

Macromolecular Synthesis in *Saccharomyces cerevisiae* in Different Growth Media

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Synthesis of ribonucleic acid (RNA), deoxyribonucleic acid (DNA), and protein was determined in *Saccharomyces cerevisiae* during amino acid and pyrimidine starvation and during shift-up and shift-down conditions. During amino acid starvation, cell mass, cell number, and RNA continued to increase for varying periods. During amino acid and pyrimidine starvation, cell mass and RNA showed little increase, whereas total DNA increased 11 to 17%. After a shift from broth medium to a minimal defined medium, increase in RNA and protein remained at the preshift rate before assuming a lower rate. DNA increase remained at an intermediate rate during shift-down, and then dropped to a low rate. During shift-up from minimal to broth medium, increase in cell number, protein, and DNA showed varying lag periods before increasing to the new rate characteristic of broth medium; each of these quantities exhibited a step sometime in the first 2 hr after transfer to rich medium, suggesting a partial synchronous division. Immediately after shift-up, RNA synthesis assumed a high rate, and then dropped to a rate characteristic of growth in the rich medium after about 1 hr.

In the past decade, much work has been done on the dynamics of bacterial growth and the control of macromolecular synthesis in bacteria. For cells in balanced growth, the content of various cellular constituents and their relationships to each other vary as a function of the growth rate; the cell may show a continuum of physiological states, depending on the growth rate supported by the medium. Schaechter et al. (12) have shown that in *Salmonella typhimurium*, cell mass, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) content, and the number of nuclei per cell during balanced growth can be expressed as exponential functions of the growth rate.

During a transition between different states of balanced growth, the close relationship maintained among the syntheses of various macromolecular constituents of the cell is temporarily disrupted. In this period, the rates of synthesis of the different classes of macromolecules are dissociated until their quantitative relationships are reestablished at a ratio characteristic of balanced growth in the new environment. By observing the pattern of synthesis of cell components in a transition between different steady-state conditions, some information can be obtained about the manner in which their synthesis is controlled. During a shift-up from a medium supporting a low growth rate to one supporting a higher rate,

the synthesis of RNA, DNA, protein, and cell mass exhibits a characteristic pattern which is independent of the magnitude of the shift (4). The rate of RNA synthesis is immediately increased to a high value and subsequently is reestablished at a rate characteristic of the new medium (3); there is a preferential synthesis of ribosomal protein during this period (13). Increases in DNA, protein, and colony counts exhibit varying lag periods during which they remain at the preshift rate. In a shift-down from a medium supporting a high growth rate to one supporting a low rate, net increases in cell mass and RNA immediately cease, while cell number and DNA continue to increase at the preshift rate for a period before dropping to a lower rate (4). The amount of increase in DNA during conditions under which protein and RNA synthesis is inhibited is consistent with the idea that, upon shift-down, all rounds of DNA replication in progress are completed at the preshift rate and that protein synthesis is required for the initiation of new rounds. Maaløe and Hanawalt (8) and Hanawalt et al. (1) have shown that during amino acid starvation there is at least a 40% increase in total DNA; in this period the fraction of cells synthesizing DNA gradually decreases. Moreover, incubation under conditions which inhibit RNA and protein synthesis is accompanied by a gradual increase in the

fraction of cells immune to thymineless death, suggesting that cells complete replication rounds in progress but require the addition of amino acids to reinitiate DNA replication. The experiments of Lark et al. (6) confirmed the hypothesis that, in *Escherichia coli*, DNA synthesis during amino acid starvation represents continuation of rounds in progress at the time of amino acid deprivation to a definite terminus; upon readdition of amino acids, new rounds are initiated at a definite origin which is recognized after subsequent periods of amino acid starvation.

Saccharomyces cerevisiae provides an opportunity to study the relationships among the macromolecular components of the cell under varying growth conditions in a eukaryotic system. In contrast to bacteria, yeasts possess a defined nucleus with as many as 18 genetic linkage groups (9). In addition, the generation time can be varied by the selection of different growth media. It was the purpose of this investigation to observe the effects of amino acid starvation and shift-up and shift-down conditions on DNA, RNA, and protein synthesis and cell growth by using a strain of *S. cerevisiae* requiring two amino acids, a purine, and a pyrimidine.

MATERIALS AND METHODS

Organism and growth conditions. *S. cerevisiae* strain 5015-D, a haploid yeast requiring methionine, tryptophan, adenine, and uracil, was used in all experiments. Cells were grown aerobically in Wickerham's complete medium (15), Wickerham's minimal medium (15) supplemented with the various requirements, or in broth medium (2% glucose, 1% tryptone, 0.5% yeast extract). For measurement of adenine incorporation, the medium was supplemented with 5 μg of adenine-8- ^{14}C /ml (specific activity 0.0125 $\mu\text{C}/\mu\text{g}$; New England Nuclear Corp., Boston, Mass.) Cells were grown from a loop inoculum in 100 ml of medium in a 250-ml flask for 18 hr on a reciprocal shaker at 30 C, then harvested by centrifugation, and washed with 0.1 M phosphate buffer (pH 6.6). For growth studies, cells were resuspended in 50 to 300 ml of fresh medium in 250-ml or 1-liter flasks and incubated at 30 C on a Dubnoff metabolic shaking incubator. Transfer of cultures to different media was accomplished by collecting the cells on Bac-T-Flex membrane filters (Carl Schleicher and Schuell Co., Keene, N.H.), then washing the cells with 1 liter of prewarmed medium, and suspending them in new medium.

Measurement of macromolecular constituents. For determination of total protein, cell samples were washed with 0.1 M phosphate buffer (pH 6.6), hot 5% perchloric acid, and finally with 95% ethyl alcohol. Protein was extracted from the residue with 1 N NaOH for 10 min at 100 C. Protein in the extract was measured by the method of Lowry et al. (7).

For determination of total DNA, cell samples were washed with phosphate buffer (pH 6.6) followed by

cold 5% trichloroacetic acid. Lipid was extracted twice with hot 95% ethyl alcohol, and DNA was determined in the residue by using a modified version of the Kissane and Robins fluorometric procedure (2).

Total RNA was determined either colorimetrically or by incorporation of adenine-8- ^{14}C . For colorimetric determinations, cell samples were washed twice with cold 0.1 M phosphate buffer (pH 6.6), three times with cold 5% trichloroacetic acid, and twice with 95% ETOH. Nucleic acids were extracted from the residue with 5% perchloric acid at 70 C for 15 min; RNA in the extract was determined by the orcinol method. For measurement of the adenine- ^{14}C incorporated, samples were suspended in cold trichloroacetic acid to a final concentration of 5%; cells were collected on membrane filters and washed with cold 5% trichloroacetic acid. Filters were placed in scintillation vials and dried; 5 ml of scintillation fluid [3.0 g of 2,5-diphenyloxazole + 0.1 g of 1,4-bis-2-(5-phenyloxazole)-benzene per liter of toluene] was added, and radioactivity was determined by use of a Packard Tri-Carb liquid scintillation spectrometer.

Growth of cultures was followed by determination of total cell number by using a model F Coulter counter; cell clumps were broken up by sonic treatment of samples for 30 sec with a Branson cell disruptor. In some experiments, growth was followed by the increase in optical density at 520 nm with a Beckman DU spectrophotometer. All chemicals were reagent grade obtained from commercial sources.

RESULTS

Total adenine- ^{14}C incorporation as a measure of total RNA synthesis. Since some difficulty was encountered in achieving reproducible results by using the orcinol method to determine total RNA content in growth studies, it was decided to use incorporation of adenine-8- ^{14}C as a measure of total RNA synthesis. An overnight culture of strain 5015-D grown in Wickerham's minimal medium supplemented with methionine, tryptophan, uracil, and adenine was transferred to medium of the same composition but containing 5 μg of adenine- ^{14}C /ml. After 3 and 6 hr of aerobic incubation, samples were taken to determine total trichloroacetic acid-precipitable radioactivity and radioactivity released by 12 hr of incubation in 1 N NaOH. The results showed that 2.9% of the trichloroacetic acid-precipitable radioactivity was resistant to 1 N NaOH. The ratio of NaOH-resistant radioactivity to acid-precipitable radioactivity is in good agreement with the DNA/RNA ratio of cells grown in supplemented Wickerham's minimal medium as determined by colorimetric determinations of RNA and DNA. In a second experiment, an overnight culture of 5015-D grown in Wickerham's complete medium was transferred to Wickerham's complete medium with adenine- ^{14}C and incubated at 30 C. After 4 hr of incubation,

samples were taken to determine total trichloroacetic acid-precipitable radioactivity and the radioactivity released by incubation in 5% perchloric acid at 90 C for 20 min. Results showed that 0.9% of the total cold acid-precipitable counts were resistant to hot perchloric acid. These experiments suggest that about 98% of the total cold acid-precipitable radioactivity can be accounted for as RNA; in the remaining experiments, incorporation of adenine- ^{14}C into the cold acid-precipitable fraction was considered to reflect total RNA synthesis. Measurement of DNA synthesis by NaOH-resistant incorporation of labeled adenine gave ambiguous results either by acidification of the NaOH digestion or by neutralization followed by adsorption of DNA to membrane filters in the presence of $6\times$ saline-sodium citrate. The values obtained by the fluorimetric procedure of Kissane and Robins were more consistent, and this method was used in succeeding experiments.

Macromolecular constituents of strain 5015-D at different growth rates. To determine the RNA, DNA, and protein content of strain 5015-D at different growth rates, overnight cultures of yeast were grown in Wickerham's minimal medium supplemented with methionine, tryptophan, adenine, and uracil, Wickerham's complete medium, or broth medium; they were then washed and resuspended in fresh medium. The cultures were monitored until well into logarithmic growth, and samples were taken for measurement of the various constituents and for total cell counts (Table 1). The total amount of RNA, DNA, and protein per cell, as measured by the procedures described above, increased with increasing growth rate (except for protein content of cells grown in the synthetic media). The values reported here are higher than those of Ogur et al. (10), perhaps owing to strain differences and different methods of analysis.

Amino acid and pyrimidine starvation of strain 5015-D. To determine the effects of amino acid and pyrimidine starvation on various cellular constituents of cultures in logarithmic growth in complete medium, a series of experiments was carried out in which increases in absorbancy, cell numbers, and RNA, DNA, and protein were measured during starvation for methionine, tryptophan, and uracil. Figure 1 shows the effect on increase in optical density at 520 nm of starvation for one or more of the required amino acids and uracil. Overnight cultures of 5015-D grown in Wickerham's complete medium containing 0.1% Casamino Acids were washed and resuspended in fresh medium. When cultures were in logarithmic growth, the cells were rapidly transferred to flasks of prewarmed Wickerham's

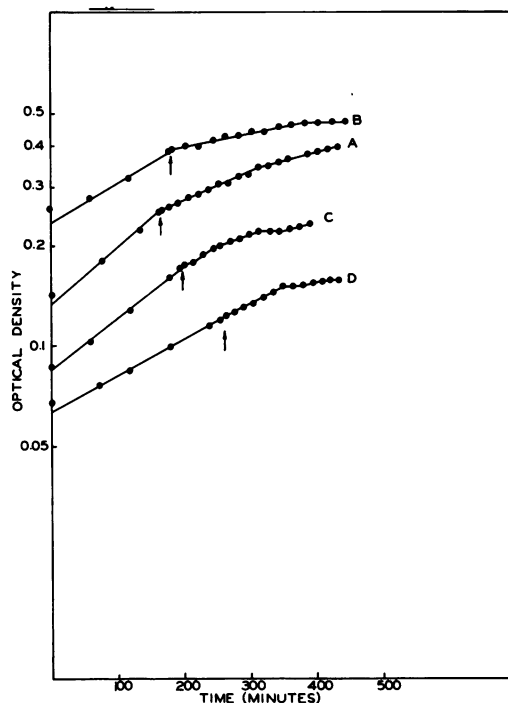


FIG. 1. Effect of amino acid and pyrimidine starvation on the growth of strain 5015-D. (A) Transfer to Wickerham's minimal + adenine + tryptophan (starvation for methionine and uracil). (B) Transfer to Wickerham's minimal + adenine (starvation for methionine, tryptophan, and uracil). (C) Transfer to Wickerham's minimal + adenine + uracil (starvation for methionine and tryptophan). (D) Transfer to Wickerham's minimal + uracil + adenine + tryptophan (starvation for methionine). Arrow indicates time of transfer from Wickerham's complete + 0.1% Casamino Acids to supplemented Wickerham's minimal medium.

minimal medium supplemented with: adenine, adenine + tryptophan, adenine + uracil, or adenine + uracil + tryptophan. In all cases, cell mass continued to increase for a considerable period after transfer. Immediately after transfer, the rate of increase dropped by 50% in the cultures starved for methionine + uracil and for methionine + tryptophan; during methionine starvation the rate showed only a slight decrease initially, whereas the rate dropped 66% in the culture starved for uracil + tryptophan. After various periods of incubation (1.5 to 2.5 hr) in the deficient media, the rates of increase again dropped abruptly; the final rates were about the same in all cultures, except in the culture starved for methionine, tryptophan, and uracil, in which increase in cell mass ceased after 3 hr.

In a second experiment, the effect of amino

acid starvation on increase in total cell number and total adenine incorporation was investigated. Overnight cultures of 5015-D grown in Wickerham's complete medium were washed and resuspended in fresh medium; when cultures were in logarithmic growth, they were rapidly transferred to Wickerham's minimal medium supplemented with all requirements except methionine, tryptophan, or methionine and tryptophan (Fig. 2). During methionine starvation, the rate of increase of total cell number dropped about 20% immediately after transfer to the deficient medium; after 1.5 hr of incubation the rate again decreased about 80%. Total adenine incorporation continued at a high rate for about 45 min, then gradually leveled off until, after 2.5 hr of incubation, adenine incorporation ceased. Total counts incorporated during 4 hr of methionine starvation increased by 140%. During tryptophan starvation, increase in total cell number dropped to a low rate after 1 hr of starvation. Similarly, adenine incorporation continued at a high rate for about 30 min and then assumed a low rate for the balance of the experiment. During 4 hr of tryptophan starvation, total counts incorporated increased by about 30%. During starvation for both methionine and tryptophan, increase in cell number assumed a low rate immediately after transfer; the rate was again reduced after 2 hr of starvation. The rate of adenine incorporation

continued at a high rate initially; after 30 min of incubation, adenine incorporation ceased.

Figure 3 shows the effect of tryptophan, adenine, and uracil starvation of a culture of 5015-D grown on broth medium. An overnight culture of yeast grown in broth medium was washed and resuspended in fresh medium; when the culture was in logarithmic growth, cells were rapidly transferred to Wickerham's minimal medium with methionine. Increases in optical density and total RNA immediately ceased after transfer. However, DNA continued to increase at approximately the same rate during the first 30 min of starvation and showed no further increase for the remainder of the incubation period. Total increase in DNA during starvation for tryptophan, adenine, and uracil was about 11%.

The effect of starvation of 5015-D for methionine, tryptophan, and uracil on RNA, DNA, and protein synthesis is shown in Fig. 4. An overnight culture of yeast grown in Wickerham's complete medium was washed and resuspended in fresh medium; when the culture was in logarithmic growth it was rapidly transferred to Wickerham's minimal medium containing adenine. After transfer to the deficient medium, increase in RNA and protein ceased immediately; cell mass increased at a lower rate. For the first 10 to 15 min after transfer, DNA increase seemed to continue at about the same rate; it then

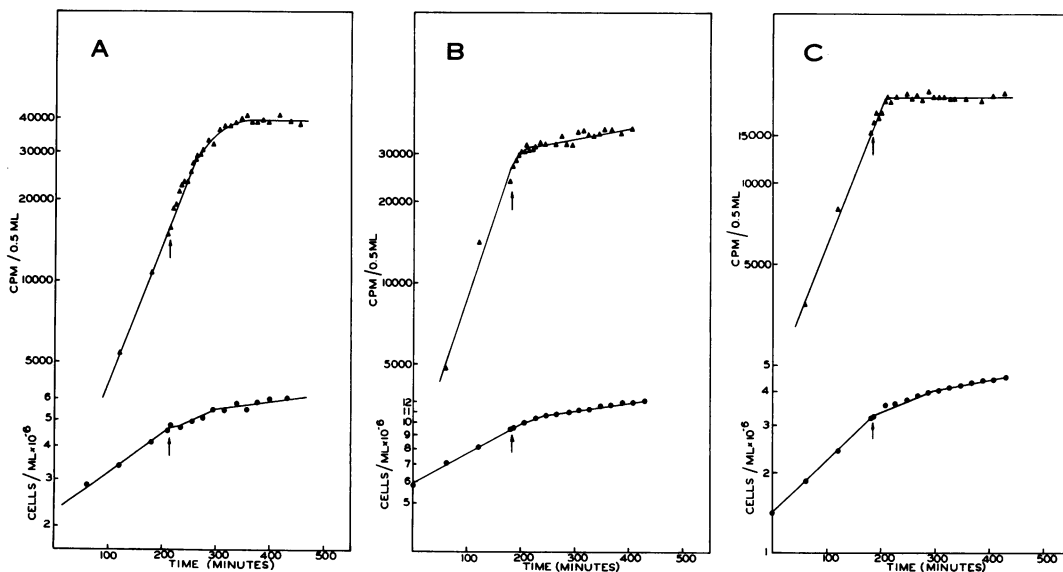


FIG. 2. Effect of amino acid starvation on cell count and RNA synthesis. (A) Transfer to Wickerham's minimal medium + adenine + uracil + tryptophan (starvation for methionine). (B) Transfer to Wickerham's minimal + adenine + uracil + methionine (starvation for tryptophan). (C) Transfer to Wickerham's minimal + adenine + uracil (starvation for methionine and tryptophan). Symbols: (●) cell count, (▲) RNA synthesis. Arrow indicates time of transfer from Wickerham's complete to supplemented Wickerham's minimal medium.

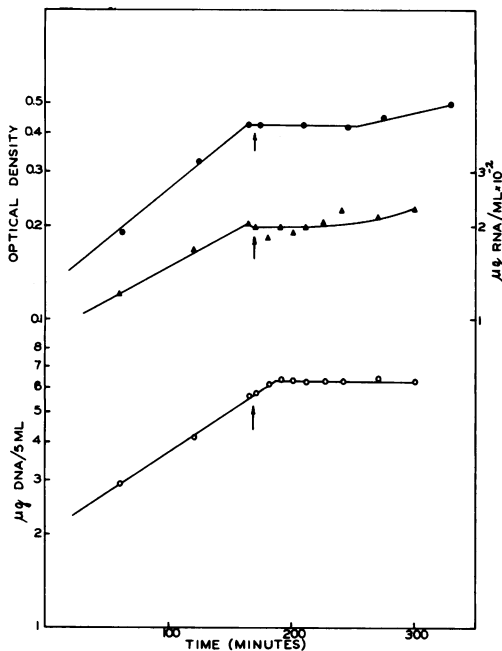


FIG. 3. Synthesis of macromolecular constituents during starvation for tryptophan, adenine, and uracil. Symbols: (●) optical density, (▲) RNA synthesis, (○) DNA synthesis. Arrow indicates time of transfer from broth medium to Wickerham's minimal medium + methionine.

dropped to a lower rate for the rest of the incubation period. Total increase in DNA during the incubation period was about 17%.

Macromolecular synthesis during shift-down conditions. The amount of DNA, RNA, and protein per cell varied as a function of the growth rate (Table 1). On the basis of these results, the effect of macromolecular synthesis of shifting a culture growing logarithmically at one rate to a medium supporting a different growth rate was investigated. In the first case, the effect on RNA, DNA, and protein synthesis was observed during a shift-down from broth medium to Wickerham's minimal medium supplemented with methionine, adenine, tryptophan, and uracil. An overnight culture of 5015-D grown in broth medium was washed and resuspended in fresh medium. When the culture was in logarithmic growth, cells were rapidly transferred to Wickerham's minimal + methionine, tryptophan, adenine, and uracil (Fig. 5). Increase in total cell counts showed a consistent pattern similar to that observed in the amino acid starvation experiments; the rate of increase of cell number dropped 60%, remained constant for the first 120 min after shift-down, and then assumed a lower rate. The rate of ade-

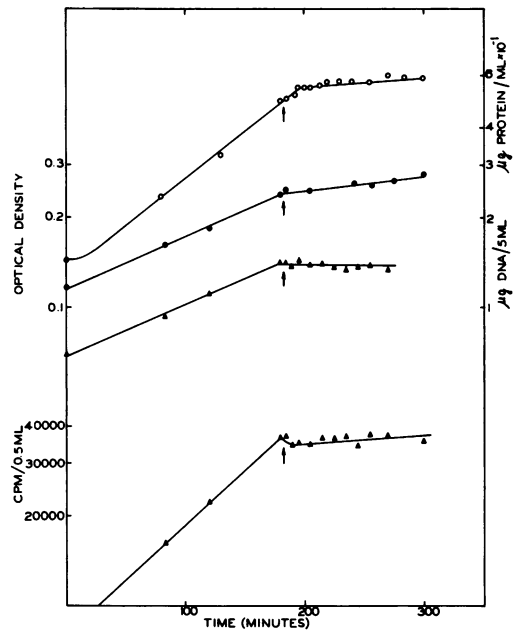


FIG. 4. Synthesis of macromolecular constituents during starvation for methionine, tryptophan, and uracil. Symbols: (●) optical density, (▲) protein synthesis, (▲) RNA synthesis, (○) DNA synthesis. Arrow indicates time of transfer from Wickerham's complete to Wickerham's minimal + adenine.

nine incorporation remained close to the preshift rate for the first 30 min after transfer and then gradually decreased over the next 60 min of incubation in minimal medium. During the remainder of the incubation time, adenine incorporation was roughly linear at a low rate. After transfer to minimal medium, the rate of increase in total DNA immediately dropped to about 14% of the rate in broth medium; after 60 min of incubation, increase in DNA exhibited a brief lag and then continued at a reduced rate during the rest of the incubation period. Increase in total protein during shift-down conditions resembles increase in total cell number; the rate of increase dropped immediately upon transfer and was again reduced after 2 hr of incubation.

Macromolecular synthesis during shift-up conditions. In a similar set of experiments, the effect on RNA, DNA, and protein synthesis was investigated during transfer of yeast growing at a low growth rate to media supporting a rapid growth rate. Figure 6 shows the results of a shift-up from Wickerham's minimal medium supplemented with methionine, tryptophan, adenine, and uracil to Wickerham's complete medium. Increase in cell numbers showed a very brief lag, if any, before being reestablished at a slightly higher rate.

TABLE 1. *Macromolecular constituents of strain 5015-D at different growth rates*

Growth medium	Generation time	Protein		RNA ^a		DNA	
		Amt	Dry wt ^b	Amt	Dry wt ^b	Amt	Dry wt ^b
	min	μg/cell	%	μg/cell	%	μg/cell	%
Broth	95	5.12×10^{-6}	32.8	56.02×10^{-7}	35.8	1.17×10^{-7}	0.75
Wickerham's complete	200	3.78×10^{-6}	24.2	30.89×10^{-7}	19.8	0.88×10^{-7}	0.56
Wickerham's minimal + methionine, tryptophan, adenine, and uracil	296	3.83×10^{-6}	24.5	23.19×10^{-7}	14.9	0.61×10^{-7}	0.39

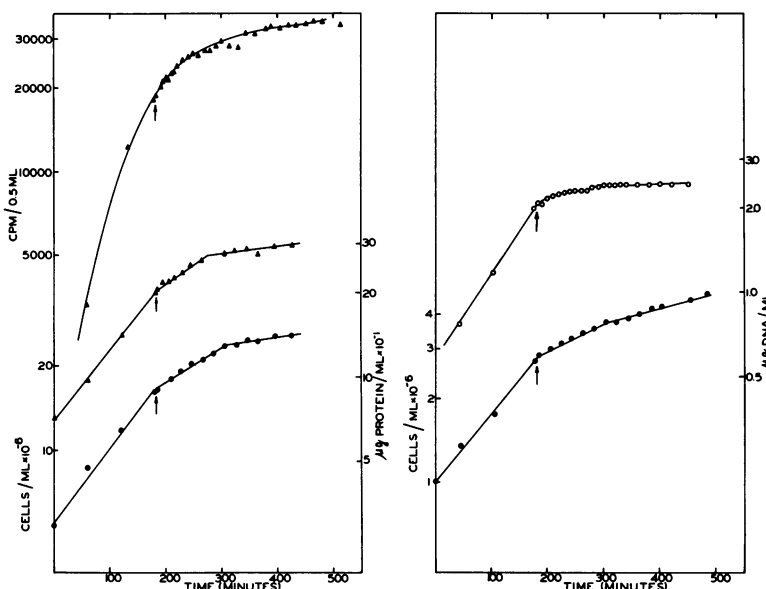
^a Corrected for DNA.^b Based on 1 mg (dry weight) of cells = 6.41×10^7 cells.

FIG. 5. Shift-down from broth medium to Wickerham's minimal medium + adenine + uracil + methionine + tryptophan. Symbols: (●) cell count, (Δ) protein synthesis, (▲) RNA synthesis, (○) DNA synthesis. Arrow indicates time of transfer.

There is some suggestion of a plateau 55 min after transfer, but the results are not unequivocal. In general, the small differences in generation times and rate of increase of cell constituents suggest that the shift in growth conditions is small. Upon transfer to Wickerham's complete medium, the rate of adenine incorporation immediately shifted to a slightly higher rate and remained at that rate during the rest of the experiment; no lag in the rate of adenine incorporation during shift-up was observed. There seemed to be a 40-min period, after transfer to complete medium, during which the rate of protein synthesis lagged; it then increased to a higher rate.

The rate of DNA synthesis showed a definite 30-min lag after transfer to Wickerham's complete medium and then abruptly resumed at a higher rate.

After shift-up from Wickerham's minimal + methionine, tryptophan, adenine, and uracil to broth medium, increase in protein, DNA, and cell number showed varying lag periods before assuming a higher rate, and all exhibited a stepwise increase during the first 1.5 hr after transfer to broth medium (Fig. 7). Increase in total protein gradually increased during the first 40 min after transfer and then became linear. At 1 hr after shift-up, there was a 30-min lag in protein

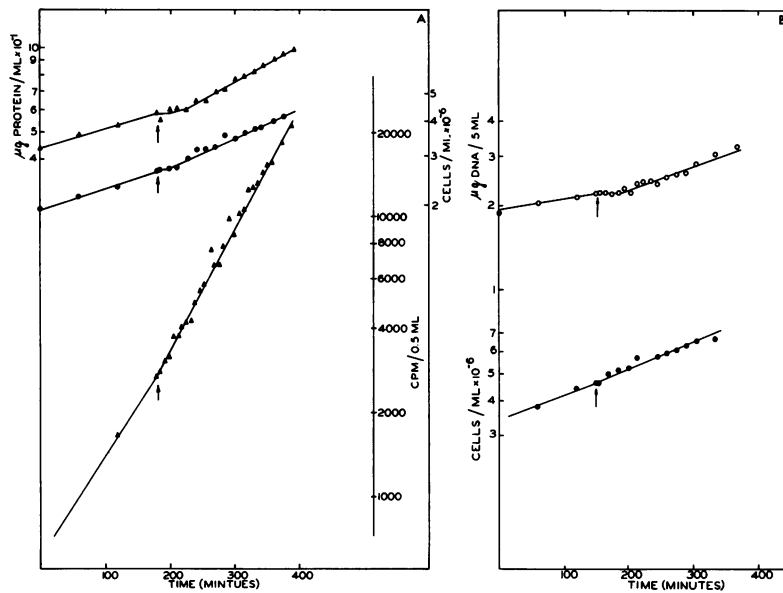


FIG. 6. Shift-up from Wickerham's minimal medium + methionine + tryptophan + adenine + uracil to Wickerham's complete medium. Symbols: (●) cell count, (△) protein synthesis, (▲) RNA synthesis, (○) DNA synthesis. Arrow indicates time of transfer.

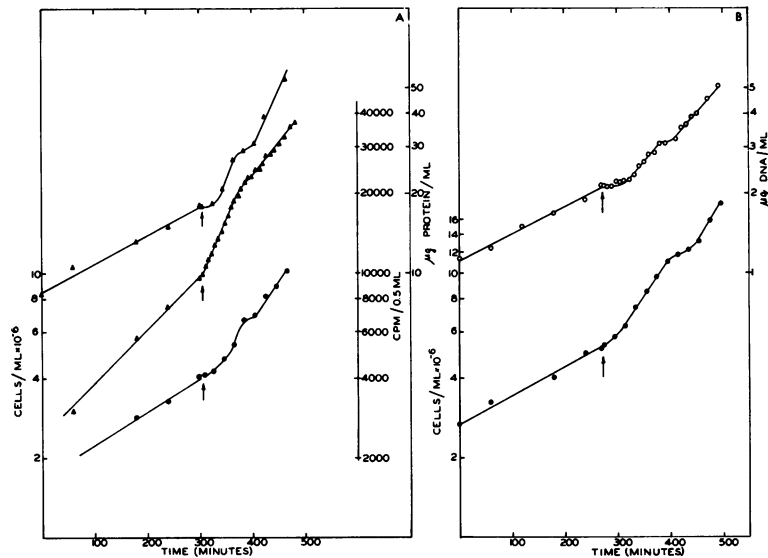


FIG. 7. Shift-up from Wickerham's minimal medium + methionine + tryptophan + adenine + uracil to broth medium. Symbols: (●) cell count, (△) protein synthesis, (▲) RNA synthesis, (○) DNA synthesis. Arrow indicates time of transfer.

synthesis after which it resumed at the previous rate. Similarly, increase in cell count showed a gradual increase to the post-shift rate; 75 min after transfer, it leveled off for 20 min and then resumed at the previous rate. Increase in DNA ex-

hibited a 50-min lag after shift-up (perhaps remaining at the preshift rate) and assumed a higher rate for the next 50 min. After 100 min of incubation in broth medium, there was a 30-min lag in the rate of DNA increase after which it

assumed the earlier rate. In contrast to the behavior of other cellular constituents during shift-up, the rate of RNA synthesis increased dramatically and remained at a higher rate for the first hour after shift-up. After this time the rate abruptly dropped about 50% and remained constant for the rest of the experiment. The decrease in the rate of adenine incorporation occurred simultaneously with the step in cell increase. The final rate of adenine incorporation in broth medium was only slightly higher than that in supplemented Wickerham's minimal; this was probably caused by dilution of the label in the broth medium.

DISCUSSION

During the starvation of *S. cerevisiae* for amino acids or amino acids and uracil, cell mass increased for a considerable period (as much as a 50% increase over 4.5 hr of incubation). Similarly, striking increases in cell count and adenine incorporation were noted during amino acid and uracil starvation. Yeasts are known to possess large intracellular amino acid pools and may under some circumstances accumulate exogenous amino acids up to 1000-fold (14); the continued synthesis of RNA during amino acid starvation may represent exhaustion of such intracellular pools. It is of interest that the greatest residual RNA synthesis (140% increase in adenine incorporated during the first 135 min of incubation) was observed during methionine starvation. It is known that yeast can accumulate high levels of methionine as *S*-adenosylmethionine, which is thought to be unavailable to the cell under normal growth conditions (11). The results would suggest that the cell has a relatively larger available pool of methionine than of tryptophan. During starvation for methionine, tryptophan, and uracil, however, adenine incorporation immediately ceased; the amount of total RNA may, in fact, have dropped slightly (see Fig. 4). During starvation for tryptophan, uracil, and adenine, cell mass and RNA showed little increase; on the other hand, DNA increased at the original rate for a brief period before leveling off. Total increase in DNA was about 11% during starvation for adenine, uracil, and tryptophan, and about 17% during starvation for uracil, methionine, and tryptophan. Williamson (17) showed that, in yeast growing at 25 C with a doubling time of about 2 hr, DNA synthesis occupies about the first 27% of the cell cycle. If roughly 30% of the cells in a random population were engaged in DNA synthesis at the time of transfer to the amino acid-deficient media, the observed increase in DNA during amino acid and pyrimidine starvation would be consistent with the idea that

all cells engaged in DNA synthesis will continue, whereas older cells in the population will not initiate a new cycle of DNA synthesis. It cannot be determined from the data whether cells actively synthesizing DNA at the time of amino acid starvation continue to the completion of a full complement of DNA or complete only a fraction of the genome.

In a shift-down from broth medium to supplemented Wickerham's minimal medium, adenine incorporation and increase in total protein remained at the preshift rate but gradually leveled off to a rate lower than that characteristic for cells in balanced growth in minimal medium. The initial high rate may, again, represent a large intracellular amino acid pool so that, within the cell, actual shift-down conditions are not reached until the pools have been depleted by incubation in minimal medium. Upon transfer to minimal medium, the rate of increase in cell number showed an immediate drop to a rate comparable to that of cells in balanced growth in minimal medium, but after 2 hr of incubation abruptly underwent a second drop to a much lower rate. In general, there seems to be a lag in response among all three quantities, after which the rates of increase drop to a low value; the condition of balanced growth in minimal medium was not re-established during the course of the experiments. In contrast to the results of the amino acid starvation experiments, the rate of DNA synthesis during shift-down from broth medium to minimal medium immediately dropped to a low value. When total DNA increased about 10%, there was a brief lag before DNA synthesis resumed at a lower rate. Here again, the rate characteristic of balanced growth in minimal medium was not attained. However, the increase in total DNA prior to assumption of final low rate was the same as that observed during tryptophan, adenine, and uracil starvation (Fig. 3).

Although there was little difference between the pre- and post-shift rates of increase in RNA, DNA, protein, and cell number during a shift-up from supplemented Wickerham's minimal to supplemented Wickerham's complete medium, it is of interest that all quantities except RNA exhibited a slight lag period after shift-up. The rate of RNA synthesis immediately assumed a higher rate which was maintained for the balance of the experiment. This phenomenon was much more evident in a shift-up from supplemented Wickerham's minimal to broth medium. Here RNA synthesis assumed a high rate which was maintained for the first 70 min after the shift. Kudo and Imahori (5) showed that, upon a shift-up of nitrogen-starved yeast cells to a rich medium, newly synthesized RNA appears in the polysome fraction

and later moves to the 80S fraction, and that the base composition of this RNA is similar to that of ribosomal RNA. Thus the increased rate of RNA synthesis during a shift-up from minimal to rich medium may represent a synthesis of ribosomal RNA. The stepwise increases in total DNA and cell count following shift-up to broth medium resemble a partially synchronous division, and suggest that shift-up conditions may bring a portion of the cell population into phase with regard to DNA synthesis and cell division. However, a stepwise increase in total protein was also observed; in a synchronous culture of yeast, protein increases continuously throughout the cell cycle (16).

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