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Chromosomal DNA Replication Initiates at the Same Origins in Meiosis and Mitosis

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Autonomously replicating sequence (*ARS*) elements are identified by their ability to promote high-frequency transformation and extrachromosomal replication of plasmids in the yeast *Saccharomyces cerevisiae*. Six of the 14 *ARS* elements present in a 200-kb region of *Saccharomyces cerevisiae* chromosome III are mitotic chromosomal replication origins. The unexpected observation that eight *ARS* elements do not function at detectable levels as chromosomal replication origins during mitotic growth suggested that these *ARS* elements may function as chromosomal origins during premeiotic S phase. Two-dimensional agarose gel electrophoresis was used to map premeiotic replication origins in a 100-kb segment of chromosome III between *HML* and *CEN3*. The pattern of origin usage in premeiotic S phase was identical to that in mitotic S phase, with the possible exception of *ARS308*, which is an inefficient mitotic origin associated with *CEN3*. *CEN3* was found to replicate during premeiotic S phase, demonstrating that the failure of sister chromatids to disjoin during the meiosis I division is not due to unreplicated centromeres. No origins were found in the DNA fragments without *ARS* function. Thus, in both mitosis and meiosis, chromosomal replication origins are coincident with *ARS* elements but not all *ARS* elements have chromosomal origin function. The efficiency of origin use and the patterns of replication termination are similar in meiosis and in mitosis. DNA replication termination occurs over a broad distance between active origins.

All higher eukaryotes studied have S phases that differ in length during development. The S phase is short during early embryogenesis, longer in somatic cells, and quite extended during premeiotic S (reviewed in reference 7). For example, in the newt *Triturus cristatus*, S phase is 1 h long in the blastula, 4 h long in the neurula, about 40 h long in somatic cells, and 200 h long in premeiotic cells. Interestingly, in *T. cristatus*, the spacing between active meiotic origins is several times longer than between mitotic origins, suggesting that only a subset of mitotic origins or altogether different replication origins are used in meiosis.

In *Saccharomyces cerevisiae*, mitotic S phase and premeiotic S phase appear to be regulated differently. Premeiotic S phase is approximately 65 min in length (53), two to three times longer than mitotic S phase (3, 41, 46, 51). In addition, different genes appear to be required for entry into the two S phases. In the mitotic cell cycle, the functions of the *CDC4*, *CDC28*, *CDC36*, *CDC39*, and *CDC7* gene products are required prior to DNA replication (reviewed in reference 39), while in the meiotic cell cycle, these gene products are required after DNA replication and before the meiosis I division (19, 42, 44, 45). Conversely, the products of *SPO7*, *SPO8*, *SPO9*, *MEI1*, and *MEI2,3* are required for premeiotic DNA replication but not for mitotic DNA replication (reviewed in reference 15).

Despite these differences in regulation, both the average spacing between origins and the rate of replication fork movement in premeiotic S phase (24) are similar to those measured in mitotic S phase (reviewed in reference 33). However, the methods used in these studies, DNA fiber autoradiography and sedimentation analysis, could not be used to determine whether the same DNA sequences were used as origins in both cases. It is possible that the origins used during meiosis and mitosis are identical in yeast cells. Alternatively, different DNA sequences may function as meiotic origins but have a spacing similar to that of mitotic origins.

The 200-kb region of chromosome III between the left telomere and the *MAT* locus contains 14 autonomously replicating sequence (*ARS*) elements, *ARS300* to *ARS313*, identified by their ability to promote high-frequency transformation and extrachromosomal maintenance of plasmids in yeast cells (35). Two-dimensional (2-D) gel analysis demonstrated that this region of chromosome III is replicated from six replication origins during mitotic S phase (reviewed in reference 36). These origins are all coincident with *ARS* elements. Five of the six mitotic origins, *ARS305* (22), *ARS306* (13, 54), *ARS307* (13, 17), *ARS309* (17), and *ARS310* (34), are active in most cell cycles. The sixth mitotic origin, associated with *ARS308* and *CEN3*, is active in only 15 to 20% of the cell cycles (17, 34). It is not clear why certain *ARS* elements do not function as chromosomal origins. Perhaps these inactive *ARS* elements are conserved on the chromosome because they are used as origins under other growth conditions. Another possibility is that origin activity is controlled by chromosome position effects (reviewed in reference 36).

The unexpected observation that only 6 of the 14 *ARS* elements present in the 200-kb region (35) are detectably active as chromosomal origins of replication in mitotic S phase (14, 34) led us to consider the possibility that the inactive *ARS* elements might function as chromosomal origins during premeiotic S phase. In order to make a comparison of the mitotic and meiotic origins, we used 2-D agarose gel electrophoresis (5) to map premeiotic replication intermediates in a 100-kb segment that includes most of the left arm of chromosome III. The three active meiotic replication origins in this 100-kb region are coincident with mitotic replication origins. In addition, the two known mitotic replication origins on the right arm of chromosome III are also active as meiotic origins. Thus, the

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highly active origins used in premeiotic S phase are identical to those used in mitotic S phase.

MATERIALS AND METHODS

Strain, plasmids, and media. *S. cerevisiae* NKY730 [*MATa leu2::hisG/MATα leu2::hisG lys2/lys2 HO/HO ura3ΔhisG/ura3ΔhisG*], derived from strain SK1, was provided by Nancy Kleckner. The *leu2::hisG* allele is described in reference 8. Plasmids containing chromosome III fragments have been described previously (35). The YPG plates, YPD plates, YPD liquid medium, and YPAc liquid medium (presporulation medium) were standard (43). The sporulation medium SPM is described in reference 1.

Sporulation protocol. The sporulation protocol provided by R. Padmore and N. Kleckner has been described in detail elsewhere (10). For the time course experiments, cells from 200 to 900 ml of culture were harvested from YPAc just before (time zero) and at various times after transfer to SPM. For mapping of replication intermediates along chromosome III, cells were harvested 3 h after transfer to SPM (see Results).

DAPI staining. The method described in reference 52 for staining with 4′,6-diamidino-2-phenylindole (DAPI) was used. Cells with a single nucleus, cells with two nuclei, cells with four nuclei, and mature asci were scored in each sample.

Isolation of replicating yeast DNA. To arrest DNA replication, samples of the sporulating culture were mixed with 0.5 volume of an ice-cold slurry of azide stop solution (0.5 M NaOH, 0.4 M EDTA, 0.2% sodium azide [pH 8.65] [30]), and placed in an ice bath. Cells were harvested by filtration (1.2-μm Magna nylon filter; MSI, Westboro, Mass.) and washed twice with water. DNA was isolated as described previously (17).

Restriction enzyme digestion. The restriction enzyme buffer of Mirkovitch et al. (31) was used. From 25 to 50 μg of DNA was digested with 200 U of restriction endonuclease (New England Biolabs) for 2 h at 37°C, an additional 200 U of enzyme was added, and the digest was incubated for another 2 h. After ethanol precipitation, the DNA was resuspended in 50 μl of TE (10 mM Tris, 1 mM EDTA [pH 8.0]), and the entire digest was loaded in one well of an agarose gel for 2-D analysis.

2-D agarose gel electrophoresis. DNA fragments and probes used for 2-D gel analysis are listed in Table 1. DNA replication intermediates were analyzed by the 2-D gel method of Brewer and Fangman (5). A model H1 horizontal gel box (Bethesda Research Laboratories) was used for gels 20 cm across and 25 cm long. For the first-dimension gel, a 3-mm-thick 20-well comb was used. With molecular weight standards as a guide, a 12-cm gel slice was cut from the first-dimension gel, starting about 1 kb below the size of the fragment to be examined and turned 90°, and a second-dimension gel was poured around it. The conditions used for running the 1-D and 2-D agarose gels depended on the size of the DNA fragment of interest. For the first dimension, 2-kb fragments were run in a 0.8% agarose gel at 1.2 V/cm for 42 h, and in the second dimension, the fragments were run in a 1.5% agarose gel at 5 V/cm for 13.5 h. Fragments of from 3 to 5 kb were run first in a 0.4% agarose gel at 1.2 V/cm for 26 h and then in a 1% agarose gel at 5 V/cm for 8 h. Fragments of from 5 to 7 kb were run in a 0.3% agarose gel at 1 V/cm for 44 h and then in a 0.8% agarose gel at 5 V/cm for 8 h. Gels were blotted and hybridized as described previously (10).

TABLE 1. DNA fragments and probes used for 2-D gel analysis[a]

Fragment no.	Test fragment and size (kb)	Probe fragment and size (kb)
1	*Eco*RI (5.4)	*Hin*dIII-*Bam*HI (1.4)
2	*Hin*dIII (2.2)	*Hin*dIII-*Bam*HI (1.4)
3	*Bam*HI-*Xba*I (3.9)	*Eco*RV (1.6)
4	*Eco*RI (3.8)	*Eco*RV (1.6)
5	*Eco*RV (4.1)	*Eco*RV (4.1)
6	*Eco*RI-*Bam*HI (4.9)	*Eco*RV-*Bam*HI (2.8)
7	*Xba*I (4.9)	*Eco*RV-*Bam*HI (2.8)
8	*Bam*HI (5.3)	*Pst*I (1.2)
9	*Xba*I (1.9)	*Bam*HI-*Pst*I (0.6)
10	*Hin*dIII (3.4)	*Bam*HI-*Pst*I (0.6)
11	*Xba*I (5.2)	*Pst*I-*Bam*HI (2.6)
12	*Pst*I (4.7)	*Pst*I-*Bam*HI (2.6)
13	*Eco*RI (5.9)	*Bam*HI-*Pst*I (2.1)
14	*Eco*RV (4.2)	*Bam*HI-*Pst*I (2.1)
15	*Pst*I-*Bam*HI (4.2)	*Pst*I-*Bam*HI (4.2)
16	*Xho*I (3.6)	*Xho*I-*Bam*HI (1.7)
17	*Bam*HI-*Eco*RV (3.4)	*Bam*HI-*Eco*RV (3.4)
18	*Xho*I-*Hin*dIII (2.9)	*Eco*RV (1.3)
19	*Pst*I-*Eco*RI (5.0)	*Eco*RV (2.6)
20	*Hin*dIII (4.8)	*Eco*RV-*Bam*HI (1.6)
21	*Eco*RV (4.7)	*Eco*RV-*Bam*HI (1.6)
22	*Bam*HI (4.4)	*Bam*HI (4.4)
23	*Eco*RI (3.5)	*Bam*HI-*Eco*RI (1.9)
24	*Bam*HI-*Eco*RV (3.7)	*Bam*HI-*Eco*RI (1.9)
25	*Eco*RI (4.2)	*Eco*RV-*Eco*RI (2.4)
26	*Eco*RV-*Xba*I (3.6)	*Eco*RV-*Eco*RI (2.4)
27	*Dra*I-*Xho*I (3.7)	*Xba*I-*Eco*RI (1.6)
28	*Bgl*II (3.0)	*Xho*I (1.4)
29	*Hin*dIII-*Pst*I (3.8)	*Xho*I (1.4)
30	*Eco*RV-*Xho*I (4.2)	*Eco*RI-*Xho*I (1.9)
31	*Pst*I (5.4)	*Eco*RI-*Xho*I (1.9)
32	*Hin*dIII (4.8)	*Xba*I-*Hin*dIII (1.6)
33	*Xba*I-*Xho*I (5.1)	*Xba*I-*Hin*dIII (1.6)
34	*Pst*I (7.5)	*Eco*RI-*Pst*I (2.2)
35	*Bam*HI (4.9)	*Eco*RI-*Pst*I (2.2)
36	*Bgl*II (3.4)	*Bam*HI-*Bgl*II (1.1)
37	*Eco*RV-*Xho*I (4.9)	*Dra*I-*Xho*I (1.5)
38	*Bgl*II (5.3)	*Dra*I-*Xho*I (1.5)
39	*Xho*I-*Eco*RV (4.1)	*Dra*I-*Bgl*II (0.9)
40	*Eco*RI-*Bam*HI (5.7)	*Dra*I-*Bgl*II (0.9)
41	*Dra*I (5.0)	*Dra*I-*Bgl*II (0.9)
A	*Bam*HI-*Pst*I (3.7)	*Eco*RI-*Pst*I (1.3)
42	*Eco*RV (3.3)	*Eco*RI-*Pst*I (1.3)
43	*Eco*RI (3.5)	*Eco*RI-*Pst*I (1.3)
44	*Xba*I (5.6)	*Eco*RV-*Bam*HI (2.5)
45	*Eco*RV (5.1)	*Eco*RV-*Bam*HI (2.5)
46	*Bam*HI-*Eco*RV (4.9)	*Bgl*II-*Pst*I (2.1)
47	*Hin*dIII (5.5)	*Eco*RV (0.8)

[a] The positions of the fragments are shown on the chromosome III map in Fig. 4.

RESULTS

Sporulation kinetics. Mitotic cells enter the meiotic cell cycle in response to nitrogen starvation only at the major G_1 control point known as Start. Premeiotic DNA synthesis is followed by recombination, the meiosis I and II divisions, and finally tetrad maturation (reviewed in reference 15). As a first step in mapping premeiotic DNA replication origins, the kinetics of sporulation were examined in order to determine when premeiotic DNA replication was occurring. Sporulation kinetics were assessed by two different techniques. First, samples were removed from a sporulating culture at various times and stained with DAPI to monitor the kinetics of the meiosis I and II divisions and tetrad development. Premeiotic S phase must occur during the time before the meiosis I division. Second, 2-D gel analysis was used to directly visualize replication intermediates.

The time courses of completion of landmark events in

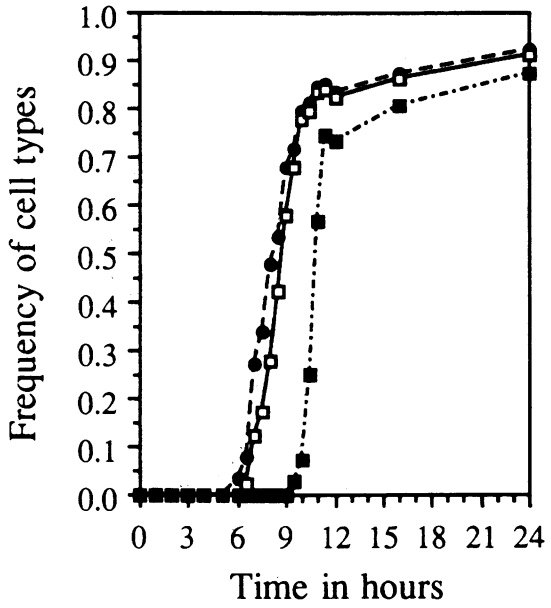

FIG. 1. Sporulation kinetics. Samples from a sporulating culture were stained with DAPI and counted as described in Materials and Methods. A total of 200 cells were scored for each time point. ●, cells with two nuclei; □, cells with four nuclei; ■, mature asci. Accumulation curves, calculated as the sum of cells at a particular stage plus cells at later stages, are shown.

meiosis for strain NKY730, which is a derivative of SK1, are shown in Fig. 1. The first cells with two nuclei (meiosis I) were seen at 6 h, and by 10 h, 80% of the population had completed meiosis I. Cells with four nuclei (meiosis II) appeared at 6.5 h, and this division was essentially complete by 10 h. Mature asci first appeared at 9.5 h, and by 12 h, 74% of the population had completed sporulation. Consistent with earlier observations (25, 38, 53), these data demonstrate efficient and relatively synchronous sporulation by this strain. The finding that approximately 80% of the population had completed the meiotic divisions by 10 h indicates that premeiotic replication and recombination must occur during the first 6 to 8 h of sporulation and that mitotic cells could not contribute significantly to the replication intermediates observed. Previous observations have suggested that cells in the population which do not undergo the meiotic divisions also fail to undergo premeiotic DNA replication or additional rounds of mitotic replication (38).

A time course experiment was undertaken to determine the peak of premeiotic DNA replication and to assess whether different regions of chromosome III are replicated at different times. DNA was prepared from samples taken from YPAc medium just before transfer to SPM and from SPM every hour up to 6 h. DNA from each sample was cut with *Eco*RV, run on a 2-D gel, transferred to a hybridization membrane, and probed to reveal the fragment of interest. In this 2-D gel system (5), replication intermediates and recombination intermediates of a given restriction fragment fall along characteristic arcs, as diagrammed in Fig. 2.

The results of such a time course experiment are presented in Fig. 3. The filters were probed to visualize the *ARS305*-containing fragment shown in Fig. 4 (fragment 14) and then stripped and reprobed sequentially to visualize fragments 21 and 5. Fragment 42, containing *ARS307*, was also examined (data not shown). As described in more detail below, the shapes of the arcs of replication intermediates provide information about how the fragments examined replicate. The bubble-to-Y arc transition seen for fragment 14 (Fig. 3A to G) and the patterns visualized for overlapping fragments (see below) demonstrate that *ARS305* is active as a meiotic origin. The simple Y arcs visualized for fragments 21 (Fig. 3H to N) and 5 (Fig. 3O to U) indicate that each fragment is replicated by a single fork that initiates at an origin external to the fragment.

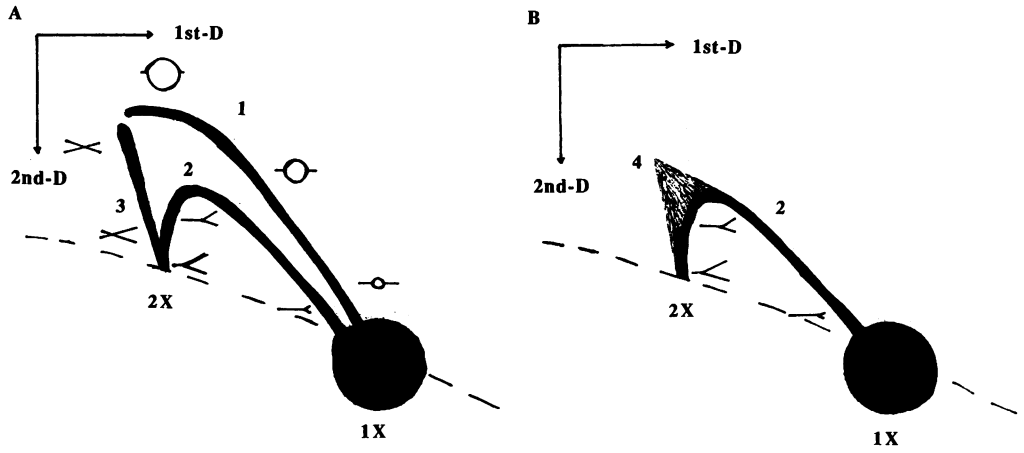

FIG. 2. Diagrams of 2-D gel patterns. The arcs of DNA replication intermediates and recombination intermediates expected from 2-D gel analysis are shown along with drawings of the molecules that are present in each arc. The dark spot in the lower right corner is the position where nonreplicating monomer-length molecules migrate. Linear DNA molecules fall along the dashed line. Molecules in arc 1 contain replication bubbles which result from DNA replication initiation within the fragment. Arc 2 contains Y-shaped molecules. A complete Y arc in the absence of a bubble arc demonstrates that replication initiates outside of the fragment. Spike 3 has been shown to contain X-shaped recombination intermediates (10). The diffuse triangular signal labeled 4 contains a collection of double-Y-shaped replication intermediates and results from random DNA replication termination within a fragment. (A) 1, bubble arc; 2, Y arc; 3, recombination spike. (B) 2, Y arc; 4, triangular signal due to random termination.

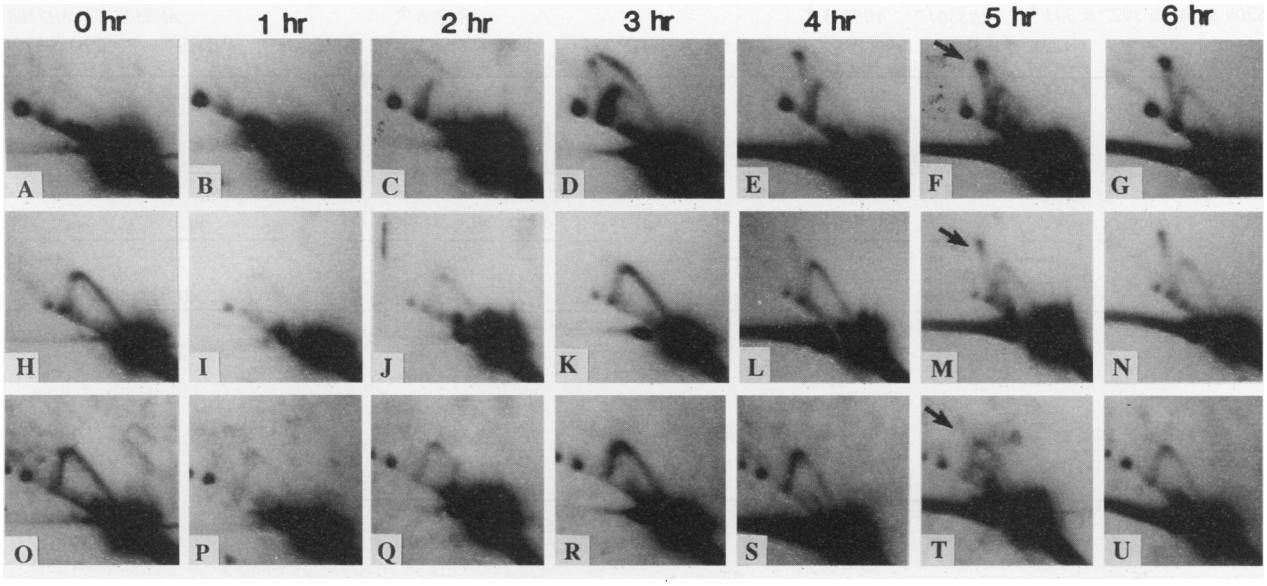

FIG. 3. Kinetics of premeiotic DNA replication. Samples were taken from YPAc and from SPM every hour for 6 h. The first row (A to G) presents the 2-D gel analysis of fragment 14, which contains *ARS305*; the second row (H to N) shows fragment 21; and the third row (O to U) shows the analysis of fragment 5. The arrows indicate recombination spikes. Refer to Fig. 4 for a map showing the chromosomal positions of the fragments and to Table 1 for the restriction enzyme sites defining these fragments and the fragments used as probes.

For each of the fragments shown in Fig. 3, and also for fragment 42 (data not shown), the time course of replication was similar. Because the same amount of DNA was analyzed in each sample, the blots were hybridized at the same time, and the films were exposed for the same length of time, the intensity of the replication signal reflects the amount of replication. Mitotic replication intermediates, which are very weak for fragment 14, can be seen in the time-zero samples for fragments 21 and 5 (Fig. 3, leftmost column). The replication signals decrease or remain very weak for the first 2 h in SPM before they reach their maximum intensity at 3 h and then decrease steadily through the last sample at 6 h. These results are consistent with those of previous studies which showed by pulse labeling (53) or fluorescence-activated cell sorting (FACS) analysis (8, 38) that the peak of premeiotic DNA replication occurs approximately 3 h after transfer to SPM. We conclude that 3 h after transfer to SPM is the best time for visualizing premeiotic replication intermediates.

The observation that DNA replication intermediates were seen at each time point in the time course experiment (Fig. 3) demonstrates that the culture did not undergo premeiotic S phase with tight synchrony. At least some of the asynchrony (Fig. 1 and 3) must have resulted from asynchrony in the starting YPAc cultures, which contained cells at each stage in the mitotic cell cycle. When these cells were transferred from YPAc to SPM, cells in early G_1, which were not yet committed to the mitotic cell cycle, entered the meiotic cell cycle. In contrast, cells in late G_1, S, G_2, and M, which were already committed to the mitotic cell cycle, completed the mitotic cell division before entering the meiotic cell cycle. Therefore, the sporulating culture contained cells at different stages in the meiotic cell cycle. Another factor contributing to the asynchrony is that mother cells, which are larger than their daughters (buds) at the time of cell division, were probably able to initiate sporulation before their daughters. Daughters are known to have a longer mitotic cell cycle than mothers, resulting from an expanded G_1 in daughter cells, which presumably reflects the need for daughters to reach a critical size before committing to a division cycle (3).

In addition to the replication intermediates, all the fragments examined in the time course experiment (Fig. 3) showed a spike (indicated by an arrow) that extends upward from the arc of linear DNA fragments at a position to the left of the replication intermediates (see diagram in Fig. 2A). This spike was faint at 4 h and strong at 5 and 6 h. In experiments reported elsewhere, we have demonstrated that these spikes contain joint molecules which are meiotic homologous recombination intermediates (10).

Kinetics of *CEN3* replication. Longstanding cytological observations have shown that sister chromatids remain associated at their centromeres until the anaphase of mitosis and throughout the meiosis I division (11). These observations were the basis for a proposal that replication of centromeric DNA is delayed until the time of sister chromatid segregation at anaphase of mitosis or anaphase of the meiosis II division (32, 47). It has been shown that *S. cerevisiae* centromeres replicate during the first half of mitotic S phase, substantially before the mitotic division (30), but the possibility that centromere replication might be delayed in meiosis has not been studied. We therefore examined the time course of replication of *CEN3* (Fig. 5). In this experiment, DNA was prepared from samples taken at 2-h intervals from the time that cells were transferred to SPM for 12 h. It is apparent that the most intense replication signal occurred at 4 h (Fig. 5C), a time indistinguishable from the time of peak replication of other chromosome III fragments and 2 h before the first cells completed the meiosis I division (Fig. 1). We conclude that *CEN3* replicates during premeiotic S phase and therefore that sister chromatids must not be prevented from segregating precociously in meiosis I by an unreplicated centromere.

Another feature of meiotic centromere replication is that replication forks pause at the centromere as they do in mitotic S phase (18). This is apparent in both mitosis (Fig. 5A) and meiosis (Fig. 5B and C) as a dark spot (indicated by an arrow)

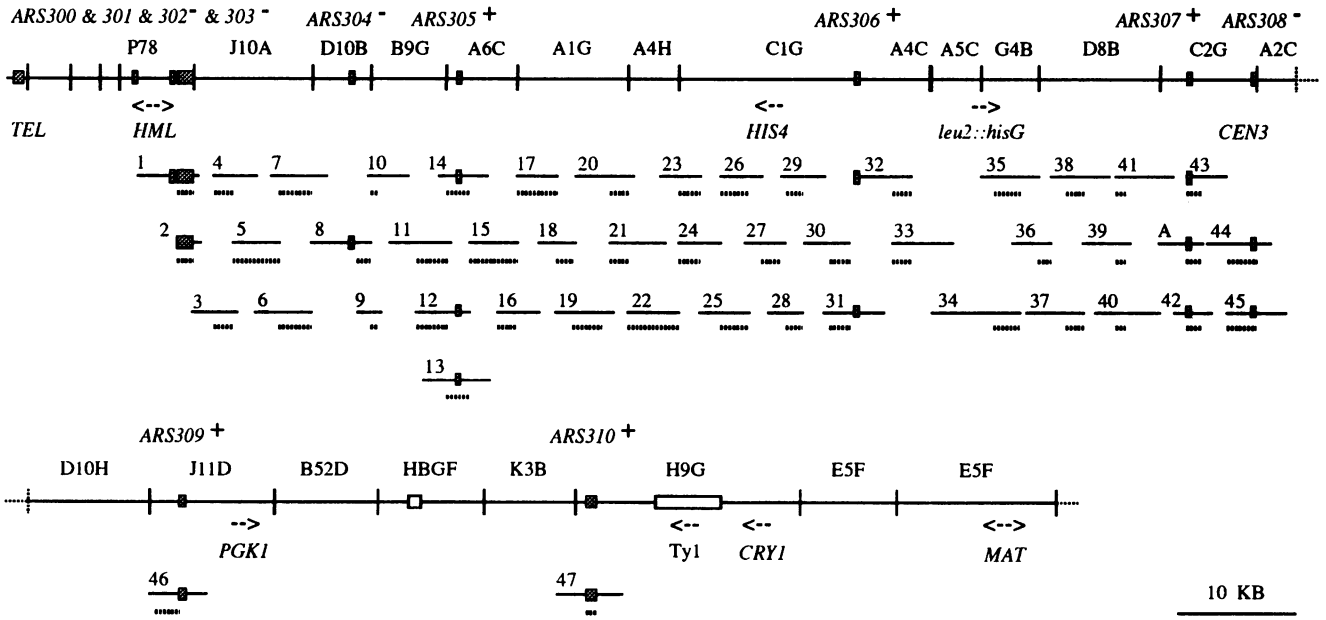

FIG. 4. Map of chromosome III. The 200-kb region from the left telomere to the *MAT* locus is shown. *Bam*HI sites are indicated by vertical lines. The names of the chromosome III clones (35) are given above the horizontal line. *ARS* elements (*ARS300* to *ARS310*) are represented as stippled boxes along the horizontal line. *ARS311*, *ARS312*, and *ARS313* are not indicated on the chromosome III map because their positions have not been precisely defined (35). A plus sign after an *ARS* element indicates that it acts as a meiotic chromosomal DNA replication origin, whereas a minus sign indicates that the *ARS* element has no detectable meiotic origin activity. The arrows below the horizontal line depict the locations of particular genes. The map shown is for strain NKY730, which differs from the published sequence for *S. cerevisiae* chromosome III in a number of regions (37). Fragments A5C and G4B differ because in strain NKY730, the Ty1-17 (Ty2) element is absent and *LEU2* is disrupted by the insertion of a 1.1-kb *Salmonella hisG* fragment which contains a *Bam*HI site and a *Dra*I site. In addition, in NKY730, the *Bam*HI site between A2C and D10H is absent and therefore is represented as a dotted vertical line. Fragment HBGF in NKY730 contains a 1.1-kb insertion located within the right-arm hot spot for Ty transposition (49). Also, in NKY730, the H9G fragment contains a 6.0-kb Ty1 element (2), which is absent in the strain used for the published sequence (37). The position of the Ty in H9G represents a new location for Ty insertion (23, 48–50). These two insertions are indicated as open boxes. The E5F region of NKY730 contains an extra *Bam*HI site not found in the published sequence (37). The solid lines labeled 1 through 47 indicate the fragments examined by 2-D gel analysis, and the dotted lines below them indicate the probe fragments used. The restriction enzyme sites that define each of these fragments are listed in Table 1.

along the ascending portion of the Y arc due to the accumulation of Y-shaped replication intermediates. The replication fork pause in mitosis correlated with centromere function and the ability of mutant centromeres to form a DNase I-resistant protein-DNA complex (18). Extending these observations to premeiotic S phase suggests that the protein-DNA complex necessary for centromere function is present during premeiotic DNA replication.

Analysis of premeiotic DNA replication intermediates of chromosome III. Our approach to mapping premeiotic replication origins was to examine replication intermediates of overlapping fragments covering a 100-kb segment of chromosome III. In this way it was possible not only to determine whether the *ARS* elements known to function as mitotic DNA replication origins are premeiotic DNA replication origins, but also to determine whether any of the *ARS* elements inactive as mitotic DNA replication origins are active as premeiotic replication origins and whether any DNA fragments without *ARS* function contain premeiotic origins.

When this work was begun, the sequence of chromosome III was not yet available (37). Thus, as a first step in planning these experiments, the restriction enzyme map of plasmids carrying sequences covering a 200-kb region of chromosome III (35) was determined for nine restriction enzymes: *Bam*HI, *Bgl*II, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Pst*I, *Xba*I, and *Xho*I (50). Southern analysis was used to make a comparison of the sites in the clones and in the strain used for these studies in the 190-kb region between the *Bam*HI site 9.7 kb from the left telomere and the *Bam*HI site to the right of the *MAT* locus.

Two classes of changes were identified. First, there were several insertions and deletions that changed the length of *Bam*HI fragments, and these changes are reflected in the chromosome III map shown in Fig. 4 and detailed in the figure legend. Second, there were individual restriction site polymorphisms which probably result from single-base-pair mutations that create or abolish a restriction site. The resolution of the Southern analysis used in these studies was several hundred base pairs. Therefore, fragments of less than 200 to 300 bp in length that were released by the digestion of adjacent restriction sites were not detected. In addition, a particular site could not be assayed in some cases because a probe for that region was not used. These sites were excluded from the analysis. Of the 437 resolvable restriction sites present in the 190-kb cloned region studied (9), 13 sites were absent in SK1, and 10 additional sites were present in SK1 in comparison with the published sequence (37).

For the analysis of overlapping chromosome III fragments, DNA was isolated from cultures 3 h after transfer to SPM, digested with one or more restriction enzymes, run on 2-D gels (5), and transferred to hybridization membranes, and the blots were probed to reveal replication intermediates in the fragments of interest. Mapping of origins requires the examination of overlapping restriction fragments for two reasons. First, analysis of overlapping fragments allows the position of an

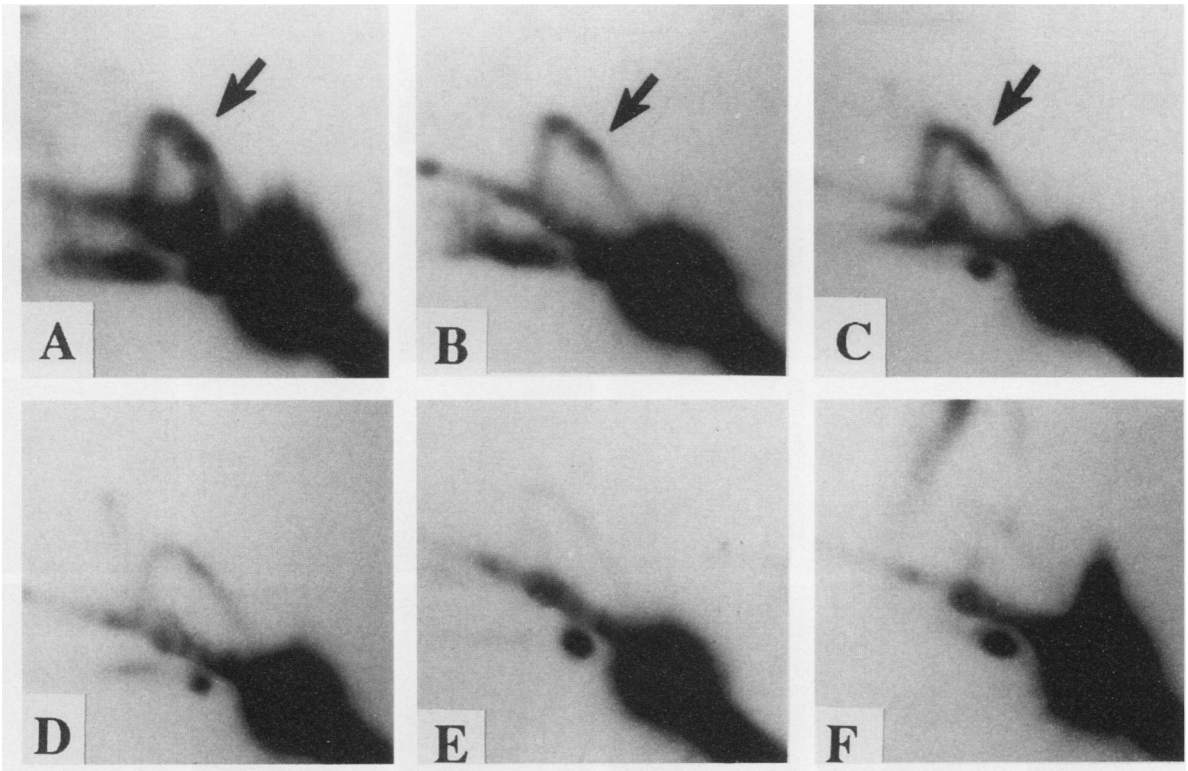

FIG. 5. Replication of *CEN3*. Samples were taken from YPAc and from SPM every 2 h for 12 h (12-h sample is not shown). 2-D gel analysis of fragment 45, containing *ARS308* and *CEN3*, is shown. The dark spot, indicated by the arrow, results from the accumulation of DNA replication intermediates due to replication forks pausing at *CEN3*. (A) YPAc sample at time zero; (B) SPM sample at 2 h; (C) SPM sample at 4 h; (D) SPM sample at 6 h; (E) SPM sample at 8 h; (F) SPM sample at 10 h. Refer to Fig. 4 for a map indicating the position of fragment 45 and to Table 1 for the restriction enzyme sites defining the fragment.

origin within a fragment to be determined. A DNA fragment replicated from an origin located in the middle of the fragment gives a bubble arc pattern on a 2-D gel. If the origin is off-center in a fragment, the pattern is discontinuous, with a transition from a bubble arc to a Y arc. Assuming that the forks emanating from an origin move at equal rates, the mass at which the bubble arc to Y arc transition occurs can be used to estimate the position of the origin relative to one end of the fragment. The analysis of overlapping fragments allows unambiguous placement of the origin. Second, overlapping restriction fragments must be examined because replication intermediates containing small bubbles are not well separated from those containing small Y's under the 2-D gel conditions used (Fig. 2). Therefore, an origin near one end of a fragment may not be detected because the bubble-to-Y transition occurs in the region of the pattern where the arcs are not well separated. Thus, many overlapping DNA fragments were analyzed in order to map origins in the 100-kb region of *S. cerevisiae* chromosome III studied (Fig. 4).

Premeiotic DNA replication origins. The 2-D gel analysis of 45 overlapping DNA fragments that cover the left arm of chromosome III between *HML* and *CEN3* is shown in Fig. 6. Inspection of the patterns reveals the presence of three efficient meiotic replication origins, each of which coincides with a mitotic replication origin.

The fragments shown in panels 12, 13, and 14 of Fig. 6 all contain *ARS305*. The clear bubble-to-Y arc transitions seen in panels 13 and 14 demonstrate the presence of a meiotic replication origin. The mass at which the transition occurs can be estimated by comparing the position of the transition with the migration of molecular weight standards run with the first-dimension gel. The positions of the transitions map the replication origin to *ARS305*. In panel 12 of Fig. 6, which shows the analysis of a 4.7-kb *Pst*I fragment in which *ARS305* is 1.1 kb from one end, it is difficult to see the bubble-to-Y arc transition (indicated by an arrow) for two reasons. First, the bubble and Y arcs containing early replication intermediates (i.e., the least-replicated molecules) are not as well resolved as the arcs containing late replication intermediates. Second, the probe used to detect the fragment is homologous to the region of the fragment that replicates last, so late replication intermediates in which the probe sequence has replicated hybridize more strongly than early replication intermediates.

The second meiotic origin, coincident with *ARS306*, is located in the middle of fragment 31 and at the very left end of fragment 32. The position of the bubble-to-Y transition and the observation that fragments 30 and 32 are both replicated as simple Y-shaped intermediates map the meiotic replication origin to *ARS306*.

The bubble-to-Y arc transition that identifies the third meiotic replication origin is shown in panel 42 of Fig. 6. The position of the transition in panel 42, the observation that fragment 43 is replicated as a simple Y, and the position of the transition in the fragment labeled A in Fig. 4 (data not shown) map the origin to *ARS307*.

ARS308, the fourth mitotic origin contained in this region, has been shown previously to initiate mitotic DNA replication 15 to 20% of the time in two other strains (17, 34). The presence of only Y-shaped DNA replication intermediates in the dark exposures of the 2-D gels of the *ARS308*-containing

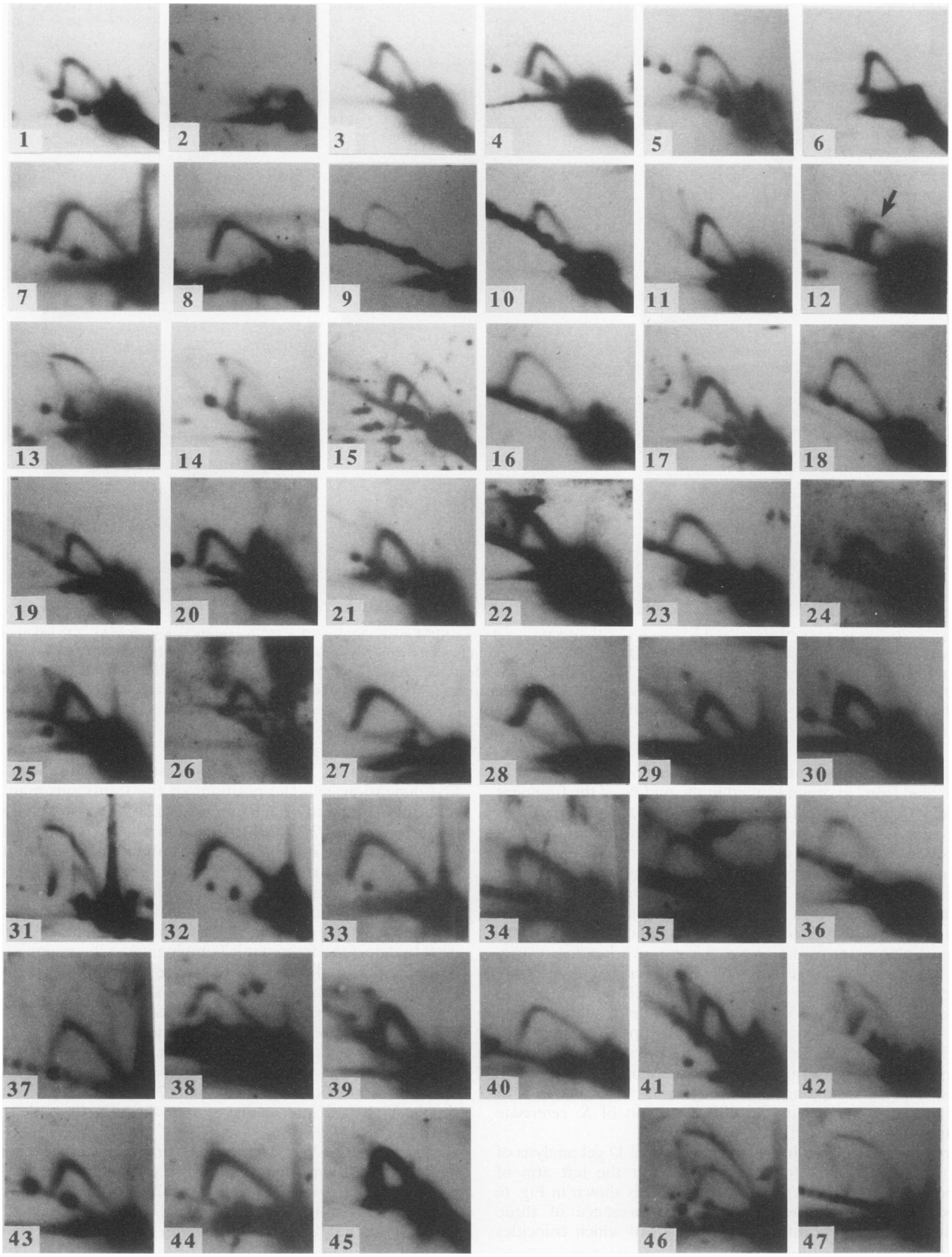

FIG. 6. 2-D gel analysis of chromosome III fragments. Forty-five overlapping DNA fragments (panels 1 to 45) in the 100-kb region between *HML* and *CEN3* were examined. Two additional fragments, which contain *ARS309* (panel 46) and *ARS310* (panel 47), were also analyzed. The DNA analyzed was prepared from samples harvested 3 h after the cells were transferred to SPM except for the samples in panels 5, 21, and 42, which were harvested after 4 h in SPM. Refer to Fig. 4 for a map indicating the positions of these DNA fragments and to Table 1 for a list of the restriction enzyme sites defining these fragments.

fragments (fragments 44 and 45) indicates that *ARS308* is not a meiotic origin. In addition, the fact that only a Y arc pattern was seen in the analysis of fragment 45 in mitotic cells (Fig. 5A) indicates that *ARS308* is not a strong mitotic origin in strain SK1, but a darker exposure is needed to determine whether *ARS308* functions as a weak mitotic origin. Thus, further analysis of *ARS308* in DNA isolated from mitotic cells will be required to determine whether the failure of *ARS308* to function as a meiotic replication origin reflects a difference between mitotic and meiotic replication or whether it is due to a difference in strain background.

Fragments containing the other two known mitotic origins (17, 34) were also examined. Fragment 46, which contains *ARS309*, and fragment 47, which contains *ARS310*, both gave bubble arc patterns, indicating that *ARS309* and *ARS310* are active as premeiotic origins (Fig. 6, panels 46 and 47).

Are other sequences active as meiotic replication origins? Inspection of the 2-D gel patterns obtained for all of the other chromosome III fragments analyzed reveals that none contains a bubble arc (Fig. 6). These results demonstrate that none of these fragments contains a premeiotic chromosomal replication origin. Of particular interest in this region were three *ARS* elements which are inactive as mitotic chromosomal replication origins (14, 34). Fragment 1, which contains both *ARS302* and *ARS303*, gives only a Y arc 2-D gel pattern, confirming that these *ARS* elements are not chromosomal origins in meiosis. In addition, the analysis of another fragment containing *ARS303* alone (fragment 2) revealed a faint arc of Y-shaped DNA replication intermediates, again suggesting that *ARS303* is not an origin in meiosis. Finally, only a Y arc pattern was seen for fragment 8, which contains *ARS304*, demonstrating that *ARS304* is not a premeiotic chromosomal origin. Therefore, neither *ARS302*, *ARS303*, nor *ARS304* is active as a chromosomal replication origin in either mitosis or meiosis. We conclude that in meiosis as well as mitosis, chromosomal replication origins are coincident with *ARS* elements, but not all *ARS* elements have chromosomal origin function.

DNA replication termination intermediates. DNA replication terminates when adjacent replication forks, moving toward each other, converge. Therefore, a fragment in which replication terminates is replicated by forks moving in from each end, giving rise to a double Y or H-shaped replication intermediate. If replication terminates at a specific site within a fragment, the replication intermediates fall along a single double-Y arc that arises either from the spot of linear nonreplicating fragments or from a specific site along the Y arc, giving rise to a Y-to-double-Y transition (5, 6, 26, 28, 29). On the other hand, if DNA replication forks meet at random throughout a fragment, as has been found for yeast mitotic DNA replication, then DNA termination would occur at various sites within the fragment, giving rise to a collection of double-Y-shaped replication intermediates. Nonspecific termination is seen as a diffuse triangle of hybridization extending from the late region of the Y arc, as shown in Fig. 2B (17, 54). The nonspecific termination signals are less intense than the Y arcs both because the signal is spread out over a larger area and because termination occurs in a region that spans several restriction fragments (see below).

The 2-D gel patterns presented in Fig. 6 show no evidence of specific termination signals, suggesting that replication must terminate nonspecifically. In the case of these meiotic DNA samples, the analysis of nonspecific termination is complicated somewhat by the presence of a spike of recombination intermediates in some samples (Fig. 2A and B). For example, panels 19 and 25 of Fig. 6 show a diffuse triangular termination signal extending upward and leftward from the late portion of the Y arc with no evident recombination spike. In contrast, fragment 11 shows a Y arc pattern and a recombination spike with no apparent termination signal. A few of the fragments show a Y arc and a termination signal along with a recombination spike. In this case, the left side of the termination triangle is more intense than the right side because of the added contribution of the recombination signal (fragments 17, 29, and 41).

Triangular termination signals are seen for many of the fragments located between replication origins (Fig. 6). Termination intermediates in fragments between *ARS305* and *ARS306* can be seen in panels 19 to 22 and 24 to 29 of Fig. 6. Termination intermediates in fragments between *ARS306* and *ARS307* can be seen in panels 34, 39, and 41. In every case, there is a strong complete Y arc along with the termination signal, indicating that, in most cells, each of these fragments is replicated by a single replication fork. Therefore, replication terminates within a given fragment in only a fraction of the population.

Interestingly, weak termination signals are apparent even in fragments adjacent to active origins, including fragment 15, which is positioned 1.0 kb to the right of *ARS305*; fragment 29, which is positioned 2.8 kb to the left of *ARS306*; and possibly fragment 30, which is positioned 0.4 kb to the left of *ARS306*. Therefore, a low level of nonspecific replication termination occurs over a very broad distance between two active origins. These results are consistent with observations made with mitotic yeast cells, in which termination occurred over a large region of the chromosome (17, 54).

Termination signals are not apparent in the DNA fragments located to the left of *ARS305* (Fig. 6, panels 1 to 11). These results suggest that this region is replicated by a fork that moves leftward from *ARS305* and that replication forks do not travel rightward between *HML* and *ARS305*. Although the region of chromosome III from the left telomere to *ARS301* was not examined directly in our study, it is likely that *ARS305*, the leftmost origin detected, is the leftmost origin in the chromosome. The absence of termination signals suggests that there are no premeiotic origins to the left of *ARS305*. Alternatively, if an origin exists in the unstudied region, then there must be a barrier which blocks rightward fork movement into the *HML* region.

DISCUSSION

Replication patterns in mitosis and meiosis. The data presented here demonstrate that the pattern of DNA replication origin usage on chromosome III in premeiotic S phase is identical to that found in mitotic S phase, with the possible exception of *ARS308*. *ARS305*, *ARS306*, *ARS307*, *ARS309*, and *ARS310* are chromosomal origins, while *ARS302*, *ARS303*, and *ARS304* do not function as chromosomal origins at detectable levels. Although *ARS300* and *ARS301* have not been examined directly, the failure to find termination intermediates to the left of *ARS305* suggests that these two *ARS* elements are also not meiotic origins. In neither mitosis nor meiosis have origins been found in DNA fragments without *ARS* function. Thus, chromosomal replication origins are coincident with *ARS* elements, but not all *ARS* elements have chromosomal origin function.

These results demonstrate that the proposal that *ARS* elements that are not active as chromosomal origins in the mitotic cell cycle might be meiotic origins is incorrect. Thus, it is still not clear what distinguishes *ARS* elements that are active as chromosomal origins from those that are not. Neither proximity to a telomere nor association with a silent mating

type cassette is sufficient to repress origin function (14). Because the *ARS* elements that are inactive as chromosomal replication origins are active as origins in plasmids, the *ARS* elements must contain sequences sufficient for origin function. Perhaps chromosomal context is the important determinant of origin function.

Not only is the pattern of origin usage the same in mitosis and meiosis, the apparent efficiency of origin use is also similar. The strong bubble arcs and weak early Y arcs seen in the analysis of *ARS305*, *ARS306*, *ARS307*, and *ARS310* (Fig. 6) confirm that they are efficient meiotic origins. These *ARS* elements function as efficient mitotic origins as well (12, 13, 17, 22, 54). In contrast, the meiotic analysis of *ARS309*, which is an efficient mitotic origin (12, 17), revealed a relatively intense complete Y arc along with the bubble arc (Fig. 6). The complete Y arc might be present because *ARS309* does not initiate DNA replication in every cell during meiosis. Alternatively, the Y arc could have arisen from the breakage at one fork of bubble-shaped replication intermediates, yielding Y-shaped molecules (16, 27). These two possibilities can be distinguished by determining the direction of replication fork movement through the regions flanking *ARS309* (16, 17).

Our study of nine chromosome III *ARS* elements revealed a coincidence between origins efficient in mitosis and meiosis. Consistent with our observations, a recent study in which an indirect assay of origin function was used revealed that *ARS1* is active as a meiotic origin as well as a mitotic origin (19). However, since only a small number of origins in meiosis have been studied, it is too early to conclude that all mitotic and meiotic origins are identical.

The similarity between meiotic and mitotic replication patterns also extends to replication termination. The broad regions of termination observed suggest that there are cell-to-cell differences in origin usage, the timing of initiation, the rate of replication fork movement, or all of these. For example, if a particular premeiotic origin does not initiate replication in a fraction of the population, then termination in these cells would occur at a novel position between the origins that are active, giving rise to faint termination signals for specific DNA fragments. This explanation is not likely to account for the broad regions of termination on the left arm of chromosome III because each of the origins on the left arm appears to be used efficiently. However, if the timing of initiation and/or the replication fork rate varies from cell to cell, then the position between the active origins where two replication forks converge would vary widely, producing a broad zone in which termination occurs in the population.

Finally, the analysis of overlapping fragments covering most of the left arm of chromosome III demonstrates that, as in mitotic S phase, there are few significant barriers to replication fork movement. Replication fork pause sites and replication fork barriers are seen as strong spots of accumulated replication intermediates at specific positions along Y arcs (6, 17, 18). The pause site associated with *CEN3* in mitotic S phase (18) is the only significant pause site apparent in meiotic S phase (Fig. 5 and 6). The Ty-associated pause site found on the left arm of chromosome III in mitotic S phase (17) was not examined because Ty1-17 is not present in the strain used for this study.

The fact that termination signals were not seen in the 2-D gel analysis of fragments to the left of *ARS305* suggests that replication forks do not travel rightward in the region from *HML* to *ARS305*. This is consistent with the idea that *ARS305*, the leftmost origin detected in our survey of meiotic origins along chromosome III from *HML* to *CEN3*, is indeed the leftmost origin. It is clear that *ARS305* is the leftmost mitotic origin on chromosome III. The mitotic replication timing data demonstrate increasingly later times of replication for fragments to the left of *ARS305* (40). In addition, neutral-alkaline 2-D gel analysis (21), which determines the direction of replication fork movement, revealed only forks moving leftward from *ARS305* toward the telomere during mitotic S phase (20). Finally, direct examination with neutral-neutral 2-D gels of *ARS301*, *ARS302*, *ARS303*, and *ARS304* revealed that none of these *ARS* elements is detectably active as a mitotic origin (14, 34).

Why is premeiotic S phase longer than mitotic S phase? Several hypotheses could account for the longer duration of meiotic S phase than of mitotic S phase in *S. cerevisiae*. If the DNA replication fork rate were slower in meiosis, a longer S phase would be required. This explanation is not correct, because the rates of replication fork movement are indistinguishable in mitotic S phase (reviewed in reference 33) and meiotic S phase (24), and forks move bidirectionally from replication origins in both cases.

A second hypothesis suggests that the increased length of meiotic S phase is the result of an increased distance between active origins in meiosis. Previous studies have shown that the average spacing between origins is the same in mitosis and meiosis (reviewed in references 24 and 33). The analysis presented here confirms these conclusions and further demonstrates that the same DNA sequences are used as origins during mitosis and meiosis. Therefore, the proposal that there are fewer meiotic origins is not supported by these data.

A third idea is that the longer meiotic S phase could result from a staggering of replication initiation in meiosis, either of origins on a single chromosome or of initiation of different chromosomes. It is clear that there is a temporal order of chromosomal replication initiation in mitotic S phase, with origins initiating throughout S phase (reviewed in reference 4). The time interval between the initiation of the earliest origin and the initiation of the latest origin studied on chromosome III is 12 min (40), but the time interval between the earliest origin studied (*ARS305*) and the latest origin studied (the *KEX2 ARS*) is 28 min (4). The intervals between samples in the meiotic time course presented here were not sufficiently short to detect differences in the time of replication of any sequences on chromosome III. However, our observations that the efficiency of origin use and the pattern of termination are similar in meiosis and mitosis suggest that replication initiation events on chromosome III are not more staggered in meiosis than in mitosis. If initiation events were more staggered, then in the absence of specific replication terminators, forks from the early-initiating origins would be expected to replicate more DNA before encountering a fork emanating from an adjacent late-initiating origin. This would be expected to result in a shift in replication termination patterns. In addition, if a fork from an early-initiating origin replicated through an adjacent origin before it initiated, then a decreased efficiency of use would be expected for the late-activated origin. Since four of the five meiotic origins studied are clearly utilized efficiently, it is unlikely that replication initiation events are more staggered in meiosis than in mitosis. If our results for chromosome III are applicable to the rest of the genome, then the replication of different chromosomes rather than initiation events on a single chromosome must be staggered to account for a longer meiotic S phase.

An alternative explanation for the failure to find clear evidence supporting a longer meiotic S phase is that the estimate of the length of the meiotic S phase is incorrect. The single estimate available is based on an analysis of the frequency of cells incorporating a radioactive precursor into DNA

as a function of time during sporulation and reasonable but untested assumptions about the constancy of the length of S phase and the rate of entry into and exit from S phase (53).

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