to 5784. However, our results from the *CeORC2* cDNA sequence revealed that the authentic protein begins at position 2618 and the first exon ends at 2793 and excludes the first five predicted exons. Before termination at 5784, the DNA from 5781 to 5882 is spliced out and the last exon terminates at 5998. The GenBank accession number is U40270.

29. A 340-bp partial cDNA encoding amino acids related to *S. cerevisiae ORC2* was isolated by RT-PCR reaction using human HeLa cell mRNA. First-strand cDNA was synthesized from 2 μg of mRNA primed with oligo(dT). The resulting cDNA was used as a template for PCR with degenerate primers based on amino acids conserved among ScORC2, AtORC2, and CeORC2 (5′-[C/T]A[C/T]GGIGTIGGITCIAA[A/G][A/C]G-3′ and 5′-ATGTGGTCIA[C/T]IG[A/T]IGCIA[C/T]IA-3′). An amplified fragment was sequenced and found to be related to *S. cerevisiae ORC2*. This combination of primers also amplified a DNA fragment from *K. lactis* that was sequenced and found to be related to *S. cerevisiae ORC2* (*23*). The human DNA fragment was used as a probe to isolate a full-length human cDNA [see (*21*)]. Positive clones were plaque-purified and sequenced on both strands. The GenBank accession number is U40268.
30. M. Gossen, D. T. S. Pak, S. Hansen, J. Acharya, M. Botchan, *Science* **270**, 1674 (1995); A. E. Ehrenhofer-Murray, M. Gossen, D. T. S. Pak, M. R. Botchan, J. Rine, *ibid.*, p. 167.
31. pKG28 contains the full-length cDNA encoding HsORC1 under the control of the cytomegalovirus promoter fused to a single copy of the T7 epitope in pCGT [A. Wilson, M. G. Peterson, W. Herr, *Genes Dev.* **9**, 2445 (1995)]. Xba I and Bam HI sites were introduced into the cDNA encoding HsORC1 by PCR immediately upstream of the in-frame initiator methionine and immediately downstream of the stop codon, respectively. The entire coding region except for the 3′-most 120 nucleotides was replaced with a native DNA fragment, and sequencing of the 120 bp at the 3′ end revealed no mutations generated during PCR. Human 293 cells were transfected or mock-transfected with 10 μg of pKG28 by electroporation and were harvested 24 hours later. Whole-cell extracts were prepared and used for immunoprecipitation with rabbit antibody to amino acids 1 to 296 of HsOrc2p fused to GST. Immune complexes were collected on protein A–Sepharose (Pharmacia), washed, and separated by SDS–polyacrylamide gel electrophoresis (SDS-PAGE). The proteins were transferred to nitrocellulose, and immunoblot analysis was done with a monoclonal antibody to the T7 epitope (Novagen). The ECL system was used for detection (Amersham).
32. A. Kornberg and T. A. Baker, *DNA Replication* (Freeman, New York, 1991), vol. 2.
33. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
34. We thank M. Hengartner, N. Kaplan, R. Martienssen, L. Rodgers, and J. Skowronski for providing various cDNA and genomic libraries; D. Casso and M. Caligiuri for *S. pombe* strains and vectors; A. Stenlund and M. Berg for help with transfections; M. Ockler for help with artwork; M. Weinreich and P. Kaufman for helpful discussions; and P. Kaufman for reading of the manuscript. Supported by the Japan Society for the Promotion of Science Postdoctoral Fellowship for Research Abroad to M.H. and by NIH grant CA13106 to B.S.

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Separation of Origin Recognition Complex Functions by Cross-Species Complementation

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Transcriptional silencing at the *HMR***a** locus of *Saccharomyces cerevisiae* requires the function of the origin recognition complex (ORC), the replication initiator of yeast. Expression of a *Drosophila melanogaster Orc2* complementary DNA in the yeast *orc2-1* strain, which is defective for replication and silencing, complemented the silencing defect but not the replication defect; this result indicated that the replication and silencing functions of ORC were separable. The *orc2-1* mutation mapped to the region of greatest homology between the *Drosophila* and yeast proteins. The silent state mediated by *DmOrc2* was epigenetic; it was propagated during mitotic divisions in a relatively stable way, whereas the nonsilent state was metastable. In contrast, the silent state was erased during meiosis.

Position effects are influences on the expression of a gene as a function of its location within the genome. Classical position effects include the inactivation of X chromosomes in female mammals (*1*) and position effect variegation in *Drosophila* (*2*). The inheritance of position effects in dividing cells is epigenetic, that is, genetically identical cells can display different phenotypes. At present, the mechanism of establishing and maintaining epigenetic states of gene expression is unknown. In *S. cerevisiae*, a form of position effect occurs at the cryptic mating-type loci *HMR* and *HML* (*3*). These loci serve as donors of mating-type information in mating-type interconversion. Although the genes at *HMR* and *HML* are identical to the expressed sequences at the mating-type locus *MAT*, they are kept silent throughout the cell cycle. Silencing at *HMR* and *HML* requires the combined action of nearby regulatory sequences, called silencers, and multiple proteins, at least some of which function at the silencers. Interestingly, some silencers also act as chromosomal origins of replication (*4*). Altering the autonomously replicating sequence (ARS) consensus sites of the *HMR*-E silencer results in a loss of replication initiation at the silencer and a concomitant loss of silencing at *HMR* (*5*). Moreover, mutations in two subunits of ORC decrease silencing at *HMR* and reduce replication initiation at *HMR*-E (*6*, *7*). These observations have led to the hypothesis of some link between replication and silencing. We have challenged this hypothesis by investigating the cross-species complementation of ORC functions. If a homolog is capable of complementing both functions, the association between the two processes must be ancient and conserved. In contrast, if heterologous ORC proteins complement only the silencing function or only the replication function, then the two roles of ORC are experimentally separable and may have been separate in evolution.

To test the cross-species complementation of ORC functions, we determined whether the *Drosophila Orc2* gene (*DmOrc2*) (*8*) was able to complement the *orc2-1* mutation in the yeast *ORC2* gene, which encodes the second largest subunit of ORC (*9*, *10*). A complementary DNA (cDNA) encoding DmOrc2 was placed under the control of the strong constitutive yeast glyceraldehyde-3-phosphate dehydrogenase (GPD) promoter and the phosphoglycerate kinase terminator (PGK) (*11*). The recombinant gene was inserted into an integrative yeast–*Escherichia coli* shuttle vector and then integrated into the yeast genome (*12*). To test whether the protein encoded by *DmOrc2* was expressed in yeast, we prepared whole-cell extracts from strains that either did or did not contain the *DmOrc2* overexpression construct and analyzed them by immunoblotting with an antibody to DmOrc2 (Fig. 1). Immunoreactive DmOrc2 protein was present in the extracts from strains containing the GPD-*DmOrc2*-PGK hybrid gene, and it was not detected in strains lacking the construct. The electrophoretic mobility of the DmOrc2 protein expressed in yeast was consistent with its predicted molecular mass of 68.5 kD.

We next determined whether *DmOrc2* was capable of complementing the defects of the *orc2-1* mutation. By cloning and sequencing the *orc2-1* allele, we found that this mutation resulted in a change of a proline to a leucine residue at position 603 of the Orc2 protein, a residue that was conserved between *Drosophila* and yeast. The *orc2-1* mutation affects both the replication initiation function and the silencing function of the Orc2 protein (*6*). The rep-

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lication defect causes a temperature-sensitive cell-cycle defect, presumably because essential parts of the genome fail to be replicated at the nonpermissive temperature. Moreover, *orc2-1* strains exhibit reduced replication initiation at chromosomal origins, and they have a defect in the maintenance of both centromere-based and 2μ plasmids, even at the permissive temperature (6). The silencing defect of *orc2-1* is most evident at a sensitized *HMR***a** allele (*HMR-SSΔI*) (6). In a *MAT*α strain, this defect leads to the simultaneous expression of **a** and α information, which produces a nonmating phenotype. This loss of silencing at *HMR***a** correlates with a drastically reduced ability of the *HMR*-E silencer to act as a chromosomal origin of replication.

To assay the complementation of the *orc2-1* mutation by *DmOrc2*, we introduced the GPD-*DmOrc2*-PGK vector into the *orc2-1* strain and compared the temperature sensitivity and mating ability of these transformants to that of the *orc2-1* strain (Fig. 2). Two types of *DmOrc2*-expressing *orc2-1* transformants were recovered. In some transformants, the mating ability was restored to nearly that of a wild-type strain, indicating that *DmOrc2* was able to restore silencing at *HMR***a** in these strains (JRY5213; Fig. 2C, c). In other transformants, the mating ability was not restored (JRY5212; Fig. 2C, d), indicating that *DmOrc2* was unable to restore silencing of *HMR***a** in these strains. However, neither of the two types of transformants was able to grow at the nonpermissive temperature (Fig. 2B); this result indicated that *DmOrc2* could not provide the essential replication function that is missing in an *orc2-1* mutant. Therefore, *DmOrc2* was capable of complementing the silencing defect, but not the replication defect, of *orc2-1*. The sequence homology between the *Drosophila* and yeast Orc2 proteins thus reflected a functional homology. Moreover, this result suggested that the roles of ORC in silencing and in replication could be separated.

We performed several additional tests to determine whether DmOrc2 was providing any replication function in yeast: (i) *DmOrc2*-expressing *orc2-1* strains had no detectable growth advantage over *orc2-1* strains at the permissive temperature or at any semipermissive temperature tested, regardless of whether the strains were repressed or derepressed at *HMR* (*13*). (ii) *DmOrc2* was unable to relieve the plasmid maintenance defect of *orc2-1* (*14*). (iii) *DmOrc2* was unable to rescue the inviability of a strain containing an *orc2* null allele (*15*). In principle, *DmOrc2* could have restored silencing at *HMR* by restoring replication initiation at *HMR*-E in *orc2-1* strains without restoring sufficient replication function to allow viability. To test this possibility, we performed two-dimensional origin-mapping assays (Fig. 3). As observed earlier (6), even at the permissive temperature, the *orc2-1* mutation caused a decrease in replication initiation at the *HMR*-E silencer relative to that of a wild-type *ORC2* strain (Fig. 3, A and B). However, we were unable to detect any appreciable increase in replication initiation at the silencer in *orc2-1* strains containing the GPD-*DmOrc2*-PGK hybrid gene (Fig. 3C). As a second measure of the silencer origin efficiency, we determined the loss rate of plasmids that carried *HMR*-E as the sole origin. The presence of *DmOrc2* was unable to stabilize the extreme plasmid loss in *orc2-1* strains (*14*). Thus, these findings provided genetic and physical evidence that the DmOrc2 protein did not support replication initiation in yeast.

It was surprising that *DmOrc2* complemented the silencing defect of *orc2-1* in some transformants, but not in others (Fig. 2). These strains were genetically identical, and yet they displayed different phenotypes. Therefore, this complementation had an epigenetic component, which prompted further investigation. Testing whether the two different classes of *DmOrc2* transformants had different amounts of expression of DmOrc2 (Fig. 1, lanes 2 and 3) revealed no difference in the amounts of DmOrc2 expressed in strains in which *HMR***a** was or was not silenced. Thus, differences in *DmOrc2* expression were not the source of the variation. Chromosomal replication initiation at the *HMR*-E silencer was also compared in the two different cell types

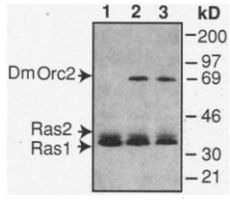

Fig. 1. Detection of DmOrc2 expressed in yeast. Cell extracts of the *orc2-1* strain JRY4475 (lane 1) and the *DmOrc2*-expressing strains JRY5212 (lane 2) and JRY5213 (lane 3) were analyzed for the presence of DmOrc2. JRY5212 and JRY5213, which are transformants of JRY4475 with pJR1722, contained a derepressed and a repressed *HMR***a** allele, respectively. The strains were grown to mid-log phase, and whole cell lysates were prepared by glass bead lysis [(*22*), except that phosphate-buffered saline containing protease inhibitors was used as the lysis buffer]. The protein equivalent of 0.5 OD_{600} units of cells was separated on an 8% SDS–polyacrylamide gel and immunoblotted with a polyclonal antibody to DmOrc2 (*8*). A Ras monoclonal antibody (Y13-259, Oncogene Science), which recognizes both Ras1p and Ras2p, was used as a loading control. Immunocomplexes were visualized with the enhanced chemiluminescence system ECL (Amersham).

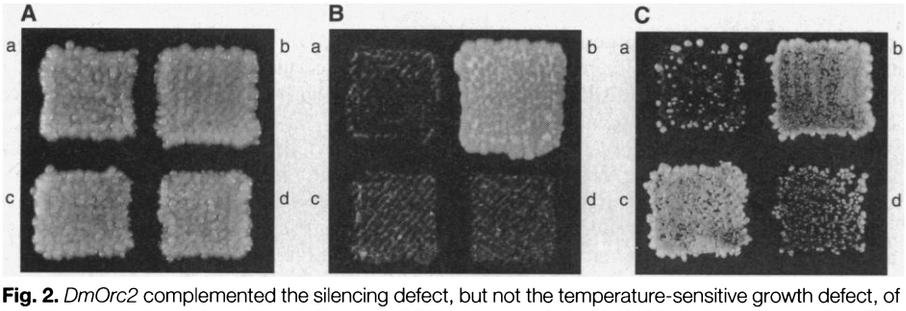

Fig. 2. *DmOrc2* complemented the silencing defect, but not the temperature-sensitive growth defect, of the yeast *orc2-1* mutation. The *MAT*α *HMR-SSΔI* strains *orc2-1* (JRY4475) [(a) in each panel], *ORC2* (JRY4473) [(b) in each panel], and two *DmOrc2*-expressing *orc2-1* strains (JRY5213 and JRY5212, respectively) [(c) and (d) in each panel] were assayed for their growth at the permissive temperature (23°C) (**A**) and at the restrictive temperature (37°C) (**B**), as well as for their ability to mate with a *MAT***a** *his4* tester strain (JRY2726) at the permissive temperature (**C**).

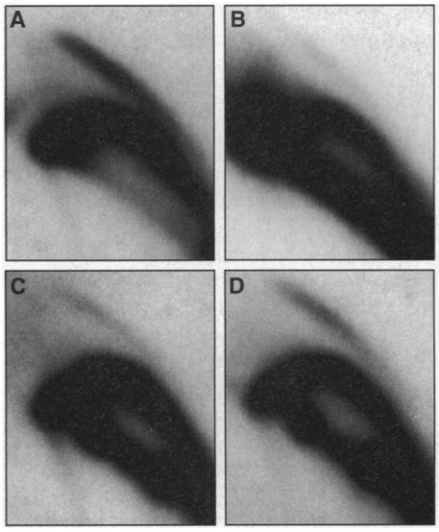

Fig. 3. *DmOrc2* did not increase replication initiation at the *HMR*-E silencer in *orc2-1* strains. A Hind III–Bgl II *HMR* fragment was analyzed for the presence of replication intermediates with the use of two-dimensional origin-mapping gels (*23*), as described (6). The *MAT*α *HMR-SSΔI* strains were (**A**) a wild-type *ORC2* strain (JRY4473), (**B**) an *orc2-1* strain (JRY4475), (**C**) a *DmOrc2*-expressing *orc2-1* strain with a repressed *HMR* locus (JRY5213), and (**D**) a *DmOrc2*-expressing *orc2-1* strain with a derepressed *HMR* locus (JRY5212).

(Fig. 3, C and D). However, regardless of the amount of silencing at *HMR***a** in these strains, no difference in replication initiation from the silencer was detected in either cell type.

Because individual colonies could be isolated that were either silenced or not silenced at *HMR*, both of these states were stable enough to form a colony of a single type. However, from colonies of either type, we were able to recover clonal isolates with the characteristics of the opposite type. For example, a transformant colony in which *HMR***a** was silenced contained a subset of cells in which *HMR***a** was not silenced. Conversely, a transformant colony in which *HMR***a** was not silenced also harbored cells in which *HMR***a** was silenced. Prolonged propagation of a strain in which *HMR***a** was not silenced led to an increase in the percentage of cells with a silenced *HMR* locus (Fig. 4). This increase in silencing was *DmOrc2*-dependent, because a similar increase was not observed with the *orc2-1* strain alone. The accumulation of clones with a silenced *HMR* was not the result of a selective growth advantage, because strains with repressed or derepressed *HMR* had the same growth rates. Moreover, the ratio of cells with silenced versus nonsilenced *HMR* loci in a transformant colony that was predominantly silenced at *HMR* varied only slightly during mitotic growth. These results indicated that the *DmOrc2*-dependent silent state was more stably propagated in mitosis than was the nonsilent state. The nonsilent state was metastable in the presence of *DmOrc2*, such that the fraction of cells silenced at *HMR* increased over time. These data were consistent with a role of the DmOrc2 protein in the establishment of the silent state, the maintenance of the silent state, or both.

The persistence of the silent state at *HMR* mediated by *DmOrc2* was tested during meiosis. For this purpose, strains with either a repressed or a derepressed *HMR* locus were mated to an isogenic wild-type strain, and the mating phenotype of the *orc2-1* progeny containing *DmOrc2* was evaluated. In a cross in which the *DmOrc2*-containing parent harbored a derepressed *HMR* locus, the majority of the *orc2-1* offspring carrying *DmOrc2* also were derepressed at *HMR* (33 of 41). A cross with a parent containing a repressed *HMR* locus also yielded a majority of progeny in which *HMR***a** was derepressed (40 of 45). Therefore, the *DmOrc2*-dependent silent phenotype was much less stable during meiosis than it was during mitosis. These observations indicated an erasure of the silent phenotype during meiosis, and thus they suggest that mitotic inheritance is mechanistically distinct from meiotic inheritance.

The ability of *DmOrc2* to complement the silencing defect provides the first evidence that the structural similarity between the *Drosophila* and yeast proteins reflects a functional homology. However, as measured by genetic and physical methods, *DmOrc2* was unable to promote replication in yeast. Nonetheless, Gossen *et al.* and Gavin *et al.* (*8*, *16*) strongly suggest that *DmOrc2* does play a role in replication in *Drosophila* because of the highly conserved structure of the ORC complex and the existence of homologs in a variety of eukaryotes. The inability of *DmOrc2* to complement the replication defect in yeast could be explained in several ways. Perhaps a yeast ORC complex containing DmOrc2 was unable to recognize enough yeast origins of replication to replicate the genome. Alternatively, the DmOrc2-containing complex may be unable to interact with other key replication proteins, such as Cdc7p (*17*) or Cdc6p (*18*).

The apparent lack of replication function provided by *DmOrc2* in yeast indicated that repression at the *HMR* locus could be restored independently of replication initiation at the *HMR*-E silencer. Thus, *DmOrc2* can be considered an *orc2* allele that separates the replication and silencing functions of ORC. The ability of *DmOrc2* to provide the silencing function indicates that ORC complexes containing DmOrc2 are able to communicate with other silencing proteins. Could these data reflect a role of ORC in silencing in *Drosophila*? It is possible that ORC plays no role in silencing in *Drosophila*; the ability of *DmOrc2* to restore silencing in yeast would then merely reflect the role of the yeast ORC complex in silencing and the ability of *DmOrc2* to stabilize or restore the function of the complex in *orc2-1* mutants. Alternatively, at face value, the data are also compatible with a role for *Drosophila* ORC in the control of gene expression in *Drosophila*. This model would imply an interaction between *Drosophila* ORC and regulatory proteins in *Drosophila* that would be equivalent to an interaction between yeast ORC and silencing proteins. However, if *Drosophila* ORC is involved in controlling gene expression, there must be at least some differences in the silencing mechanism between yeast and other eukaryotes, because a portion of Orc1p that is required for silencing in *Saccharomyces* is not conserved in other eukaryotes (*16*).

The epigenetic component of *DmOrc2*'s silencing complementation in yeast bears similarities to the variegated position effects in *Drosophila* (*19*). Therefore, it will be of interest to learn whether *Drosophila* ORC plays a role in these position effects. The epigenetic behavior of silencing conferred by *DmOrc2* might also reflect a decreased ability of the DmOrc2-containing complex to interact with the yeast silencing proteins, in particular with the Sir1 protein (*20*). The erasure of the silent phenotype in cells during meiosis suggests that such a multiprotein complex dissociates during meiosis or within a few mitotic divisions immediately after meiosis. The epigenetic inheritance of silencing is not restricted to the silencing brought about by *DmOrc2*, because *sir1* mutants behave in a similar way. On the basis of these parallels, we predict an intimate relation between ORC and the Sir1 protein in silencing.

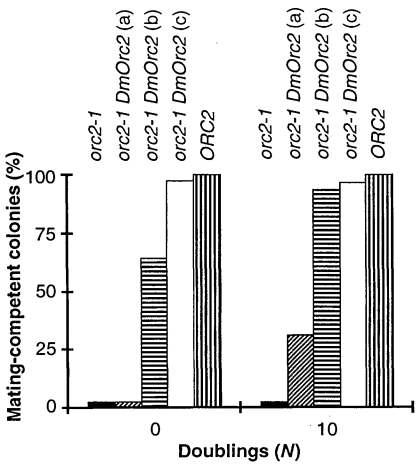

Fig. 4. *DmOrc2* increased the percentage of *orc2-1* clones with a repressed *HMR* locus. Three independent *MATα HMR-SSΔI DmOrc2 orc2-1* strains that initially contained varying amounts of derepressed *HMR* loci were analyzed for changes in the number of clones within the culture that were repressed at *HMR*. The *DmOrc2* strains initially contained *HMR* loci that were (a) predominantly derepressed, (b) 65% repressed, or (c) predominantly repressed. The strains were grown in liquid culture during 10 doubling times, samples were plated on complete medium, and the repression state at *HMR* of individual colonies within the culture was tested by determining their mating ability. The colonies were rated either as mating-competent (*HMR* repressed) or as mating-incompetent (*HMR* derepressed), and this rating is given as the percentage of mating-competent colonies among all colonies tested. The sample sizes were 120 to 160 individual colonies per strain and time point. The *orc2-1* strain JRY4475 and the *ORC2* strain JRY4473 served as controls.

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12. The coding sequence for *DmOrc2* was excised as a 1.9-kb Bsp HI–Sal I fragment from a pNB40-based plasmid [N. H. Brown and F. C. Kafatos, *J. Mol. Biol.* **203**, 425 (1988)] containing the cDNA (*8*), treated with Klenow polymerase and inserted into the Sma I site of pG-3 (*11*) such that the *DmOrc2* transcription start was next to the GPD promoter. This GPD-*DmOrc2*-PGK expression construct was then inserted as a Hind III–Xba I cassette into YIplac128 (*21*) to create pJR1722. The yeast strain JRY4475 (*MATα*, *HMR-SSΔI, ade2-1, his3-11,15, trp1-1, leu2-3,112, ura3-1, can1-100, orc2-1*) was transformed to leucine prototrophy with Eco RV–linearized pJR1722 [R. J. Klebe, J. V. Harriss, Z. D. Sharp, M. G. Douglas, *Gene* **25**, 333 (1983)].
13. The growth of the *DmOrc2*-expressing *orc2-1* strains JRY5212 and JRY5213 was compared to that of JRY4475 (*orc2-1*) and, as a reference, to the growth of the isogenic *ORC2* strain JRY4473. The strains were streaked on complete medium and incubated at 23°, 26°, 30°, 34°, and 37°C for 3 days, and their growth rates in liquid culture at 23°C were compared.
14. The loss rates (*5*) for plasmids with ARS1 (pJR1469), ARSH4 (pRS316), or the synthetic *HMR*-E silencer (pJR950) as the sole origins of replication were determined in an *ORC2* strain (JRY3009), in an *orc2-1* strain (JRY4475), and in two *DmOrc2*-expressing *orc2-1* strains (JRY5212 and JRY5213) (*5*). The loss rates, expressed as plasmid loss events per cell division, of plasmids replicated by ARS1 or ARSH4 were low (<0.001) in the wild-type strain but were increased in the *orc2-1* strains (0.060 and 0.155 for ARS1 and ARSH4, respectively). The *orc2-1* mutation increased the loss rate for plasmids replicated by the synthetic silencer from 0.024 in the *ORC2* strain to 0.190. The loss rates for *DmOrc2*-expressing *orc2-1* strains were 0.039 ± 0.025, 0.146 ± 0.022, and 0.197 ± 0.069 for ARS1, ARSH4, and the synthetic silencer, respectively.
15. A null allele of *ORC2* was constructed by replacing an internal Msc I fragment of a 5.5-kb Sac I–Sal I *ORC2* fragment by the 1.8-kb Bam HI fragment of *HIS3*. In that *ORC2* is an essential gene, a diploid heterozygous for *orc2-Δ2::HIS3* was constructed by transforming the wild-type diploid JRY4014 to histidine prototrophy with the disruption construct. JRY4014 is a W303-based diploid strain heterozygous for the *HMR-SSΔI* allele. An *ORC2/orc2-Δ2::HIS3* diploid was then transformed with a *URA3*-marked yeast–*E. coli* shuttle vector carrying *ORC2*, pJR1263 (*9*). Tetrads were dissected from the transformed diploid, and a haploid *MATα HMR-SSΔI orc2-Δ2::HIS3* strain containing pJR1263 (JRY5010) was recovered. JRY5010 was transformed to leucine prototrophy with YCplac111 (*21*) containing the GPD-*DmOrc2*-PGK cassette (pJR1720). Several transformants were then tested for their ability to lose the *URA3-ORC2* plasmid by assaying their resistance to 5-fluoroorotic acid (FOA) [J. D. Boeke, F. LaCroute, G. R. Fink, *Mol. Gen. Genet.* **197**, 345 (1984)]. In contrast to JRY5010 cells transformed with a *LEU2-ORC2* plasmid, JRY5010 cells transformed with pJR1720 were unable to grow on FOA, which indicated that they could not lose the *URA3-ORC2* plasmid. Thus, *DmOrc2* expression was unable to rescue the inviability of the *orc2* deletion.
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24. We thank C. Zucker for providing a clone containing partial *DmOrc2* and alerting us to its existence; C. A. Fox, C. Trueblood, R. Kamakaka, A. Dillin, S. Okamura, and the other members of our laboratories for materials, advice, and discussions; and C. A. Fox, R. Kamakaka, and S. Linn for comments on the manuscript. Supported by a Junior Fellowship from the Swiss National Foundation (A.E.), NIH grant GM31105 (J.R.), and a Mutagenesis Center grant from the National Institute of Environmental Health Sciences for the support of core facilities (P30ESO1896-12, B. Ames).

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A *Drosophila* Homolog of the Yeast Origin Recognition Complex

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Genes from *Drosophila melanogaster* have been identified that encode proteins homologous to Orc2p and Orc5p of the *Saccharomyces cerevisiae* origin recognition complex (ORC). The abundance of the *Drosophila* Orc2p homolog DmORC2 is developmentally regulated and is greatest during the earliest stages of embryogenesis, concomitant with the highest rate of DNA replication. Fractionation of embryo nuclear extracts revealed that DmORC2 is found in a tightly associated complex with five additional polypeptides, much like the yeast ORC. These studies will enable direct testing of the initiator-based model of replication in a metazoan.

DNA replication in higher eukaryotes is intricately regulated, both temporally and spatially, within each cell cycle and throughout development. The mechanisms underlying this regulation remain largely obscure, primarily because the sites and proteins involved in initiation have not been clearly identified. In the classic replicon model (*1*), a positive-acting factor (the initiator) acts at a specific DNA sequence (the replicator) to direct DNA synthesis to a nearby start site, the origin of replication. This concept has proven to be valid for prokaryotes as well as for eukaryotic DNA viruses (*2*). However, metazoan initiators that recognize chromosomal DNA have not been described. Moreover, the exact nature of the metazoan replicon is subject to controversy; start sites of DNA replication have been mapped to relatively short DNA sequences or to widespread initiation zones, depending on the origin region under investigation and on the techniques used (*3*).

The current state of knowledge concerning the initiation of chromosomal DNA replication in eukaryotes derives mainly from studies in *S. cerevisiae*, for which both an initiator and replicators have been identified. The ORC is a six-subunit assembly that binds in a site-specific, adenosine triphosphate (ATP)–dependent manner to autonomously replicating sequence (ARS) consensus sites, which define budding yeast origins of DNA replication (*4*). Despite the large number of replicator sites in a yeast chromosome and the sequence variations within these sites, it appears as if one vital initiator complex is central to the orchestration of the events that lead to DNA synthesis. The ORC influences the rate of firing at all active origins that have been examined (*5*) and also participates in transcriptional silencing at ARS elements of the yeast mating type loci (*6, 7*). Three ORC subunits (Orc2p, Orc5p, and Orc6p) have been described; they have been shown to be essential for viability and, in the case of Orc2p and Orc5p, for plasmid maintenance as well (*6, 8, 9*).

A genomic DNA sequence in the region of the *Drosophila* genome proximal to the *inositol polyphosphate-1-phosphatase* gene (*IPP*) showed homology to *S. cerevisiae ORC2* (*10*). We used the initially defined region of sequence homology to generate hybridization probes, and we identified complementary DNA (cDNA) clones in two independent early embryonic libraries (*11*). The sequences of these cDNAs predicted a protein that showed ~30% amino acid identity and ~56% homology to the COOH-terminus (residues 430 to 547) of *S. cerevisiae* Orc2p (Fig. 1B). Because the homology was less pronounced at the NH_2-termini of the two proteins, the average amino acid identity was 21% (37% homology).

Antibodies to this *Drosophila* Orc2p homolog, DmORC2 (*12*), were used to analyze protein extracts from *Drosophila* embryos col-

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