JOURNAL OF BACTERIOLOGY, Nov. 1969, p. 679-686

Vol. 100, No. 2
Printed in U.S.A.

Methyl-Deficient Transfer Ribonucleic Acid and Macromolecular Synthesis in Methionine-Starved *Saccharomyces cerevisiae*

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Received for publication 15 September 1969

Haploid methionine auxotrophs of *Saccharomyces cerevisiae* continue to multiply for several hours after withdrawal of a required amino acid from the medium. Macromolecular synthesis continues during this period of residual growth, although the net ribonucleic acid (RNA) and protein content is constant during the later part of this period. In this study, growth after withdrawal of methionine was in some cases accompanied by accumulation of transfer RNA (tRNA), which was shown by methylation in vitro to be deficient in methyl groups. This phenomenon was shown by only four of nine methionine auxotrophs tested, but no evidence could be found that these four strains had "relaxed" control of RNA synthesis. The nine methionine-requiring strains represent mutations in five different positions in the methionine biosynthesis pathway, and only mutants blocked at two of these five positions accumulated methyl-deficient tRNA. This accumulation therefore appears to be correlated with the position of the strain's block in the pathway of methionine biosynthesis.

Methyl-deficient transfer (t) ribonucleic acid (RNA) accumulates in a methionine auxotroph of the yeast *Saccharomyces cerevisiae* when this organism is starved for methionine (11), and what may be a similar phenomenon has been observed in *Neurospora crassa* (B. J. Salmon and F. F. Davis, Fed. Proc. **22**:229, 1963). These observations were somewhat unexpected, in view of the fact that an analogous accumulation of methyl-deficient tRNA occurs in methionineless strains of *Escherichia coli* only if the organism has lost the normal "stringent" control of ribosomal and tRNA synthesis (13). In the "relaxed" (RC^{rel}) strains, withdrawal of a required amino acid from the intracellular pool fails to stop bulk RNA synthesis (4) and, if the amino acid is methionine, the accumulating RNA tends to be methyl-deficient as a consequence of the lack of *S*-adenosyl-L-methionine, the methyl donor (2).

We have further investigated the phenomenon previously observed in the eucaryote *S. cerevisiae*. This paper reports the results of a study of the effect of methionine starvation on the tRNA of nine methionine-requiring strains. The RNA synthesized during starvation of some of the strains was found to be methyl-deficient. Possible reasons for this finding are presented in this and the following paper (10), and it is concluded that the phenomenon is not analogous to that in relaxed strains of *E. coli*.

We first characterized the phenomenon of accumulation of methyl-deficient tRNA by correlation with the residual growth of the cells after methionine withdrawal from the medium. In view of the known relationship between protein and RNA synthesis in yeast (19), macromolecular synthesis was then investigated, with particular emphasis on the possibility that relaxed control of RNA synthesis occurred in certain strains. In the accompanying paper, we report the results of a parallel study of the relationship between methyl deficiency and the composition of the intracellular methionine pool in these strains.

MATERIALS AND METHODS

Materials. [^{14}C-*methyl*]*S*-adenosyl-L-methionine (50.2, 53.9, or 55.0 mc/mmole) and [2-^{14}C]uracil (30 mc/mmole) were obtained from New England Nuclear Corp., Boston, Mass. [2,8-^{3}H]adenine (7.7 c/mmole), [5,6-^{3}H]uracil (4.8 c/mmole), L-[^{14}C-*methyl*]methionine (25.0, 29.5, and 56.8 mc/mmole),

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and [2-^{14}C]uracil (50.0 mc/mmole) were obtained from the Radiochemical Centre, Amersham, England. DL-[2-^{14}C]methionine (0.57 mc/mmole) was obtained from Calbiochem, Los Angeles, Calif.

Strains and growth conditions. All strains used were haploid methionine auxotrophs of *S. cerevisiae*, isolated and characterized by M. Grenson. They are listed, together with their growth responses to precursors and derivatives of methionine, in Table 1. The strains are listed in this table in arbitrarily numbered groups based on nutritional response and complementation tests (14). (Most feeding tests and all complementation tests were performed by M. Grenson.) The genealogy of seven of the nine mutants has been presented previously (14); D72 and D73 were both isolated after X-ray treatment of D1 (14) and are sister strains of D74. Mutants D41, D72, and D74 were phenotypically identical, but D41 gave complementation with both D72 and D74. The probable blocks in methionine biosynthesis of these groups of strains are discussed in the accompanying paper (10).

Cells were grown at 30 C on a rotatory shaker, and increase in cell density was followed by a Klett-Summerson photometer as described previously (11). Minimal media were supplemented with L-methionine (50 μg/ml), L-histidine (20 μg/ml; D84), adenine (100 μg/ml; D38 and D41), or uracil (100 μg/ml; D6, D72, D73, D74, and D84) as required. Methionine withdrawal was usually performed two to four generations before the onset of the late growth phase (12).

Incorporation of metabolites in vivo. Supplementation of media with radioactive metabolites was as described previously (9, 12). Growth was followed on a nonradioactive portion of the culture unless otherwise stated. Estimations of incorporation into protein and RNA were made as described previously (12), except that in some experiments a Panax windowless scintillation counter (type C.0111) was used for determination of radioactivity. The determination of the relative size of methionine metabolites in the acid-soluble pool has been described (12).

Methylating enzymes, tRNA, and assay conditions. Preparation of tRNA and crude methylating enzyme extracts, assay of methyl group incorporation into tRNA by using [^{14}*C-methyl*]*S*-adenosyl-L-methionine as methyl donor, and analysis of methylated components of tRNA have been described previously (9, 11, 14). Incubation mixtures for the determination of methyl group acceptance of tRNA contained in 1 ml: 100 μmoles of tris(hydroxymethyl)aminomethane-hydrochloride (*p*H 8.0), 10 μmoles of $MgSO_4$, 20 μmoles of NH_4Cl, 0.1 μmole of ethylenediaminetetraacetic acid, 2 μmoles of glutathione and tRNA, methylating enzymes, and [^{14}*C-methyl*]*S*-adenosyl-L-methionine as specified. All values given for levels of incorporation of methyl groups into tRNA in vitro are derived from time curves corrected for enzyme blanks; all time curves were followed to plateau levels of incorporation, and from two to six different concentrations of tRNA were used. Radioactivity was determined in a windowless gas-flow counter (Nuclear-Chicago). An optical density (OD) unit is defined as the amount of material which, in 1 ml, gives $A^{1\,cm} = 1.00$ at 260 nm or 280 nm as specified.

RESULTS

Cell growth after methionine withdrawal. The form of growth curve obtained after methionine withdrawal from the medium of a methionine auxotroph of *S. cerevisiae* has been described previously (11). In all strains thus far studied, cell growth continues for several hours after methionine withdrawal. This residual growth is divided into three more or less well-defined phases. The first phase, which usually lasts about 2 hr, is characterized by the normal logarithmic growth rate. In the second phase, lasting from 4 to 6 hr, the growth rate is decreased, whereas active budding ceases during the final phase. A typical growth curve is shown in Fig. 1. The change from one phase to another is not always clearly marked, but it appears to be related to the amount of methionine present in the intracellular pool at the time of withdrawal. Both total cell count and viable count increase during the first and second phases, but a decrease of viable count may be observed soon after the cells enter the third phase.

Growth curves were followed in all experiments reported in this paper. In general, most strains underwent about 1.5 to 2 doublings after methionine withdrawal. Strain D73, however, was characterized by a slow growth rate in minimal medium (a generation time of approximately 250 min as compared to 120 to 140 min for the other strains) and by an increase in cell density of only 0.5 to 0.8 times during starvation.

Accumulation of methyl-deficient tRNA during methionine starvation. The final stage of tRNA biosynthesis is the modification of the bases of the RNA molecules (2); the base modification most amenable to experimental investigation is methylation. In logarithmically growing cells of strain D84, all tRNA species are fully methylated, but during the second phase of methionine starvation this strain accumulates methyl-deficient tRNA (11). This can be demonstrated by methylation of the tRNA in vitro, with [^{14}*C-methyl*]*S*-adenosyl-L-methionine as the methyl donor and a crude cell extract (14) as the source of tRNA methylating enzymes. The incorporation of ^{14}C-methyl groups in vitro is maximal if the D84 cells are harvested in the third phase of starvation (11). Results are presented in Table 2 for incorporation of ^{14}C-methyl groups into tRNA isolated from several methionine auxotrophs of *S. cerevisiae* harvested during the third phase of growth after methionine

withdrawal. Methylating enzyme preparations were made from cells harvested during logarithmic growth, and control incorporations into tRNA from logarithmic cells are also presented. The incubation system was in all cases homologous; i.e., tRNA and methylating enzymes were from the same strain. The values given have been calculated from plateau-level incorporation into several different amounts of each tRNA preparation.

D84 accumulated small but significant amounts of methyl-deficient tRNA during methionine starvation (Table 2), as found previously (11). Strains MG331 and D6, which contain the same methionine block as D84, both accumulated a similar amount of methyl-deficient tRNA as D84, whereas strain D73 accumulated 8 to 10 times more. Other strains maintained fully methylated tRNA throughout starvation. The extent of methyl-deficiency was found to vary from one experiment to another. (Ranges for D73 and D84 are given in Table 2.) This was due to a varying methyl-deficiency in the tRNA itself and not to the enzyme preparation used.

Only two of the five groups of mutants responded to methionine starvation by methyl-deficiency in tRNA. Thus, accumulation of methyl-deficient tRNA after withdrawal of methionine is not a general feature of all methionine auxotrophs of *S. cerevisiae*. Strains D38, D73, and D84 were selected as typifying the three responses found, and most further experiments were performed on these strains to characterize the phenomenon.

TABLE 1. *Nutritional responses of methionine auxotrophs of S. cerevisiae*[a]

Strain	Supplement to basal medium						Group
	HSer	Cys	Cysta	HCys	Met	SAM	
D73 (α me⁻ur⁻)	−	−	−	−	+	−	0
D6 (α me⁻ur⁻)	−	−	−	+	+		1
D84 (α me⁻hi⁻ur⁻)	−	−	−	+	+	+	1
MG331 (α me⁻)	−	−	−	+	+	+	1
MG334 (α me⁻)	−	+	−	+	+		2
D38 (a me⁻ad⁻)	−	+	−	+	+	+	2
D41 (a me⁻ad⁻)	−	±	−	+	+		3
D72 (α me⁻ur⁻)	−	±	−	+	+		4
D74 (α me⁻ur⁻)	−	±	−	+	+		4

[a] Supplements in liquid cultures were present at a concentration of 0.05 mg/ml. The basal medium was also supplemented with adenine, uracil, and histidine as required. Nutritional response is reported as: +, heavy growth; ±, growth response delayed and marginal; −, no growth. Cultures were incubated for 40 hr at 30 C. Mating type is indicated as (a) and (α). Abbreviations: HSer, homoserine; Cys, cysteine; Cysta, cystathionine; HCys, homocysteine; Met, methionine; SAM, *S*-adenosylmethionine.

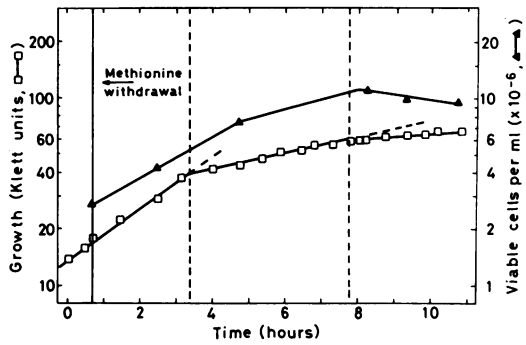

FIG. 1. *Growth (□) and viable cell count (▲) of S. cerevisiae strain D84 before and during methionine starvation. The cells were grown for 1.5 generations before methionine withdrawal. The beginning of each phase of starvation is indicated by vertical dashed lines. At intervals, 0.1 ml of cells was removed, diluted in sterile 0.5% NaCl, spread on agar plates containing 0.5% yeast extract and 2% glucose, and incubated at 30 C. Mean values of quadruple samples of each dilution are given in the figure.*

Time of accumulation of methyl-deficient tRNA. Methyl-deficient tRNA accumulates during the second phase of growth after methionine withdrawal. This has been shown for D84 (11) and was also true for the more extensive accumulation in D73 (Fig. 2). D38, which showed no accumulation of methyl-deficient tRNA by the third phase, showed no transient accumulation during any part of residual growth (Table 3).

Methylated components formed in the assay in vitro. Methyl-deficient tRNA from D73 and that from D84 were methylated in vitro, reisolated, and subjected to acid hydrolysis and two-dimensional chromatography (9) to analyze the components formed (Table 4). tRNA from our strains of *S. cerevisiae* harvested during logarithmic growth has a fixed and well-defined distribution of methyl groups, as found by analyses of cells labeled in vivo with L-[^{14}*C-methyl*]methionine (14). Analysis of this distribution of methyl groups of logarithmic tRNA from strains D73 and D84 is presented in Table 4 for comparison.

Although several methylated components were formed in vitro, the major product was methylated uracil. Other components were formed to a lesser extent, presumably reflecting a lack of sites for their formation in the tRNA preparation, although in the case of 1-methyladenine the lack of incorporation may reflect the lability of the methylating enzyme (1).

Protein and RNA synthesis during methionine

TABLE 2. *Incorporation of ^{14}C-methyl groups in vitro into tRNA from several methionine-requiring strains of S. cerevisiae*[a]

Group	Strain	^{14}C-methyl groups incorporated into tRNA (nmoles/100 OD units at 260 nm)	
		Logarithmic	Starved
0	D73	0.8	26–52
1	D6	0.7	6.4
1	D84	0.1	2.5–6.8
1	MG331	0.6	5.3
2	MG334	0.3	0.5
2	D38	0.5	0.7
3	D41	1.2	0.1
4	D72	0.6	0.3
4	D74	1.0	0.7

[a] Strains are divided into groups as in Table 1; the following are isogenic with the exception of the methionine mutation: D72, D73, and D74; MG331 and MG334; D38 and D41. tRNA was prepared from cells harvested during either logarithmic growth or during the third phase of methionine starvation. Incorporation of ^{14}C-methyl groups was measured as described, incubation mixtures containing [^{14}*C-methyl*]*S*-adenosyl-L-methionine (3 to 10 nmoles/ml; 50.2, 53.9, or 55.0 mc/mmole) as methyl donor, methylating enzymes from the various strains harvested in the logarithmic growth phase (A_{280}/A_{260} = 0.62 to 0.76; 7 to 16 OD_{280} units/ml), and various amounts of the different tRNA preparations. Samples of 0.15 or 0.20 ml were withdrawn for assay of radioactivity. The degree of incorporation into logarithmic tRNA varies with the time of harvesting of the cells (12); minimal values found are shown in the table.

starvation. Removal of a required amino acid from the medium of bacterial cells leads to immediate depletion of the intracellular pool, so that effects on macromolecular synthesis are manifested directly (4). However, in *S. cerevisiae*, the intracellular pool is scarcely reduced by the process of amino acid withdrawal. The required amino acid thus remains in the pool and is removed relatively slowly, as shown for the pool of methionine (and metabolites derived from it) in strains D38 and D84 (Fig. 3 and 4). Therefore, it is difficult to investigate amino acid-mediated control of RNA synthesis in this organism. Some form of relaxed control over RNA synthesis was considered as a possible explanation for the accumulation of methyl-deficient tRNA during methionine starvation.

The relationship between protein and RNA synthesis during starvation was therefore investigated. Before methionine withdrawal, cells were grown in the presence of radioactive metabolites for several generations to allow the cells to reach the same specific activity as the medium (Fig. 4). Net protein and RNA syntheses were followed during methionine withdrawal in strains D38, D84, and D73 (Fig. 5, 6, and 7).

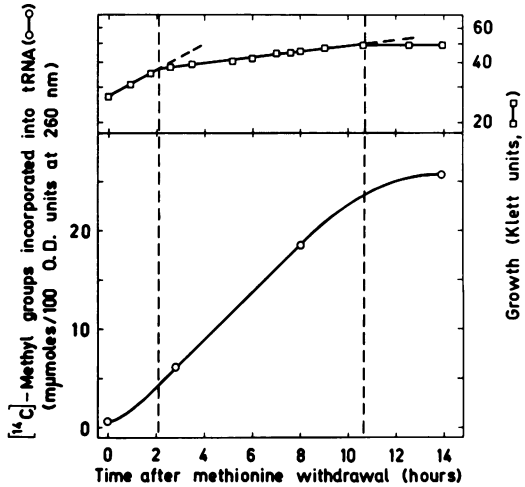

FIG. 2. *Growth* (□) *and incorporation of methyl groups in vitro into tRNA* (○) *isolated from cells during methionine starvation of S. cerevisiae strain D73. Cells were grown in 2.5-liter cultures with and without methionine. Logarithmically growing cells, harvested at 100 Klett units, were used for the preparation of methylating enzymes. At the times indicated, samples of starving cells were withdrawn for isolation of tRNA. Incubation mixtures (pH 8.0) for incorporation of methyl groups into tRNA in vitro contained* [^{14}*C-methyl*]*S-adenosyl-L-methionine (10 nmoles/ml, 55 mc/mmole) as methyl donor, methylating enzymes (A_{280}/A_{260} = 0.74, 7.4 OD_{280} units/ml), and various amounts of the different tRNA preparations. Incorporation of methyl groups to plateau levels and tRNA dependence were as described.*

TABLE 3. *Incorporation of ^{14}C-methyl groups in vitro into tRNA from methionine-starved S. cerevisiae strain D38*[a]

Time after methionine withdrawal	^{14}C-methyl groups incorporated into tRNA (nmoles/100 OD units at 260 nm)
min	
192	0.8
288	0.7
528	0.7

[a] Conditions of growth and assay were as described for strain D73 (Fig. 2), except that the methylating enzymes (A_{280}/A_{260} = 0.84, 10.2 OD_{280} units/ml) were prepared from D38 cells harvested during a late period of logarithmic growth (late growth phase; 12). The first phase of growth ended 120 min and the second phase ended 340 min after methionine withdrawal.

TABLE 4. *Distribution of ^{14}C-methyl groups in tRNA from S. cerevisiae labeled in vivo and in vitro*[a]

Methylated component	tRNA labeled in vivo		tRNA from methionine-starved cells labeled in vitro	
	D84	D73	D84	D73
1-Methyladenine	10	8.1	1.5	0.3
2-Methyladenine	0.1	0.7	0.3	0
N^6-methyladenine	0	0.2	0	0
N^6-dimethyladenine	0.9	0	0	0
1-Methylguanine	11	9.7	9.7	19
N^2-methylguanine	9.9	6.2	1.4	4.5
N^2-dimethylguanine	16	19	14	18
7-Methylguanine	4.6	4.5	0.3	0.5
1-Methylhypoxanthine	0.7	1.3	1.8	3.4
Methylcytosine	17	19	2.0	0.9
Methyluracil	18	20	52	46
2′-*O*-methylribose	12	11	17	7.2

[a] The first two columns are analyses of tRNA from strain D84 (14) and D73, labeled with methyl groups in vivo. The second and third columns are analyses of tRNA isolated from cells harvested in the third phase of methionine starvation and methylated in vitro. Incubation mixtures contained, in 1 ml: 36 nmoles of [^{14}C-*methyl*]*S*-adenosyl-L-methionine (50.2 mc/mmole) and 28 OD_{260} units of D84 tRNA with 19 OD_{280} units of D84 methylating enzyme preparation (A_{280}/A_{260} = 0.59), or 3.5 OD_{260} units of D73 tRNA with 10 OD_{280} units of D73 enzyme preparation (A_{280}/A_{260} = 0.74). Incubations (30 C) were terminated after 270 min. tRNA was reisolated from the reaction mixtures and subjected to acid hydrolysis; the methylated components were separated by two-dimensional paper chromatography. All figures are percentages of total counts/min recovered in methylated compounds. All values above 1% have been corrected to two significant figures; the experimental error involved, however, is approximately ±10% of each value. Counts/min recovered from chromatograms: D84 in vivo, 8 × 10^3; D73 in vivo, 15 × 10^3; D84 in vitro, 3 × 10^3; D73 in vitro, 6 × 10^3.

In each strain, the incorporation of radioactive methionine from the intracellular pool into protein continued after methionine withdrawal from the growth medium at a rate which decreased as the pool of free methionine became exhausted (Fig. 3 and 4). In all three strains, there was a fairly close correspondence between incorporation of methionine into protein and uracil or adenine into RNA. Protein synthesis and RNA synthesis appear to be closely linked, which may be analogous to stringent RNA control in *E. coli*.

The rate of net synthesis of macromolecules

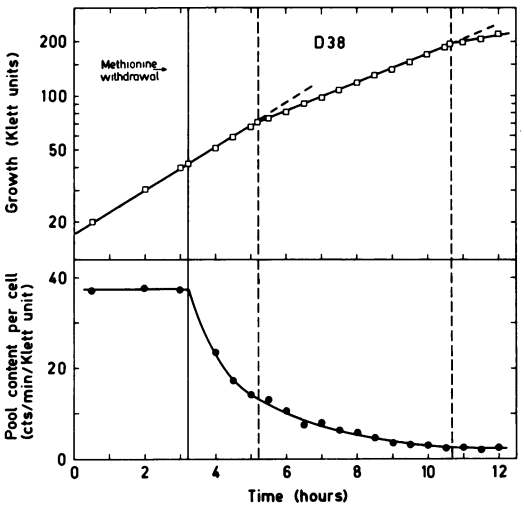

FIG. 3. *Growth (□) and pool content per cell of metabolites derived from methionine (●) during methionine starvation of S. cerevisiae strain D38. Growth medium was supplemented with* DL-[2-^{14}C] *methionine (0.16 μc/ml, 0.57 mc/mmole) for eight generations before methionine withdrawal. At the times indicated in the figure, duplicate samples of 150 μliters were withdrawn into ice-cold 0.5% NaCl (3 ml) and 5% trichloroacetic acid (3 ml). Determination of radioactivity in the samples and estimation of pool size were as described.*

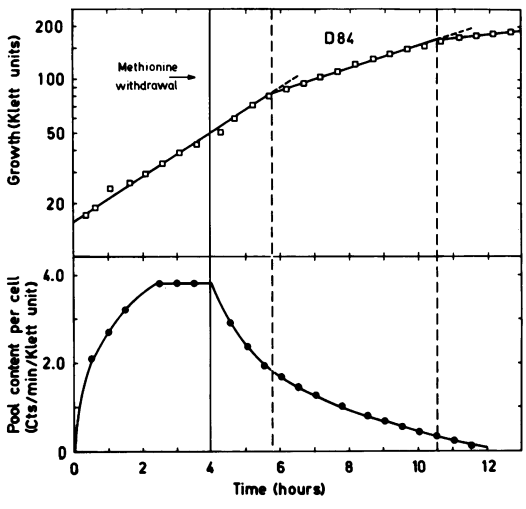

FIG. 4. *Growth (□) and pool content per cell of metabolites derived from methionine (●) during methionine starvation of S. cerevisiae strain D84. Growth medium was supplemented with* DL-[2-^{14}C] *methionine (0.067 μc/ml, 0.57 mc/mmole) at the time indicated in the figure. Duplicate samples of 150 μliters were withdrawn for treatment as in Fig. 2.*

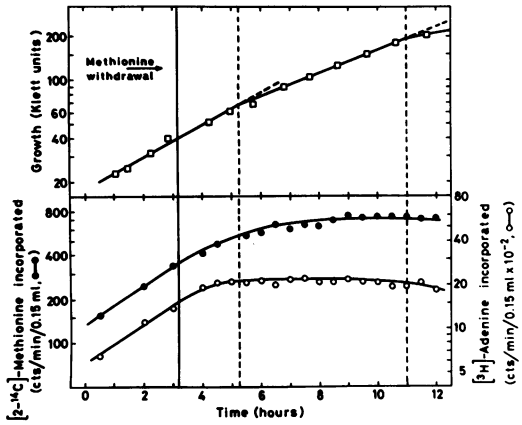

FIG. 5. *Growth of S. cerevisiae strain D38 before and during methionine starvation* (□) *and net protein* (●) *and RNA* (○) *synthesis. The beginning of each phase of starvation is indicated by the vertical dashed lines. The growth media were supplemented with [2,8-³H]adenine (39 μc/ml, 7.7 c/mmole) and with* DL-*[2-¹⁴C]methionine (0.16 μc/ml, 0.57 mc/mmole) before withdrawal. Samples of 150 μliters were withdrawn for measurement of acid-precipitable radioactivity. Radioactive metabolites were added to the culture 16 hr before commencing the experiment.*

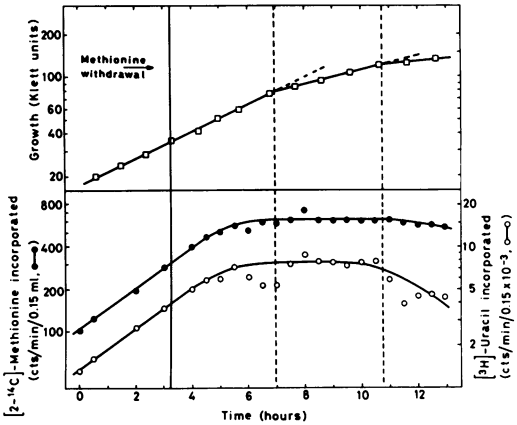

FIG. 6. *Growth of S. cerevisiae strain D84 before and during methionine starvation* (□) *and net protein* (●) *and RNA* (○) *synthesis. The beginning of each phase of starvation is indicated by the vertical dashed lines. The growth media were supplemented with [5,6-³H]uracil (33 μc/ml, 4.8 c/mmole) and with* DL-*[2-¹⁴C]methionine (0.16 μc/ml, 0.57 mc/mmole) before withdrawal. Samples of 150 μliters were withdrawn for measurement of acid-precipitable radioactivity. Radioactive metabolites were added to the culture 16 hr before commencing the experiment.*

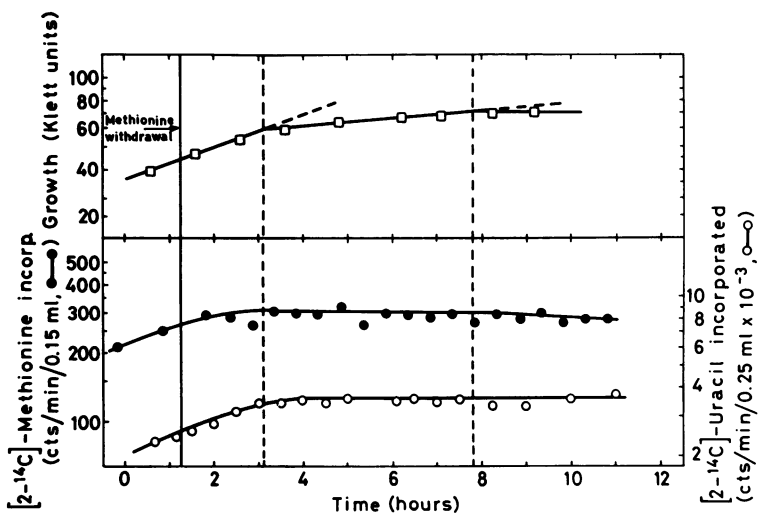

FIG. 7. *Growth of S. cerevisiae strain D73 during methionine starvation* (□) *and net protein* (●) *and RNA* (○) *synthesis. This figure is derived from two experiments: the growth curve was measured directly on the radioactive culture used for following RNA synthesis. The beginning of each phase of starvation is indicated by the vertical dashed lines. The growth media were supplemented either with [2-¹⁴C]uracil (0.35 μc/ml, 50.0 mc/mmole) or with* DL-*[2-¹⁴C]methionine (0.08 μc/ml, 0.57 mc/mmole) before withdrawal. Samples (250 μliters when following RNA synthesis; 150 μliters when following protein synthesis) were withdrawn for measurement of acid-precipitable radioactivity. ¹⁴C-uracil and ¹⁴C-methionine were added to the cultures 13 and 16 hr, respectively, before commencing the experiment.*

during the first phase of methionine starvation was almost the same as that in logarithmically growing cells. There was, however, little net RNA synthesis during the second phase, even though accumulation of methyl-deficient tRNA occurred at this time (Fig. 2). This indicates that, during the second phase, breakdown and synthesis of RNA must be taking place. This turn-

over phenomenon was investigated by adding ^{14}C-uracil at high specific activity to actively growing cultures half a generation time before methionine withdrawal. (Little uracil is taken up by the cells from the medium after removal of methionine.) The results (Fig. 8 and 9) show that in both D38 and D73 RNA was synthesized during the second phase, as it is in D84 (11).

DISCUSSION

Control mechanisms linking the synthesis of bulk cellular RNA and protein were proposed as a result of early studies on a variety of microorganisms, including yeast (19). How far RNA and protein synthesis will continue after withdrawal of an essential metabolite from the growth medium depends on the size of the pool of that metabolite inside the cell. Thus, removal of the total nitrogen source from growing yeast cells leads to complete cessation of cell growth and of protein and RNA synthesis (3, 8). Sudden removal of inorganic phosphate from a rich medium, on the other hand, is followed by an increase in the number of cells and continued RNA and protein synthesis until the intracellular inorganic polyphosphates are severely depleted (16). The present investigation shows that macromolecular synthesis and cell division continue for some time after removal of a required amino acid which is fairly abundant in the cell pool (6, 12). In spite of this macromolecular synthesis, however, it can be shown that the total RNA and protein contents per cell decrease considerably during residual growth under starvation conditions.

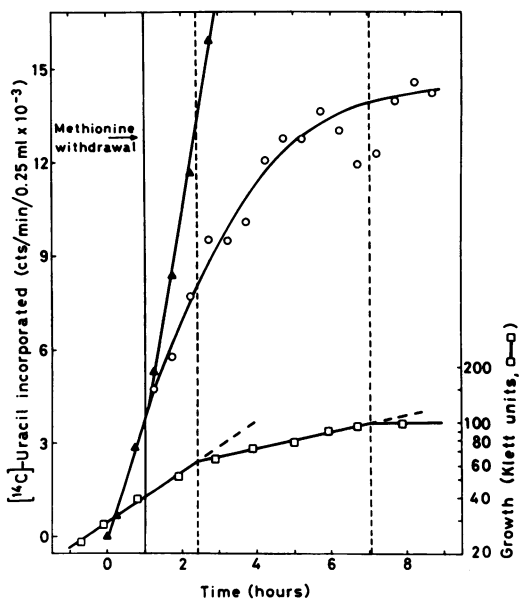

FIG. 8. *Growth (□) and RNA synthesis of S. cerevisiae strain D38. Symbols: ▲, RNA synthesis during logarithmic growth; ○, RNA synthesis during methionine starvation. All growth media were supplemented with uracil (100 μg/ml). [2-^{14}C]uracil (2.5 μc/ml, 50.0 mc/mmole) was added to the logarithmic culture at zero time and was also present in the medium without methionine. Growth was followed on the radioactive cultures. The cell density of the methionine-starved culture was identical to that of the logarithmic culture at the time of medium change. Samples of 250 μliters were withdrawn for measurement of acid-precipitable radioactivity.*

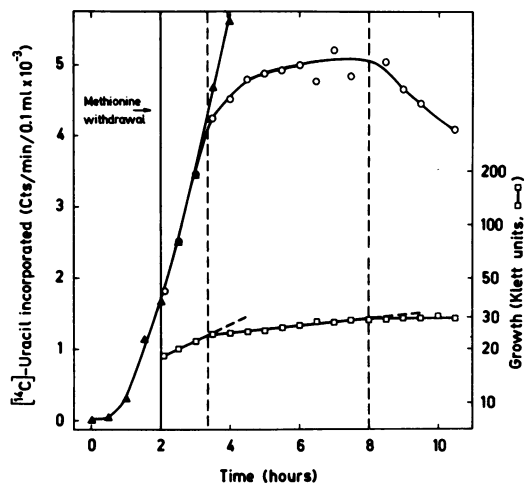

FIG. 9. *Growth (□) and RNA synthesis of S. cerevisiae strain D73. Symbols: ▲, RNA synthesis during logarithmic growth; ○, RNA synthesis during methionine starvation. Growth media were supplemented with uracil (100 μg/ml). [2-^{14}C]uracil (8.0 μc/ml, 30.0 mc/mmole) was added to the logarithmic culture at zero time and was also present in the medium without methionine. Growth was followed on the radioactive cultures. The cell density of the methionine-starved culture was identical to that of the logarithmic culture at the time of medium change. Samples of 100 μliters were withdrawn for measurement of acid-precipitable radioactivity.*

Several workers have also shown that RNA degradation occurs during starvation of yeast (5, 8); furthermore, a similar degradation of ribosomal RNA has been demonstrated during methionine starvation of *N. crassa* (18). This can also be inferred from the present study, in which it is shown that although RNA synthesis continues during the second phase of methionine starvation the net RNA content of the culture does not increase.

It has been demonstrated that, in certain of our methionine-requiring strains of *S. cerevisiae*, the tRNA synthesized during methionine starvation is methyl-deficient. Although our tRNA preparations from starved cells may be contaminated

with degraded ribosomal RNA, this degraded material does not act in vitro as a substrate for the methylating enzymes (*unpublished data*), which are specific for intact tRNA (7). Five of the nine strains investigated failed to accumulate methyl-deficient tRNA (Table 2); that this is due to a lack of sites on the tRNA (and not to labile methylase preparations) was shown by experiments in which enzyme and tRNA preparations from different strains were used (*unpublished data*). This suggested that these strains may differ in their control of RNA synthesis, by analogy with the well-known situation in *E. coli*. No clear differences could be found, however, between the patterns of RNA and protein synthesis of D73, D84, and D38, strains which accumulate relatively much, relatively little, and no methyl-deficient tRNA, respectively. We are therefore led to consider alternative mechanisms for this phenomenon, since the four mutants that accumulate methyl-deficient tRNA belong to only two of the five groups of methionine-requiring mutants tested. Furthermore, in two sets of mutants, isogenic with the exception of the methionine block, only one strain in each set contained methyl-deficient tRNA after starvation (compare MG331 and MG334 and D72, D73, and D74, Table 2). Methyl deficiency thus seems to be correlated with the block in the methionine biosynthesis pathway and appears to result from inhibition of certain methylases by accumulating metabolites of methionine (10).

The observed accumulation of methyl-deficient tRNA is an effect of moderate magnitude. The greatest in vitro methyl-acceptor activity was found for D73 tRNA (52 nmoles of ^{14}C-methyl groups per 100 OD units of tRNA), corresponding to an incorporation of one methyl group in about 35% of the tRNA molecules (assuming that the tRNA preparation was not highly contaminated with degraded RNA). This is about one-third of the incorporation that may be obtained in vitro with methyl-deficient tRNA derived from methionine-starved *E. coli* W6 (17), in which about 50% of the tRNA is devoid of all methyl groups. The major base formed in vitro in the present case is methyl-uracil, as is also the case with *E. coli* (15).

ACKNOWLEDGMENTS

We thank M. Grenson (C.E.R.I.A., Brussels) for giving us the strains of *S. cerevisiae* used in this work, and for making available to us data on their growth characteristics and on complementation tests between them. We also thank Hans G. Boman for his interest during this work, which was partly carried out while J.H.P. was the recipient of a N.A.T.O. Research Fellowship.

This investigation was supported by grant Dnr 2453 from the Swedish Natural Science Research Council (to Hans G. Boman), by grant 67-90 from the Swedish Cancer Society, and by the Makerere College Research Grants Fund.

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