among *ARS*s and appears to be organized from multiple partially redundant sequence elements (*6*). Because the ACS is the only sequence motif common to all known *ARS*s, and because single point mutations in this sequence can abolish *ARS* activity (*5*), proteins that specifically recognize the ACS are prime candidates for proteins that initiate DNA replication.

In order to identify potential yeast initiators, we developed a genetic strategy (Fig. 1), the one-hybrid system, to find proteins that recognize a target sequence of interest. This strategy was derived from the two-hybrid system for detecting protein-protein interactions (*7*). The one-hybrid system has two basic components: (i) a hybrid expression library, constructed by fusing a transcriptional activation domain to random protein segments, and (ii) a reporter gene containing a binding site of interest within its promoter region. Hybrid proteins that recognize this site are expected to induce expression of the reporter gene because of their dual ability to bind the promoter region and activate transcription (*8*). This association may be indirect because hybrids that interact with endogenous proteins already occupying the binding site can also activate transcription (*7*). Nevertheless, as long as the association is sequence-specific one may expect the protein incorporated in the hybrid to be functionally relevant.

We have used this method to look for proteins from the yeast *Saccharomyces cerevisiae* that recognize the ACS of yeast origins

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of DNA replication. The protein component of this screen was provided by a set of three complementary yeast hybrid expression libraries, YL1-3, which contain random yeast protein segments fused to the GAL4 transcriptional activation domain ($GAL4^{AD}$) (*9*). The reporter gene for our screen contained four direct repeats of the ACS in its promoter region and was integrated into the yeast strain GGY1 to form JLY363(ACS^{WT}) (Fig. 2) (*10*). To determine the dependence of *lacZ* induction on the ACS, we constructed in parallel JLY365(ACS^{MUTANT}), which harbors a reporter gene carrying four copies of a nonfunctional ACS that is multiply mutated (Fig. 2) (*10*).

With the strategy depicted (Fig. 1), we isolated nine plasmids that induced greater lacZ activity in JLY363(ACS^{WT}) than in JLY365(ACS^{MUTANT}) from a screen of 1.2 million YL1-3 transformants (*11*). Many of the plasmids that induced lacZ activity on initial screening of the library in JLY363(ACS^{WT}) failed to exhibit a dependence on the ACS when introduced into JLY365(ACS^{MUTANT}) (Fig. 3). Restriction analysis of these plasmids showed that the nine isolates represented five genomic clones, which we initially labeled AAP1-5 for ACS associated protein. AAP1 was isolated four times, AAP5 twice, and the others only once.

To examine the sequence specificity of *lacZ* induction with greater resolution, reporter constructs containing direct repeats of four ACS point mutants were each integrated into GGY1 to generate the set of reporter strains tabulated in Fig. 2 (*10*). The five AAP clones were individually examined in these strains for the ability to induce *lacZ* expression (Fig. 4). AAP1 displayed a correspondence between the induction of this set of reporter genes and the *ARS* function (*12*) of their ACS (Fig. 4, top row). The AAP5 hybrid exhibited a slightly weaker correlation, and the remaining clones showed poor correlation (*13*). These findings suggest that AAP1, and possibly AAP5, encodes a protein that recognizes the ACS in a sequence-specific manner. Constructs with deletions in the AAP1 coding sequence (*14*) were unable to induce *lacZ* expression (Fig. 4), indicating that recognition of the ACS resided in the protein segment fused to GAL4.

The genomic segments fused to the $GAL4^{AD}$ in AAP1–5 were sequenced (*15*) to determine the extent of the hybrid proteins that were made. AAP1 and AAP5 had sizable protein coding sequences of 301 and 123 amino acids, respectively, fused in frame with the $GAL4^{AD}$. In principle, these segments are large enough to direct the hybrid protein to the promoter of the reporter gene. AAP2–4 encoded hybrid proteins with only short peptide extensions (10, 22, and 38 amino acids, respectively) fused to the $GAL4^{AD}$, suggesting that these

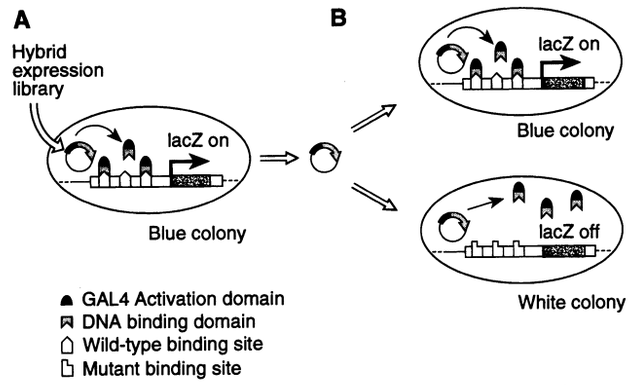

Fig. 1. Schematic of one-hybrid system screen for identifying proteins that can recognize a binding site of interest. (**A**) An expression library of hybrid proteins is transformed into a reporter strain. The hybrids contain protein coding sequences fused to the end of a constitutively expressed GAL4 activation domain. The reporter strain contains a UAS-less *lacZ* reporter gene with multiple copies of the binding site in its promoter region and a low basal transcriptional activity. Hybrid proteins that recognize the binding site act as transcriptional activators of the reporter gene and turn the cell blue in a β-galactosidase assay. (**B**) Reporter genes containing either wild-type (top) or mutant (bottom) binding sites in their promoter regions are used to test the sequence specificity of the *lacZ* induction observed in (A). Recovered plasmids are introduced into strains carrying one or the other reporter gene, and *lacZ* expression is compared.

Mutant designation	Oligonucleotide pair	Two repeats: Reporter gene	Two repeats: Reporter strain	Four repeats: Reporter gene	Four repeats: Reporter strain
WT	5'GATCcgaattCAGATTTTATGTTTA 3' 3'gcttaaGTCTAAAATACAAATCTAG 5'	pJL623	JLY360	pJL625	JLY363
Multiple	5'GATCcgaattCAGAATATTTCTATA 3' 3'gcttaaGTCTTATATAGATATCTAG 5'	pJL624	JLY361	pJL626	JLY365
A863T	5'GATCcgaattCAGATTTTTTGTTTA 3' 3'gcttaaGTCTAAAAAACAAATCTAG 5'	pJL696	JLY429	—	—
T859A	5'GATCcgaattCAGATTTTATGTATA 3' 3'gcttaaGTCTAAAATACATATCTAG 5'	pJL697	JLY431	—	—
T862C	5'GATCcgaattCAGATTTTACGTTTA 3' 3'gcttaaGTCTAAAATGCAAATCTAG 5'	JL698	JLY433	—	—
T867G	5'GATCcgaattCAGAGTTTATGTTTA 3' 3'gcttaaGTCTCAAATACAAATCTAG 5'	pJL699	JLY435	—	—

Fig. 2. Reporter genes and strains generated from mutant domain A sequences. Complementary pairs of oligonucleotides representing wild-type and mutant domain A sequences from *ARS1* are shown. Reporter gene constructs were generated by inserting two or four direct repeats of these sequences into pBgl-lacZ. Integration of each construct into GGY1 produced its corresponding reporter strain. Uppercase nucleotides match sequences found in *ARS1*. Lowercase nucleotides form the spacers between adjacent domain A segments. The *ARS* consensus sequence is boxed and mutations are shown in bold.

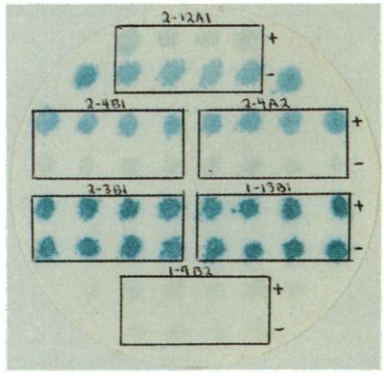

Fig. 3. Secondary screen for hybrid constructs that bind the *ARS* consensus sequence. Plasmids recovered from six positive colonies identified in the initial screen of the hybrid expression library (Fig. 1A) were each introduced into two different reporter strains (Fig. 1B): JLY363, which contains a reporter gene with four copies of the wild-type ACS, and JLY365, which contains a reporter gene bearing four copies of a multiply mutated ACS (*10*). Each plasmid is represented by eight transformants within a rectangular box. The top row (+) of each box represents four independent transformants from JL363 (wt ACS); the bottom row (−) represents four independent transformants from JLY365 (multiply mutated ACS).

hybrids were not responsible for the transcriptional induction attributed to these clones. Because of this finding and the lack of proper sequence specificity for the ACS element, AAP2–4 were not studied further.

The full-length gene for *AAP1* was cloned from a yeast genomic library and sequenced (*15*). *AAP1* contains an open reading frame for a protein 435 amino acids long with a predicted molecular weight of 50,302 daltons (Fig. 5). The hybrid GAL4^{AD}-AAP1 protein obtained from the screen was a fusion of the GAL4^{AD} to the COOH-terminal two-thirds of the predicted full-length protein, indicating that this portion of the molecule is sufficient for association with the ACS.

When we were characterizing the *AAP1* gene, the purification of a multi-protein complex that footprints the consensus sequence of several *ARS* elements in vitro was reported (*16*). This complex, the origin recognition complex (ORC), contains six protein subunits with molecular weights of 50, 53, 56, 62, 72, and 120 kD. Comparison of peptide sequences from the 50-kD subunit of ORC (*17*) with the predicted protein sequence from *AAP1* demonstrated that our gene encodes this subunit (Fig. 5) and confirmed the association between the AAP1 protein and the ACS. Because of this identity, we have renamed the *AAP1* gene *ORC6*.

Although the specific interaction of the ORC6 protein (Orc6p) with the ACS suggests that it is part of a yeast initiator, the predicted Orc6p protein sequence reveals little about its function. Orc6p shows no significant similarity to any protein or translated open reading frame in the NCBI database. No matches to nucleotide binding (*18*) or helicase (*19*) motifs are present. The predicted amino acid sequence does not resolve whether Orc6p associates directly or indirectly with the ACS, because the protein contains no apparent DNA binding motifs.

On the other hand, the sequence of *ORC6* hints at a possible connection with the regulatory machinery governing cell cycle progression. Orc6p contains four potential phosphorylation sites, (S/T)PXK, for cyclin-dependent protein kinases (*20*) clustered in the first half of the molecule (Fig. 5). Use of the more relaxed consensus site (S/T)P adds two more potential sites to this cluster. Although we have observed Orc6p phosphorylated in vivo on serine and threonine residues (*13*), we do not yet know the functional consequences of these modifications. However, because the initiation of yeast DNA replication commences promptly in response to the activation of this protein kinase in G1, it is tempting to speculate that Orc6p and possibly other ORC subunits are regulated substrates of this kinase. Finally, as expected for a protein participating in nuclear events, Orc6p contains a potential nuclear localization signal (NLS) within the (S/T)PXK cluster and one within the COOH-terminal domain (Fig. 5). Orc6p can be detected in the nucleus when examined by immunofluorescence, but so far, only when the protein is significantly overexpressed (*13*).

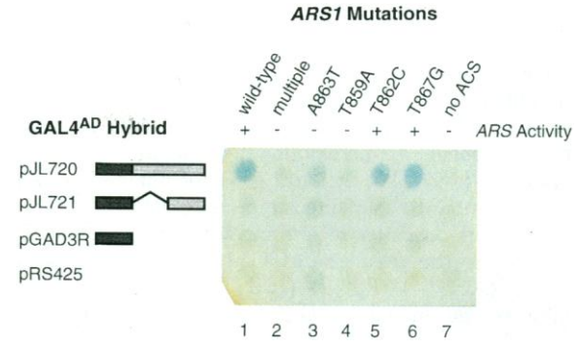

Fig. 4. The induction of *lacZ* by the *AAP1* hybrid clone is dependent on a functional ACS and the AAP1 hybrid protein. Four hybrid protein constructs (rows) were transformed into a set of reporter strains (columns) and assayed for β-galactosidase activity (*11*). The reporter genes in these strains contain two direct repeats of the ACS sequences listed in Fig. 2. Columns 1 to 7 correspond to JLY360, JLY361, JLY429, JLY431, JLY433, JLY435, and pBgl-*lacZ*. *ARS* activity (12): + (50 to 100% of wild-type) or − (unmeasurable activity). The slightly higher level of *lacZ* activity associated with A863T is independent of the hybrid protein construct and probably represents background activity.

Many yeast genes involved in DNA replication possess transcriptional control elements [MCB boxes (*20a*)] that direct their periodic expression in late G1. These elements are uniformly found within 250 nucleotides of the translational start codons for these genes. *ORC6* exhibits one perfect and one near match to the MCB element 5′ of its coding sequence, but the closest element is approximately 450 nucleotides from the predicted translational start for *ORC6*. Hence, though vaguely suggestive, the nucleotide sequence gives no strong indication that *ORC6* belongs to the MCB class of genes.

We anticipated that if *ORC6* was indeed involved in replication, it would be essential for viability. A marked deletion of the *ORC6* gene (pJL731) (*21*) that removes all but 13 codons from the open reading frame was introduced into diploids from three different strain backgrounds. The resulting heterozygous *ORC6* deletion strains, JLY481, JLY475, and JLY469, were induced to undergo meiosis, and 20 tetrads of each strain were dissected (*21*). In all backgrounds, the *ORC6* disruption cosegregated with inviability, demonstrating that *ORC6* is essential for cell growth. Microscopic examination revealed that mutant spores from JLY481 and JLY475 germinated, completed one to two rounds of cell division, and then arrested with a uniform large bud morphology reminiscent of cell division cycle mutants defective in DNA replication or nuclear division (*22*). The position of cell cycle arrest could not be established because the DNA content of these cells could not be readily measured. For unknown reasons, mutant spores derived from JLY469 germinated poorly.

The interpretation of these *ORC6* deletion experiments was complicated by the presence of a second open reading frame (ORF2) of 250 amino acids on the antisense strand of the *ORC6* gene. ORF2 spans nucleotides 1617 to 868 of the GenBank sequence and overlaps the COOH-terminal two-thirds of the *ORC6* coding sequence. A marked deletion that removed the NH_2-terminal third of the *ORC6* coding sequence without affecting ORF2

Fig. 5. Predicted amino acid sequence of *AAP1-ORC6*. Shown in bold are the amino acid matches to peptide sequences from the ORC6 subunit (*17*). Matches to the consensus phosphorylation site (S/T)PXK of cyclin-dependent protein kinases (*20*) are underlined. The GAL4^{AD}-ORC6 hybrid pJL720 contains amino acid residues 135 to 435; hybrid pJL721 contains residues 349 to 435 (*14*). Potential nuclear localization signals are at amino acid residues 117 to 122 and 263 to 279.

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        10         20         30         40         50
MSMQQVQHCV AEVLRLDPQE KPDWSSGYLK KLTNATSILY NTSLNKVMLK

        60         70         80         90        100
QDEEVARCHI CAYIASQKMN EKHMPDLCYY IDSIPLEPKK AKHLMNLFRQ

       110        120        130        140        150
SLSNSSPMKQ FAWTPSPKKN KRSPVKNGGR FTSSDPKELR NQLFGTPTKV

       160        170        180        190        200
RKSQNNDSFV IPELPPMQTN ESPSITRRKL AFEEDEDEDE EEPGNDGLSL

       210        220        230        240        250
KSHSNKSITG TRNVDSDEYE NHESDPTSEE EPLGVQESRS GRTKQNKAVG

       260        270        280        290        300
KPQSELKTAK ALRKRGRIPN SLLVKKYCKM TTEEIIRLCN DFELPREVAY

       310        320        330        340        350
KIVDEYNINA SRLVCPWQLV CGLVLNCTFI VFNERRRKDP RIDHFIVSKM

       360        370        380        390        400
CSLMLTSKVD DVIECVKLVK ELIIGEKWFR DLQIRYDDFD GIRYDEIIFR

       410        420        430   435
KLGSMLQTTN ILVTDDQYNI WKKRIEMDLA LTEPL
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Table 1. Viability of *cdc* mutants in the presence of high levels of *ORC6* expression. JL749 (GALp-HA-ORC6), JL772 (GALp-HA), and RS425 were introduced into each *cdc* mutant, and examined for growth at various temperatures under conditions that induce expression of ORC6 (*28, 29*). Plus indicates mutants whose restrictive temperature remains unchanged in the presence of JL749 relative to JL772 and RS425. Minus indicates mutants whose restrictive temperature is lowered 5° to 7°C when JL749 is present.

Strain	*cdc* Mutant	Viability
RDY488	Wild-type	+
RDY501	*cdc28-1*	+
RDY510	*cdc4-1*	+
RDY664	*cdc34-2*	+
RDY543	*cdc7-4*	+
JLY310	*cdc6-1*	−
JLY179	*cdc46-1*	−
JLY338	*cdc2-1*	+
JLY353	*cdc17-1*	+
RDY619	*cdc15-2*	+

(pJL733) was introduced into diploids (*21*). Tetrad analysis again showed that the *ORC6* deletion cosegregated with cell death. Finally, an *ORC6* gene was constructed that contains a silent codon change for the *ORC6* ORF but introduces a UGA stop codon in ORF2 (Fig. 5) (*23*). This gene was able to rescue a haploid strain containing a full deletion of the *ORC6* ORF. We conclude that *ORC6* is essential for cell viability.

Our results validate the one-hybrid system screen as a method to identify and clone genes encoding proteins that recognize a DNA sequence of interest. An independent development of this screen has also been successful in identifying DNA-binding proteins (*24*), and a variation of this screen has been used to identify a binding site for a suspected DNA-binding protein (*25*). The one-hybrid approach is particularly useful for proteins that are difficult to detect biochemically or for which starting material for a purification is difficult to obtain.

Although ORC is a prime candidate for a yeast initiator protein because of its specific recognition of the ACS, biochemical analysis of ORC has yet to reveal additional properties that are characteristic of established initiator complexes from viral and bacterial systems (for example, adenosine triphosphate hydrolysis, DNA unwinding, and DNA helicase activity) (*1*). On the basis of our current biochemical understanding of ORC, one cannot rule out an alternative role for ORC, such as the repression of unscheduled initiation of DNA replication. Our isolation of the gene for ORC6p, as well as that reported for ORC2p (*17, 26*) should facilitate the functional analysis of ORC. We have demonstrated that ORC6 is essential for viability and is required at a specific stage of the cell cycle, as expected for a gene involved in the initiation of DNA replication. Further insight into the function of ORC will arise from studies on conditional *orc6* mutants, similar to those reported for *orc2* (*17*).

The identification of ORC6 through the one-hybrid screen was based on the premise that the $GAL4^{AD}$-ORC6 hybrid can interact with the *ARS* consensus sequence in vivo. This interaction may reflect the incorporation of the hybrid into an origin recognition complex and the association of the entire complex with the ACS, because protein-DNA crosslinking experiments performed with ORC (*16*) suggest that Orc6p may not directly participate in DNA recognition. If so, the behavior of the ORC6 hybrid in the screen provides the most compelling evidence that ORC binds to the origin of replication in vivo, a result first indicated by deoxyribonuclease protection studies on yeast origins in isolated nuclei (*27*).

Ultimately, definitive proof for a direct role of ORC in the initiation of DNA replication will require the establishment of an in vitro DNA replication system dependent on yeast origins, ORC protein, and quite possibly additional proteins. We have begun a search for such additional proteins by looking for genes that interact genetically with *ORC6*. Because germinating spores bearing an *ORC6* deletion appeared to exhibit a cell division cycle phenotype, we initially focused our attention on established *cdc* mutants. pJL749 (*28*), a plasmid that overexpresses Orc6p several hundredfold (*13*), was introduced into a virtually isogenic set of temperature-sensitive *cdc* mutants arresting at various points in the cell cycle (*29*). Overexpression of *ORC6* selectively affected *cdc6* and *cdc46* mutants, lowering their restrictive temperature by 5° to 7°C; there was no significant effect on the other mutants examined or on the wild-type strain (Table 1).

Both *CDC6* and *CDC46* are required late in G1 for entry into S phase (*30*). Mutations in these genes interfere with the autonomous maintenance of *ARS*-containing plasmids, presumably by perturbing the replication of these plasmids (*31*). This plasmid-loss phenotype can be suppressed in *cdc6* by the addition of *ARS* elements to the plasmid (*31*). These results support the notion that *CDC6* and *CDC46* play a role in the proper function of origins early in replication. The specific interaction between these genes and *ORC6* suggests that ORC functions at a common step with *CDC6* and *CDC46*. It seems reasonable to speculate that Cdc46p or Cdc6p or both may help trigger the activity of ORC during the initiation of DNA replication and ensure that ORC acts at the proper time in the cell cycle.

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9. Three related hybrid expression libraries, YL1-3 (*7*), were a gift from S. Fields. The NH_2-terminal portions of these hybrids consist of the SV40 nuclear localization signal and amino acids 768 to 881 of the GAL4 activation domain ($GAL4^{AD}$). The COOH-terminal portions were derived from random yeast protein segments which have been fused to the end of the $GAL4^{AD}$. These segments are encoded by short (1–3 kb) fragments from a Sau 3A partial digest of yeast genomic DNA. Together, YL1-3 ensure that all three reading frames of these fragments can be expressed.
10. pLR1Δ1 [R. W. West Jr., R. R. Rogers, M. Ptashne, *Mol. Cell. Biol.* **4**, 2467 (1984)] was a gift of R. West. We generated pBgl-lacZ from pLR1Δ1 by: (i) substituting an Xho I-Bgl II-Xho I polylinker 5′-CCTCGAGGAGATCTCCTCGAGG-3′ for the Xho I linker and (ii) precisely excising a Hind III fragment containing 2-μm sequences. The resulting vector has a unique Bgl II site approximately 100-bp upstream of the TATA box for insertion of DNA sequences in the promoter region and a unique Stu 1 site for targeted integration of the plasmid at the URA3 locus. Multiple direct repeats of *ARS1* domain A and several of its mutant derivatives were inserted into the Bgl II site of pBgl-lacZ to generate all the reporter genes used in this work. The inserted repeat elements, derived from complementary oligonucleotides shown in Fig. 2, were oriented with the TATA box to their right. Each reporter gene construct was integrated into the URA3 locus of GGY1 (*MATα Δgal4 Δgal80 ura3 leu2 his3 ade2 tyr*) [G. Gill and M. Ptashne, *Cell* **51**, 121 (1987)] to create a reporter strain (Fig. 4). Integration of pBgl-lacZ into GGY1 generated JLY387.
11. YEPD (rich complete) and SD (synthetic dropout) media are as described [J. B. Hicks and I. Herskowitz, *Genetics* **83**, 245 (1976)]. Standard methods were used for manipulation of yeast cells [C. Guthrie and G. R. Fink, Eds., *Guide to Yeast Genetics and Molecular Biology* (Academic Press, San Diego, 1991)] and DNA [F. M. Ausubel *et al.*, Eds., *Current Protocols in Molecular Biology* (Wiley, New York, 1989)]. Libraries YL1-3 were transformed [R. H. Schiestl and R. D. Geitz, *Current Genetics* **16**, 339 (1989)] into JLY363 (*10*) and plated on SD-Leu at a density of 2 to 5000 colonies per 10-cm plate. Five hundred thousand transformants were obtained for YL1 and YL2, and 200,000 for YL3. Transformants were assayed on filters for production of β-galactosidase [L. Breeden and K. Nasmyth, *Cold Spring Harbor Symp. Quant. Biol.* **47**, 643 (1985)]. Forty-nine isolates remained positive after colony purification (15 from YL-1, 22 from YL-2, and 12 from YL-3), and library plasmids were extracted from them.

These plasmids were each transformed into both JLY363 and its mutant counterpart JLY365 (*10*). Nine plasmids induced greater β-galactosidase activity in the wild-type reporter strain than the control. These plasmids were classified into five clones, *AAP1* through *AAP5*, on the basis of their Hind III restriction pattern. Each clone was retested in JLY360, JLY361, JLY387, JLY429, JLY431, JLY433, JLY435 (Fig. 4). The *AAP1* hybrid clone was called pJL720. The *AAP1* gene was later renamed *ORC6*.

12. The *ARS* function of the sequences in Fig. 4 was analyzed in the context of *ARS*1 domain B (Bgl II–Hinf I fragment, nt 853-734) in the following CEN-based URA3-containing plasmids: pJL347 (wt), pJL243 (multiple), pJL326 (A863T), pJL338 (T869A), pJL330 (T862C), and pJL316 (T867G). These plasmids were transformed into JLY106 (*MATα ura3 leu2 his3 trp1 lys2 ade2*) and its homozygous diploid counterpart JLY162. pJL243, pJL326, and pJL338 did not yield a high frequency of transformation and could not be assayed quantitatively for *ARS* function. pJL347, pJL330, and pJL316 transformed cells with high efficiency and were assayed for mitotic stability [D. T. Stinchcomb, K. Struhl, R. W. Davis, *Nature* **282**, 39 (1979)].
13. J. J. Li and I. Herskowitz, unpublished material.
14. The *ORC6* hybrid construct originally isolated from the YL3 library (pJL720) has two Bam HI sites. The 5′ site, which is created by the hybrid junction, corresponds to the Sau 3A site at nucleotide 843. Excision of the segment between the two sites generated pJL721, leaving amino acid residues 339 to 435 in frame with the $GAL4^{AD}$ (Fig. 5). pGAD3R (*11*), the parent vector for the YL3 library, contains no *ORC6* sequence. pRS425 [T. W. Christianson, R. S. Sikorski, M. Dante, J. H. Shero, P. Hieter, *Gene* **110**, 119 (1992)] contains no components of the fusion protein.
15. All sequencing was performed with Sequenase (USB) on collapsed double-stranded templates. The protein coding segments of the *AAP1* through *AAP5* hybrid clones were sequenced from their junction with the $GAL4^{AD}$ to their stop codon. Two of the *ORC6* sequencing primers were used as colony hybridization probes to screen a high copy number yeast genomic library [M. Carlson and D. Botstein, *Cell* **28**, 145 (1982)] for a clone of the full-length *ORC6* gene (pJL724). The full-length gene was sequenced on both strands with oligonucleotide primers positioned approximately 200 nucleotides apart. The accession number for the *ORC6* sequence reported in this paper is L23323.
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21. Marked *ORC6* deletions were constructed by replacing nucleotides 458-1721 (pJL731) or nucleotides 458-846 (pJL733) of the GenBank sequence with the URA3 Hind III fragment oriented in the opposite direction to that of the *ORC6* sequence. Each construct was used to generate heterozygous deletions of *ORC6* in diploid strains by one-step gene replacement. *ORC6* deletion analysis was performed in JLY461 (*MAT***a**/*MATα ura3/ura3 leu2/leu2 his3/his3 trp1/trp1 ade2/ade2 [cir°]*), JLY462 (*MAT***a**/*MATα ura3/ura3 leu2/leu2 trp1/trp1 his4/his4 can1/can1*), and JLY463 (*MAT***a**/*MATα ura3/ura3 leu2/leu2 trp1/trp1 his3/HIS3*); their respective genetic backgrounds are S288c, EG123, and A364a. Disruption of JLY461, JLY462, and JLY463 by pJL731 (full deletion) created JLY481, JLY475, and JLY469, respectively. Disruption of JLY461, JLY462, and JLY463 by pJL733 (NH_2-terminal deletion) created JLY485, JLY479, JLY473, respectively. These heterozygous marked deletion strains were sporulated, and 20 tetrads of each were dissected and grown on YEPD to assess viability.
22. J. R. Pringle and L. H. Hartwell, in *The Molecular Biology of the Yeast Saccharomyces*, J. N. Strathern, E. W. Jones, J. R. Broach, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1981), vol. 1, pp. 97–142.
23. A point mutant (pJL766) was made by replacing the Bam HI-Sph I fragment of the full-length clone with a Bam HI-Sph I fragment generated by PCR from pJL720 with the primers

    5′-CAAGGATCCAAGAATTGA**T**CATTTTATAGT-CAG-3′

    5′-GTTATAGGGCTAAAGGCATGC-3′.

    The mutation, shown in bold, changes nucleotide 1471 of the GenBank sequence from C to T and was confirmed by sequence analysis.
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27. J. F. X. Diffley and J. H. Cocker, *Nature* **357**, 169 (1992).
28. pJL749 contains the *GAL1* promoter (nucleotides 146 to 816) driving the expression of *ORC6* (nucleotides 443 to 2298) in the high-copy yeast shuttle vector RS425 [T. W. Christianson, R. S. Sikorski, M. Dante, J. H. Shero, P. Hieter, *Gene* **110**, 119 (1992)]. The sequence 5′-CCCG-GATCCC ATG GCC TAC CCA TAT GAT GTT CCA GAT TAC GCT TCT TTG GGT CCA GGG CTG CAG GAA TTC GGG CCC ATC-3′ lies between the *GAL1* promoter and *ORC6* and contains the influenza hemagglutinin (HA) epitope fused to the NH_2-terminus of ORC6. This construct complements a deletion of the *ORC6* gene. pJL772 is identical to pJL749 except that it lacks the *ORC6* sequence.
29. The *cdc* mutant strains listed in Table 1 have been backcrossed four to five times against two congenic strains derived from *A364a*, *RDY487* (*MATα leu2 ura3 trp1*) and RDY488 (*MAT***a** *leu2 ura3 trp1*). All are *ura3 leu2 trp1*. RDY510, RDY664, JLY310, and JLY179 are *MATα*; the rest are *MAT***a**. Additional markers can be found in JLY310 (*ade2*), RDY543 (*his3*), and RDY619 (*pep4Δ::TRP1 his3 ade2*). The RDY strains were a gift from R. Deshaies. pJL749, pJL772, and RS425 (*28*) were transformed into these strains and plated on SD-Leu at 22°C. Four colony-purified isolates from each transformation were patched onto SD-Leu plates and replica-plated to SGAL-Leu plates, all at 22°C. The patches on SGAL-Leu were replicaplated to a series of prewarmed SGAL-Leu plates at 22°, 25°, 27°, 30°, 32.5°, 35°, 37°, and 38°C. The viability of cdc mutants containing pJL749 was compared to those containing pJL772 and pRS425.
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32. We thank C. Peterson for preparation of library DNA, F. Banuett for oligonucleotide synthesis, and A. Lynn for superb help with the figures. We appreciate S. Fields, N. Hollingsworth, A. Sil, R. Deshaies, P. Sorger, P. Jackson, S. Sanders, A. Johnson, C. Detweiler, and A. Lynn for helpful discussions or useful suggestions on the manuscript. This work was supported by NIH grant AI18738 (to I.H.) J.J.L. is a Lucille P. Markey Scholar and this work was supported in part by a grant from the Lucille P. Markey Charitable Trust. J.J.L. also appreciates the early support from Bristol-Myers Squibb through the Life Sciences Research Foundation. The accession number for the *ORC6* sequence reported in this paper is L23323.

13 October 1993; accepted 17 November 1993

Sharing of the Interleukin-2 (IL-2) Receptor γ Chain Between Receptors for IL-2 and IL-4

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The γ chain of the interleukin-2 (IL-2) receptor is an indispensable subunit for IL-2 binding and intracellular signal transduction. A monoclonal antibody to the γ chain, TUGm2, inhibited IL-2 binding to the functional IL-2 receptors and also inhibited IL-4–induced cell growth and the high-affinity binding of IL-4 to the CTLL-2 mouse T cell line. Another monoclonal antibody, TUGm3, which reacted with the γ chain cross-linked with IL-2, also immunoprecipitated the γ chain when cross-linked with IL-4. These results suggest that the IL-2 receptor γ chain is functionally involved in the IL-4 receptor complex.

Functional high-affinity receptors for cytokines are generally complexes consisting of binding subunits (α chains) with low affinities to ligands and effector subunits (β chains) to transduce signals, both of which are members of the cytokine receptor superfamily (*1*). The same β chain is shared by receptors for IL-3, IL-5, and granulocyte-macrophage colony-stimulating factor (GM-CSF) (*1*, *2*). Another molecule, gp130, is shared as a signaling molecule by the receptors for IL-6, leukemia inhibitory factor (LIF), oncostatin M (OSM), and ciliary neurotrophic factor (CNTF) receptors (*3*). The IL-2 receptor is also a complex (*4*), but the low-affinity α chain is not a member of the cytokine receptor superfamily and the high-affinity receptor contains, in addition to the α and β chains, the γ

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