Replication of Double-Stranded RNA of the Virus-Like Particles in *Saccharomyces cerevisiae*†

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The mode of replication of the L double-stranded RNA (dsRNA) present in virus-like particles in *Saccharomyces cerevisiae* was examined by density transfer experiments. After transfer to light medium, significant amounts of fully heavy dsRNA persisted over a number of cell doublings. In addition, very little material of hybrid density was ever formed, and the accumulation of fully light material began as early as 0.5 doubling after transfer to light medium. Our results are compatible with a conservative mode of replication or with a semiconservative mode of replication carried out by a small portion of the total dsRNA population. In additional experiments the synthesis of dsRNA relative to the cell cycle was studied. This was done by determining the ratio of short-term to long-term radioactive label in size-separated cell fractions of a prelabeled exponential culture. The ratio of short-term to long-term label remained constant for all fractions, implying that dsRNA is synthesized throughout the cell cycle, increasing through the cell cycle at an exponential rate.

Virus-like particles (VLPs) containing double-stranded RNA (dsRNA) have been found in many fungi (16, 27). The yeast *Saccharomyces cerevisiae* has been shown to contain two separately encapsulated dsRNA's (14), the L and the M species. The L, the larger of the two with a molecular weight of 3.5×10^6 (17), is present in many laboratory strains of *S. cerevisiae* (8, 14) and has been shown to encode the major coat protein of the VLPs (18). The smaller M dsRNA, with a molecular weight of 1.2×10^6 to 1.7×10^6 (4, 31, 34, 29), encodes killer toxin (6), which kills sensitive strains lacking the M dsRNA (4). The VLPs, for which no infectious cycle has been demonstrated, are cytoplasmically inherited (4).

Much remains to be learned about the way in which the dsRNA of the VLPs replicates, although an RNA transcriptase has been shown to be associated with these particles (15, 32). Replication cannot be rigidly coupled with the cell cycle since the number of copies of dsRNA per cell varies with growth conditions (22).

Among other dsRNA genomes, that of the animal virus reovirus has been best studied (for review, see reference 28). Its replication has been shown to be fully conservative and sequential,

that is, one of the two strands (the plus strand) is always synthesized before the other (the minus strand). In *S. cerevisiae*, Bevan and Herring (3) have reported what they believe to be encapsulated replicative intermediates and an associated dsRNA polymerase activity. They consider their results to be compatible with a conservation mode of replication like that of reovirus. On the other hand, Buck (7) has reported semiconservative in vitro synthesis with a VLP-associated dsRNA polymerase activity in the fungus *Penicillium chrysogenum*.

In this report, the replication of L dsRNA in *S. cerevisiae* was examined in ¹⁵N-¹⁴N-labeled medium transfer experiments. Replication was also studied relative to the cell cycle by using radioactive label to determine the rate of synthesis of L dsRNA in size-separated cell fractions of an exponential culture.

MATERIALS AND METHODS

Strains. For the density transfer experiments the S7 strain of *S. cerevisiae* was used. This strain, which contains elevated amounts of L, but no M, dsRNA, has been described previously (22). For the cell cycle experiments the diploid strain SKQ2n was used. This strain has also been previously described (11).

Growth and labeling of cells. For the density transfer experiments an exponential culture of cells uniformly labeled with ¹⁵N was prepared by growing cells for at least six doublings on a rotary shaker at 23°C in yeast nitrogen base (YNB) minus amino acids

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and $(\text{NH}_4)_2\text{SO}_4$ (24; Difco Supplement, 1968), with $(^{15}\text{NH}_4)_2\text{SO}_4$ added to a concentration of 0.1 mg/ml. When the culture reached a concentration of 10^7 cells per ml (early logarithmic growth) as determined by optical density readings, $(^{14}\text{NH}_4)_2\text{SO}_4$ was added to a concentration of 5 mg/ml, and the fully heavy (HH; 0 doublings in light medium) sample was immediately chilled. Other portions of the culture were appropriately diluted in YNB plus 5 mg of $(^{14}\text{NH}_4)_2\text{SO}_4$ per ml such that they would have undergone a requisite number of doublings once they again reached a concentration of 10^7 cells per ml. Upon reaching this concentration each sample was immediately chilled by the addition of ice.

For the cell cycle experiments, cells were grown to 2×10^7 cells per ml on a rotary shaker at 23°C in a medium containing, per liter, 6.7 g of YNB minus amino acids (Difco), 21 mg each of all amino acids except methionine and cysteine, 20 g of glucose, 10 mg of adenine, and 10 mg of uracil. The medium was buffered at pH 5.8 with 10 g of succinate and 6 g of sodium hydroxide per liter. A dual-label method was used to determine the relative rate of synthesis of the dsRNA. Cells were labeled for 3 h (1.5 generations) with 0.5 μCi of $[2\text{-}^{14}\text{C}]\text{uracil}$ per ml and pulse-labeled with 5 μCi of $[5\text{-}^3\text{H}]\text{uracil}$ per ml for 10 min (0.08 generation). Incorporation was stopped by the addition of ice to the medium.

Cell cycle fractionation. Cells pulse-labeled and long-term labeled as described above were immediately separated into size classes representing different positions in the cell cycle by centrifugal elutriation as described previously (11).

RNA isolation. For the cell cycle experiments total RNA was prepared as previously described (11). The L dsRNA was separated from other RNAs on 2.6% polyacrylamide gels according to the method of Loening (20). For the density transfer experiments the replication of both total dsRNA and the dsRNA from purified VLPs was examined. For the isolation of total dsRNA, total nucleic acid was extracted with phenol-cresol essentially by the method of Kirby (19). This was then subjected to LiCl fractionation according to the method of Diaz-Ruiz and Kaper (10). This involves precipitating rRNA with 2 M LiCl, then precipitating dsRNA from the 2 M LiCl supernatant with 4 M LiCl, leaving small single-stranded RNA (ssRNA) and DNA in the supernatant. The isolated dsRNA was checked for purity on 2.6% and 8% polyacrylamide gels according to the method of Loening (20). To isolate dsRNA from purified VLPs, the VLP purification was carried out as described previously (22). This involved banding the VLPs in a CsCl gradient, followed by further purification by sucrose gradient centrifugation. The VLPs were lysed *in situ* in ultracentrifugation buffer with Sarkosyl as previously described (17), releasing the dsRNA for density analysis. The density marker tobacco mosaic virus (TMV) RNA was prepared by the method described for isolating RNA from VLPs (22).

Determination of radioactive incorporation. In the cell cycle experiments the 2.6% polyacrylamide gels upon which the RNA had been fractionated were first scanned at 260 nm to determine the positions of the various RNA peaks. They were then frozen in dry

ice and sliced into 1-mm sections. RNA in the gel slices was solubilized in 0.3 ml of 50% Protosol at 23°C for 24 h. The amount of radioactivity in the gel slices was determined by scintillation counting in 4.5 ml of toluene-based scintillation fluid. The rate of synthesis of dsRNA for each cell size fraction was determined by calculating the ratio of pulse to long-term counts in gel slices from the dsRNA peak region of the gel. This gave a measure of the relative rate of dsRNA synthesis.

Ultracentrifugation. Ultracentrifugation of density-labeled total dsRNA and of dsRNA from lysed VLPs was carried out as described previously (17). This involved equilibrium sedimentation in a Cs_2SO_4 density gradient with an average density of 1.61 g/ml in a Beckman model E analytical centrifuge equipped with a UV scanner. When a density marker was required, TMV RNA was run with the sample. This forms a sharp precipitate band with a density of 1.64 g/ml (9).

Computer analysis of model E scans. To quantitate the percentage of the dsRNA banding at the fully heavy (HH), hybrid (HL), and fully light (LL) positions in the centrifuge scans of the total cellular dsRNA, these scans were subjected to computer analysis. The scans were first projected onto a Tektronix 4956 graphics tablet (Tektronix Corp., Beaverton, Oreg.), to be digitized for analysis by a Tektronix 4051 computer. The position of the TMV marker peak, corresponding to a density of 1.640 g/ml (9), was used to determine the relation between density and position in the sample cell by using the data of Ludlum and Warner (21), which take into account the nonlinear nature of a Cs_2SO_4 gradient. The density of HH dsRNA was determined from the 0-generation sample run with TMV as a marker and the density of LL dsRNA from the 5-generation sample run with TMV. The HL dsRNA was assumed to have a density midway between these two. The 0-generation and 5-generation samples were also used to determine the widths of individual peaks within an HH-HL-LL mixture since each represented a homogeneous sample of a single density. The peaks were assumed to be Gaussian, a shape which fit both peaks well, with each having the same width. Using the densities and peak widths of the 0-generation and 5-generation samples as standards, a least-squares fit was made to all of the other samples, which had also been run with TMV, assuming that each was made up of variable amounts of dsRNA of HH, HL, and LL densities. A typical density distribution (that for 2.5 generations) is shown in Fig. 1A. This distribution, along with a superimposed "best fit" calculated density distribution, is shown in Fig. 1B. The calculated positions corresponding to material of HH, HL, and LL densities are also shown. Although the TMV peak is not shown here, its position was used in assessing the density distribution as described above.

Because of concern for a significant overlap between material at the HH position and a non-Gaussian tail of the TMV, computer analyses were also made of the same samples run in the ultracentrifuge without TMV. In this case the peak widths and peak separations determined from the runs with TMV were used. Care was taken to make the average density of these gra-

FIG. 1. Computer analysis of density profiles of 2.5-generation dsRNA in a density transfer experiment. (A) Density profile of the 2.5-generation sample centrifuged with TMV RNA; (B) this profile (solid line) with a superimposed computer fit (dotted line). For this fit the TMV peak region has been removed from the data, but the position of the TMV was used in determining the position of material of HH, HL, and LL densities as described in the text. The computer analysis indicated 29% HH, 7% HL, and 64% LL. (C) Density profile for the same material centrifuged without TMV; (D) the computer fit to this profile. As described in the text, this fit was made using parameters established from samples run with TMV. Computer analysis indicated 25% HH, 6% HL, and 69% LL. The numbers showing actual densities apply to (B) and (D) only and are those calculated by the computer analysis of (A) and (C).

dients as close as possible to those run with TMV to justify the use of peak parameters determined from runs with TMV. The absolute position of the set of peaks and their relative amplitudes were free parameters in the fit of the data without TMV. It was found that the absolute position parameter was very sharply defined by the fit, indicating that letting this be a free parameter was probably a sound procedure. A typical density distribution for a sample run without TMV (again, that for 2.5 generations), along with a calculated "best fit" density distribution, is shown in Fig. 1C and 1D.

Materials. Optical-grade Cs_2SO_4 was purchased from E. M. Laboratories, Inc. (Elmsford, N.Y.). The $(^{15}\text{NH}_4)_2\text{SO}_4$ was obtained from Merck Sharp & Dohme, Ltd. (Montreal, Canada) and contained nitrogen at 98.5 atoms % ^{15}N . $[2\text{-}^{14}\text{C}]\text{uracil}$ and $[5\text{-}^3\text{H}]\text{uracil}$ were purchased from Schwarz/Mann.

RESULTS

Pattern of replication of total dsRNA in density transfer experiments. Cells uni-

formly labeled with ^{15}N were transferred to a ^{14}N medium, and samples were taken after 0, 0.5, 1, 1.5, 2.0, 2.5, 3.0, and 5.0 generations in light medium. Total dsRNA was extracted from each of these samples, and representative samples, those for 1.5 and 2.5 generations, were checked for purity on 2.6 and 8% polyacrylamide gels (20). No detectable contamination with any other RNA species was found (data not shown). A portion of each purified sample was centrifuged to equilibrium in a Cs_2SO_4 density gradient with TMV RNA as a density marker. The results of this centrifugation can be seen in Fig. 2. The positions shown as HH and LL correspond to the positions at which the 0-generation and 5-generation samples banded, the 0-generation sample being fully heavy and the 5-generation sample being virtually fully light. It is evident that significant amounts of material banding at a fully heavy position persisted for a number of generations. It is also evident that at no time was material with a hybrid density the predominant dsRNA species. A small but significant amount of dsRNA of a hybrid density was, however, produced. This may be seen in Fig. 3, where the density distribution for the 1.5-generation

FIG. 2. Cs_2SO_4 equilibrium density centrifugation of total dsRNA from a ^{15}N - ^{14}N density transfer experiment. Number of generations, shown in the upper left of each density profile, represents the number of cellular doublings in the ^{14}N medium after transfer. TMV RNA, with a density of 1.640 g/ml, served as a density marker. In this figure all the plots have been aligned with respect to their TMV peak. The positions of the 0-generation sample and the 5-generation sample relative to TMV have been designated HH and LL, respectively.

FIG. 3. Comparison of the density profile of 1.5-generation dsRNA (solid line) with that of a mixture (dashed line) of 0-generation (HH) and 5-generation (LL) dsRNA from a density transfer experiment. The two curves have been normalized to give equal peak amplitudes.

sample is superimposed on that for a mixture of pure HH (0-generation) and pure LL (5-generation) material.

To determine the amount of dsRNA banding at the HH, HL, and LL positions in each of the samples, the model E density profiles were subjected to computer analysis as described in Materials and Methods. As described, the densities of HH and LL were determined from the 0- and 5-generation samples. These were found to be 1.623 and 1.609 g/ml, respectively. The proportion of HH, HL, and LL dsRNA as a function of number of generations in light medium, as determined by the computer fit, is shown as the dashed lines in Fig. 4.

Because there was an overlap between a non-Gaussian tail of the TMV peak and the HH region of the dsRNA, as may be seen in Fig. 2, all samples were also centrifuged without TMV, and the density distribution of the centrifuge scans was subjected to computer analysis. The conditions of centrifugation were the same as those with TMV, and the computer analysis, as described in Materials and Methods, was based on parameters established from samples run with TMV. A comparison of the sample run without TMV with that run with TMV (Fig. 1) shows the better fit of the calculated curve to the experimental data in the HH region of the curve. The results of the analysis of samples run without TMV are shown as the solid lines in Fig. 4. It may be seen that the percentages calculated are in good agreement. Because of the peak overlap it is felt that the solid line (TMV-free data) is the more reliable. As may be seen from this figure, the amount of material banding at a hybrid density was maximal between 0.5 and 1.0 generations but never rose above about 18%.

The fully heavy material disappeared gradually, and its disappearance was mirrored by a gradual rise in the fully light material.

As a test of the computer program's ability to determine the amount of material of true hybrid density, the computer was presented with a scan of the density distribution resulting from mixing 0-generation and 5-generation samples (Fig. 5). These samples represent virtually pure HH and LL material, respectively, and ideally a computer fit to the mixed distribution should indicate no hybrid component. The actual fit indicated a 4% hybrid component, giving an indication of the uncertainty in component percentages to be expected in fits to experimental data. Figure 5A shows the computer fit to the mixed sample, and Fig. 5B shows a fit to the same data constrained to 0% hybrid component. An example of the quality of the computer fit to data which may be expected to have a significant hybrid component is presented in Fig. 6. Shown in the figure are fits to the 0.5-generation data with the hybrid percentage parameter either left free (Fig. 6A) or constrained to zero (Fig. 6B). The fit indicates an 18% hybrid component. In this case no independent estimate of the true hybrid percentage is available, but it may be seen from the figure that the computer fit to the

FIG. 4. Percentages of HH, HL, and LL dsRNA calculated by computer analysis of the density profiles of total dsRNA in a density transfer experiment. Dashed and solid lines are the results for material centrifuged with and without TMV, respectively.

FIG. 5. Computer fit to density profile of a mixture of 0-generation (HH) and 5-generation material, as a test of the computer program's ability to assess the proportions of HH, HL, and LL in experimental samples. The mixed sample is one with virtually no HL material. The computer fit (A) indicated 4% HL with 48% each of HH and LL. A fit constrained to 0% HL is shown in (B). Here HH and LL were each determined to be 50%. Data are represented by a solid line, and the computer fit is indicated by a dotted line.

FIG. 6. Computer fit to density profile of 0.5-generation dsRNA from a density transfer experiment, a sample which shows significant HL material. (A) Standard computer fit; (B) fit constrained to 0% HL. Data are represented by a solid line and the computer fit is given by a dotted line. (A) HH = 71%, HL = 18%, and LL = 11%. (B) HH = 83% and LL = 17%.

shape of the experimental distribution is qualitatively excellent.

Pattern of replication of dsRNA isolated from VLPs. With the expectation that encapsulated dsRNA might represent a subset of the total dsRNA population with a different replication pattern from that shown by the dsRNA as a whole, dsRNA was extracted from purified VLPs obtained from cells which had been subjected to a ^{15}N - ^{14}N -medium transfer. Conditions of growth were the same as those described for total dsRNA isolation except that instead of YNB the similar Wickerham minimal medium (33) was used. Samples were taken after 0, 0.5, 1.0, 1.5, and 2.0 generations in light medium.

The VLPs were purified from these samples as described in Materials and Methods. The VLPs were lysed in situ in the ultracentrifuge buffer with Sarkosyl as described. With the exception of the Sarkosyl, conditions of equilibrium sedimentation were the same as those for total dsRNA. The results of this centrifugation are shown in Fig. 7. As may be seen, the density distribution of the encapsulated dsRNA closely resembled that of the dsRNA as a whole, implying that its replication pattern does not differ greatly from that of the total dsRNA. Just as with total dsRNA, computer analysis showed little hybrid material at any time and the appearance of fully light material as early as 0.5 generation after transfer to light medium.

Synthesis of dsRNA through the cell cycle. An exponential cell culture was labeled for 3 h (1.5 generations) with [^{14}C]uracil and then pulse-labeled for 10 min (0.08 generation) with [^3H]uracil. The cells were then size separated by elutriation centrifugation, and the RNA from each elutriation sample was fractionated on 2.6% polyacrylamide gels as described in Materials and Methods. A typical optical density scan at 260 nm of such a gel showing 25S and 18S rRNA as well as the dsRNA may be seen in Fig. 8. The

FIG. 7. Equilibrium density centrifugation of dsRNA isolated from VLPs in a density transfer experiment. The number of cell doublings after density transfer is shown on each profile. All plots have been aligned with respect to their TMV peaks. HH denotes the position of the 0-generation sample relative to the TMV peak. LL represents an estimation of the position of LL material relative to TMV, based on the 2-generation sample and a 5-generation sample from another experiment.

FIG. 8. Absorbance at 260 nm (A_{260}) trace of RNA on 2.6% polyacrylamide gels showing position of L dsRNA relative to 25S and 18S rRNA.

ratio of counts in short-term and long-term label for the L dsRNA region of the gel for each sample was determined as described. The ratio of counts remained constant through the cell cycle (Fig. 9). This indicates that L dsRNA is synthesized throughout the cell cycle. The fact that the ratio remained constant implies that the rate of synthesis is always proportional to the amount already present and hence that the rate of synthesis increases exponentially through the cell cycle.

DISCUSSION

Our density transfer study of the replication of the dsRNA of *S. cerevisiae* has shown (i) a persistence of significant amounts of fully heavy dsRNA over a number of cellular generations, (ii) very little material of a hybrid density at any time, and (iii) the accumulation of fully light material beginning as early as 0.5 generation after transfer to light medium. This pattern was observed for both total and encapsulated dsRNA. Radioactive labeling experiments of size-separated cells have shown that the dsRNA is replicated continuously throughout the cell cycle at a rate that increases exponentially through the cell cycle.

These results may now be compared with those expected assuming various models for the replication of dsRNA. A number of semiconservative models will be examined, as well as the possibility of conservative replication.

Semiconservative—uniform model. In this model each molecule replicates exactly once per cell cycle. If all the molecules replicate at

the same specific time within the cell cycle, then in a density transfer experiment all molecules should be of hybrid density after one cell doubling in light medium. The circular 2 μ DNA plasmid, whose synthesis is limited to the S phase, has been shown to replicate by this mode (36). Such a replication scheme is clearly inconsistent with our own data, both because synthesis of dsRNA was shown to continue throughout the cell cycle and because the amount of hybrid material we obtained was so small. A somewhat different result would be expected for a uniform model in which replication can occur at any time during the cell cycle even though each molecule replicates exactly once. Zakian et al. (36) have calculated that if the probability of synthesis is uniform throughout the cell cycle, after one generation the expected density distribution would be 11.5% HH, 77.0% HL, and 11.5% LL. This distribution is also very different from what we observed: 53% HH, 17% HL, and 30% LL. This difference would become even greater if Zakian's calculation were to be modified to take account of the observed fact that the rate of dsRNA synthesis increases through the cell cycle rather than being uniform.

Semiconservative—random model. In

FIG. 9. Synthesis of dsRNA through the cell cycle. Cells long-term labeled with [^{14}C]uracil for 3 h and pulse-labeled with [^3H]uracil for 10 min were size separated by centrifugal elutriation. The RNA in each cell size fraction was purified and then fractionated on 2.6% polyacrylamide gels. The $^3\text{H}/^{14}\text{C}$ ratio was determined for the dsRNA peak region of the polyacrylamide gel for each sample. The flow rate represented is that of the elutriation rotor. Cell cycle position is based on previous results (11). Abbreviations: ids, initiation of DNA synthesis; be, bud emergence; cds, completion of DNA synthesis; nm, nuclear migration.

this model molecules are chosen at random for replication regardless of their previous replication history. Thus, whereas there is an average of one replication per molecule for each cellular doubling, some molecules may replicate twice whereas others don't replicate at all. The random pattern of replication is that which, with the exception of 2μ circles, has been commonly observed for multiple-copy genetic elements including several bacterial plasmids (2, 13, 26) and mitochondrial DNA in mouse L cells (5). Mitochondrial DNA of *S. cerevisiae* is also believed to replicate by this mechanism, although results are somewhat obscured by what appears to be extensive recombination (35). If the dsRNA replicated by such a mechanism, and the probability per unit time for a given dsRNA to replicate were constant, then the exponential increase which we observed in the rate of dsRNA synthesis through the cell cycle would be the expected result. However, this model predicts 25% HH, 50% HL, and 25% LL after one generation in light medium (26), again a distribution which disagrees strongly with our results. Since recombination among the dsRNA molecules would, if anything, contribute to the proportion of dsRNA molecules banding at a hybrid density at one generation, any recombination taking place would serve to make the above models even more unlikely.

Semiconservative—small replicating population. In this model a small, rapidly replicating portion of the population is responsible for all the replication that takes place. One such scheme is the rolling-circle model (12). Both the late replication of phage λ (1) and the amplification replication of *Xenopus* extrachromosomal ribosomal genes (25) have been shown to take place by such a mechanism. Any scheme in which a large proportion of the progeny molecules did not participate in further replications would be characterized by small amounts of hybrid material and relatively large amounts of HH and LL in a density transfer experiment, a result like our own. The HL material should, however, be made at the expense of the HH material in any sort of semiconservative replication mechanism. We saw no evidence that our HH material was disappearing any faster than would be expected by dilution as the dsRNA population replicated in light medium, a point to which we shall return in more detail in discussing a conservative mode of replication. However, the change in HH material expected in such a model is small, and we consider the precision of our determination of fully heavy and hybrid fractions (Fig. 5) to be insufficient to definitively rule this model out. Furthermore,

recent in vitro evidence indicates that transcription of the dsRNA genomes of the bacteriophage $\phi 6$ (30) and the fungal VLP AfV-S (24) occurs by a semiconservative displacement mechanism, with the newly synthesized strand displacing one of the two parental strands. It is not known whether this simply represents messenger synthesis or is the first step in a sequential replication process in which the second strand is subsequently synthesized on the displaced parental strand. Such a mechanism of synthesis for dsRNA replication in fungi has been proposed by Buck (7). If such a semiconservative sequential replication process occurs in *S. cerevisiae*, our data indicate that only a small proportion of the VLPs could be involved in replication.

Conservative. This model would include any scheme of replication in which the parental dsRNA does not become a part of daughter molecules. The well-characterized dsRNA virus reovirus has been shown to replicate conservatively, with the two strands being synthesized asynchronously (28). If *S. cerevisiae* dsRNA replicates by a similar asynchronous mechanism, then HL material would represent dsRNA in which the first but not the second of the two strands had been synthesized before transfer to light medium. The HH, representing parental material, should simply decrease by dilution, i.e., exponentially. Figure 10 shows (i) the theoretical

FIG. 10. Percentages of HH dsRNA: (----) that predicted by a conservative mode of replication in a density transfer experiment; (Δ) that actually observed for total dsRNA in density transfer experiments; and ($\blacktriangle$) that observed, corrected for the fact that the dsRNA was not replicating as rapidly as the yeast cells as indicated by the yield data shown in Table 1.

expected percentages of HH dsRNA through successive generations based on a conservative model and (ii) the actual percentages obtained. As may be seen, the decrease in percentage of dsRNA was approximately that which would be expected by dilution, though the rate of decrease was somewhat less than would be predicted. This deviation from the expected value can be accounted for by the fact that the dsRNA was replicating somewhat more slowly than the *S. cerevisiae* cells themselves. The amount of purified dsRNA recovered from a fixed number of cells decreased slightly with each successive sample until 3 generations after transfer to light medium (Table 1). The relative amount of dsRNA has been shown to vary with growth conditions (22), and it is assumed that changing from a medium with 0.1 mg of $(^{15}\text{NH}_4)_2\text{SO}_4$ per ml to one with 5 mg of $(^{14}\text{NH}_4)_2\text{SO}_4$ per ml was responsible for the effect observed. When the percentage of HH was corrected for the fact that it was being diluted out in a smaller total dsRNA pool, the results shown by the second experimental curve (c) in Fig. 10 were obtained. It may be seen that this corrected curve agrees quite well with the theoretical expectation of a conservative model, making this seem the model which best fits our data. Since the correction for growth rate introduced here is small, it should not significantly affect arguments made about the plausibility of either the uniform or the random models of semiconservative replication.

Among other data relating to dsRNA replication in fungi, evidence has been obtained supporting both conservative and semiconservative replication. Buck (7) has found that in an in vitro system with the capsid-associated polymerase of PsV-S particles of *Penicillium chrysogenum*, replication is apparently semiconservative. In a density transfer experiment, replication of encapsulated fully heavy dsRNA associated with a small piece of ssRNA led exclusively to the production of hybrid dsRNA with no fully

heavy material remaining. On the other hand, Bevan and Herring (3) have obtained evidence that the *S. cerevisiae* L dsRNA may replicate in a manner similar to that of reovirus dsRNA. Among VLPs from logarithmically growing cells, but not stationary cells, they have found particles which sediment somewhat more slowly in sucrose gradients than the main VLP band. Polyacrylamide gel electrophoresis with and without RNase digestion of ssRNA indicated that these particles contained dsRNA of less than unit length associated with some single-stranded material. These are the sorts of replicative intermediate that would be expected if the particles were in the process of synthesizing a second strand upon the first, as in reovirus. However, such replicative intermediates would also occur during second-strand synthesis with sequential semiconservative replication. Preliminary in vitro studies of the polymerase activity associated with these particles implied a sequential mode of RNA synthesis. Whereas this is consistent with a replication mechanism like that of reovirus, it is also consistent with semiconservative sequential replication.

In conclusion, we find that our data fit well with a conservative mode of replication, but can also be interpreted in terms of a semiconservative mode of replication by a small portion of the total dsRNA population.

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TABLE 1. Recovery of purified dsRNA as a function of cell generation in density transfer experiments

Generations in ^{14}N medium	dsRNA recovered from 10^{10} cells (μg) ^a
0	49
0.5	44
1.0	43
1.5	40
2.0	36
2.5	32
3.0	28
5.0	27

^a Based on absorbancy at 260 nm of the purified samples.

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