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Exogenous dTMP Utilization by a Novel *tup* Mutant of *Saccharomyces cerevisiae*

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The rate and extent of entry of dTMP were measured in strains of *Saccharomyces cerevisiae* carrying two new *tup* mutations (*tup5* and *tup7*) and most of the other *tup* mutations which have been reported previously by others. The *tup7* mutation allowed dramatically greater accumulation of dTMP than any of the other mutations tested. Specific labeling of DNA by [CH_3-^{3}H]dTMP, fate of the dTMP pool inside of the cells, and degradation of the dTMP in the culture medium were investigated in strains carrying the *tup7* mutation. The extracellular dTMP was not appreciably degraded, and that accumulated intracellularly was readily phosphorylated to dTDP and dTTP. Under optimum labeling conditions, 60 to 80% of the total thymidylate residues in newly synthesized DNA were derived from the exogenously provided dTMP, even in the absence of a block in de novo dTMP biosynthesis. An apparent K_m for entry of 2 mM dTMP was found. The *tup7* mutation increased permeability to dTMP (and some other 5'-mononucleotides), but did not affect uptake of nucleosides and purine and pyrimidine bases. Uptake of dTMP could be almost completely inhibited by moderate concentrations of P_i. These findings and other observations suggest that entry of dTMP in strains carrying the *tup7* mutation is mediated by a permease whose function in normal cells is the transport of P_i.

Convenient and specific labeling of yeast DNA in vivo with radioactive thymine or thymidine is not possible because *Saccharomyces cerevisiae* is impermeable to these compounds (18). In addition, this yeast lacks detectable thymidine kinase (TK) activity (19; L. F. Bisson, Ph.D. thesis, University of California, Berkeley, 1980), which also accounts for the ineffectiveness of thymidine as a precursor for DNA synthesis. Normally the thymidylate synthetase reaction (3) provides the dTMP that is phosphorylated further and used for DNA replication. To circumvent these problems, many research groups have isolated mutants which are permeable to dTMP and capable of incorporating exogenously provided dTMP into DNA. Such mutants have been called *dTMP-per* by Jannsen et al. (21), *typ* by Laskowski and Lehmann-Brauns (23), *tup* by Wickner (33), and *tum* by Brendel (6). Wickner and Leibowitz (33–35) described and mapped four unlinked mutations conferring dTMP permeability (*tup1*, *tup2*, *tup3*, and *tup4*). Three other mutations, apparently different from those identified by Wickner, were isolated by Laskowski and Lehmann-Brauns (23) and were reported to be linked to each other (although their chromosomal location was not determined).

Strains carrying such *tup* mutations have been used extensively in studies requiring specific labeling of yeast DNA (13, 14, 36). However, essentially nothing is known about the mechanism(s) or specificity of the entry of dTMP into these dTMP-permeable mutants. Furthermore, the efficiency of utilization of exogenous dTMP by these strains is very low, usually requiring extensive labeling periods of 18 to 48 h (8, 33). In some cases (6, 21), the uptake and incorporation of labeled dTMP into DNA were reported to occur only for a limited period (2 to 3 h) during growth of the cells. It is not known what growth conditions or other factors impose these restrictions on the efficient utilization of dTMP. The incorporation of exogenous dTMP into DNA is enhanced in these strains if double mutants are constructed which also carry a *tmp1* mutation (7, 17, 24). We have demonstrated that the *tmp1* locus is the structural gene for thymidylate synthetase (2). Unfortunately, strains carrying a *tmp1* mutation are of limited usefulness for the study of DNA replication in normal cells because such strains segregate petites at a very high frequency unless continual selective pressure for respiratory competency is maintained (24, 26). Also, *tmp1 tup* mutants are reported to

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undergo thymineless death in the absence of dTMP (1, 10).

Recently, we isolated many different *tup* mutants from a single, genetically well-defined parental strain (L. F. Bisson and J. Thorner, Genetics, in press) and subsequently measured their relative efficiency of utilization of dTMP. During the course of these studies, we discovered a new mutation, *tup7*, which allows dTMP uptake at a rate 5- to 100-fold higher than that observed for any *tup* mutant previously described and permits the highly efficient incorporation of exogenously supplied dTMP into cellular DNA. Because of the potential usefulness of such a mutation, we have characterized in detail the utilization of dTMP by strains carrying the *tup7* mutation.

MATERIALS AND METHODS

Materials. Reagent chemicals were obtained from the following sources: dUMP from P-L Biochemicals; dTMP, 5-fluoro-dUMP (FdUMP), aminopterin, and sulfanilamide from Sigma Chemical Co.; HP scintillation fluid from Beckman Instruments, Inc.; [5-^{3}H]dUMP from Schwarz/Mann; [*methyl*-^{3}H]dTMP from Amersham Corp.; nitrocellulose filter disks (25-mm diameter, 0.45-μm pore size) from Gelman Sciences, Inc.; polyethyleneimine-cellulose thin-layer plates (Polygram Cel 300 PEI) from Brinkmann Instruments, Inc.; medium constituents from Difco Laboratories. All other chemicals were reagent grade.

Organisms. The yeast strains used in this work and their sources are listed in Table 1. Strains B111-2A, B111-2D, B114-5D, and B114-8D were derived from strains RW111 and RW114 (33), provided by R. B. Wickner, by outcrossing twice with X2180-1A or X2180-1B.

Media and growth conditions. YPD and SD media have been previously described (31). Medium A was used for the labeling experiments and has been described before (2). In all of the studies presented here, medium A was buffered with 50 mM sodium succinate and was adjusted routinely to pH 4.8 before autoclaving.

Genetic methods. Crosses, complementation tests, mating type determinations, and other genetic procedures were performed by standard methods (31).

Uptake of nucleoside monophosphates. Cells were grown to mid-exponential phase (1×10^7 to 3×10^7 cells per ml) at 30°C on a rotary shaker in medium A. To initiate an experiment, 1.5 ml of cells was placed in a test tube (20 by 150 mm) and prewarmed to 30°C, and 0.025 to 0.5 ml of a stock of radioactive transport substrate (4 mM; 5,000 to 10,000 cpm/nmol) was added. Immediately, 0.5 ml of cells was removed and filtered through a nitrocellulose disk. The collected cells were washed 10 times with 2 ml of distilled deionized water each time, maintained at 30°C. Filters were placed in scintillation vials, scintillation fluid (10 ml) was added, and radioactivity was measured in a liquid scintillation counter (Beckman 3133P). For experiments in which uptake was followed over a short period of time, samples were removed at 1-min intervals from test tubes incubated in a 30°C water bath and were treated as described above. In experiments in which uptake was followed over a much longer period (up to 8 h), samples were withdrawn at hourly intervals from cultures that were incubated with the labeled substrate in a gyratory water bath shaker at 30°C or on a roller drum in a 30°C room. Experimental values were corrected for any nonspecific adsorption of the transport substrate by subtracting the zero-time values. Cell titers at each time point were determined by cell counts in a Petroff-Hausser counting chamber and represent the average of three separate determinations.

TABLE 1. Yeast strains

Strain	Genotype	Source
X2180-1A	a *SUC2 mal gal2 CUP1 TUP rho*$^+$	YGSC[a]
X2180-1B	α *SUC2 mal gal2 CUP1 TUP rho*$^+$	YGSC[a]
RW108	a *ade2 his4 trp1 tup1-66 rho*	R. B. Wickner
RW109	α *ade2 his4 thr4 leu2 tup1-66 rho*	R. B. Wickner
B400D	a *SUC2 mal gal2 CUP1 tup1-200 rho*	This work
B527C	α *ade1 his7 lys2 gal1 SUC2 mal tup7-1 rho*	This work
B510C	α *SUC2 mal gal2 CUP1 tup7-1 tmp1 rho*	This work
B600H-6C	a *trp5-1 leu1 tup5-1 rho*$^+$	This work
B600H-6D	α *trp5-1 leu1 lys2 tup5-1 rho*$^+$	This work
B111-2A	a *ade2 tup2-6 rho*$^+$	This work
B111-2D	α *his4 tup2-6 rho*$^+$	This work
B114-5D	a *ade2 lys2 tup4-55 rho*$^+$	This work
B114-8D	α *tup4-55 rho*$^+$	This work
BT27C	α *ade1 his7 lys2 gal1 tup7-1 rho*$^+$	This work
BT27C-3A	a *ade1 his7 tup7-1 rho*$^+$	This work

[a] YGSC, Yeast Genetic Stock Center (c/o R. K. Mortimer, Department of Biophysics, Donnor Laboratory, University of California, Berkeley, CA 94720).

Labeling of DNA with dTMP. Cells were grown for various periods of time in medium A containing [*methyl*-^{3}H]dTMP (10,000 cpm/nmol) at various concentrations (0.1 to 2 mM). Samples (1 ml) were removed and 0.1 ml of 10 N NaOH was added. After alkaline hydrolysis for 1 h at 80°C, the samples were chilled on ice, and an equal volume of ice-cold 20% trichloroacetic acid was added to precipitate the DNA. After 15 min on ice, the precipitates were collected on nitrocellulose disks. The tubes and filters were washed three times with 2 ml of water each time. The filters were washed 10 times more, and their radioactivity content was determined.

The total DNA content per cell was determined by the diphenylamine method (11) for samples taken over the course of labeling experiments, as follows. At time

zero, unlabeled dTMP was added to 200 ml of the same culture from which 1.5-ml portions were removed for measurements of [^{3}H]dTMP incorporation as described above. The cells in 80-ml samples of the culture were collected by centrifugation at 8,000 × *g* for 10 min and were washed twice with water. The pellets were suspended in water and alkaline hydrolysis was performed as described above. The alkali-extracted cells were centrifuged, washed with water, resuspended in 1 ml of 0.5 N perchlorate, and heated to 80°C for 15 min to depurinate the DNA. Absorbance at 600 nm was measured after removal of cell debris by centrifugation, and roe sperm DNA was used as the standard.

Extraction of nucleotides. Cells were grown in the presence of either [*methyl*-^{3}H]dTMP or [5-^{3}H]dUMP as described for the uptake experiments. Cells in 2-ml samples of cultures were removed by filtration through a nitrocellulose filter disk and washed 10 times with 4 ml of water each time (30°C). Cells were flushed off filters with 3 ml of ice-cold 2 N formic acid and allowed to stand on ice for 30 min. The filters were rinsed twice more with 1 ml of acid each time. The combined washes were clarified by centrifugation at 8,000 × *g* for 10 min at 4°C, and the cell-free solutions were lyophilized. The dry material was suspended in 1/20 of its original volume. For experiments in which degradation of labeled 5'-mononucleotides in the medium was assessed, 1-ml samples of the culture were removed and filtered as above. In this case, however, the culture filtrate was retained, lyophilized as before, and suspended in water.

To analyze the extracted nucleotides, samples of resuspended material were spotted onto polyethyleneimine-cellulose thin-layer plates (prerun in water and dried) and subjected to ascending chromatography, using 1 M LiCl as the solvent (28). In some cases, before chromatography the plate containing the sample spotted at the origin was dipped in anhydrous methanol to remove salts. Individual sample lanes were cut into 1-cm squares and placed in scintillation vials containing 0.5 ml of 2 N HCl to elute bound nucleotides, and scintillation fluid (10 ml) was added. Standards consisting of authentic bases, nucleosides, and nucleotides were run simultaneously.

RESULTS

Isolation of dTMP-permeable mutants. Independent spontaneous mutants permeable to dTMP were selected from X2180-1A and X2180-1B as previously described (2, 21, 34). Complementation tests were done among these mutants and against strains carrying the known *tup* mutations originally isolated by Wickner (33). In addition to obtaining mutants in the same complementation groups (*tup1*, *tup2*, and *tup4*) found previously by others, two new genes were identified and designated *tup5* and *tup7*. A more detailed genetic characterization of these two novel mutations is presented elsewhere (Bisson and Thorner, Genetics, in press).

Uptake of nucleoside monophosphates by dTMP-permeable mutants. Accumulation of radioactively labeled dTMP from the medium by intact cells carrying the various *tup* mutations was measured (Table 2). Strains carrying the *tup7* mutation displayed the highest level of dTMP accumulation at both of the dTMP concentrations tested. Strikingly, uptake by the *tup7* strains was about 100-fold greater than that by the *tup2* strains and was over five times better than the next most efficient mutant (*tup4*).

The *tup7* mutation also conferred the ability to take up other nucleoside monophosphates. Strains carrying both an *ade1* and a *tup7* mutation were capable of utilizing 5'-AMP, and to a lesser extent IMP (but not cyclic AMP), to satisfy their requirement for adenine. In contrast, neither adenosine nor inosine permitted growth of the *ade1 tup7* cells. (Uptake of adenine itself was unaffected in strains carrying the *tup7* mutation or any of the other *tup* mutations [Bisson, Ph.D. thesis].) Cells bearing the *tup7* lesion were dramatically sensitive to growth inhibition by FdUMP, yet were completely resistant to equivalent concentrations of fluorodeoxyuridine, as is documented in detail elsewhere (L. F. Bisson and J. Thorner, Mol. Gen. Genet, in press). Finally, strains carrying the *tup7* mutation also were able to take up [^{3}H]dUMP (data not shown).

Growth conditions affect the uptake of dTMP. Actively growing cells of strain BT27C (*tup7*) appeared to accumulate dTMP best, as evidenced by the fact that uptake occurred mainly during the exponential phase of growth (Fig. 1).

TABLE 2. Uptake of dTMP by *tup* mutants[a]

Strain	dTMP uptake (pmol accumulated/h per 10^7 cells) with:	
	0.2 mM	1.0 mM
X2180-1A (a *TUP*)	<1	<1
X2180-1B (α *TUP*)	<1	<1
B400D (a *tup1*)	~1	18
RW109 (α *tup1*)	~1	15
B111-2A (a *tup2*)	<1	3
B111-2D (α *tup2*)	<1	4
B114-5D (a *tup4*)	10	62
B114-8D (α *tup4*)	11	56
B600H-6C (a *tup5*)	16	33
B600H-6D (α *tup5*)	10	38
BT27C-3A (a *tup7*)	28	345
BT27C (α *tup7*)	39	330

[a] Cells were grown to mid-exponential phase (1 × 10^7 to 2 × 10^7 cells per ml) in medium A at pH 4.8, and then the uptake of label at two different external concentrations of [^{3}H]dTMP (5,000 cpm/nmol) was determined over a 2-h time course.

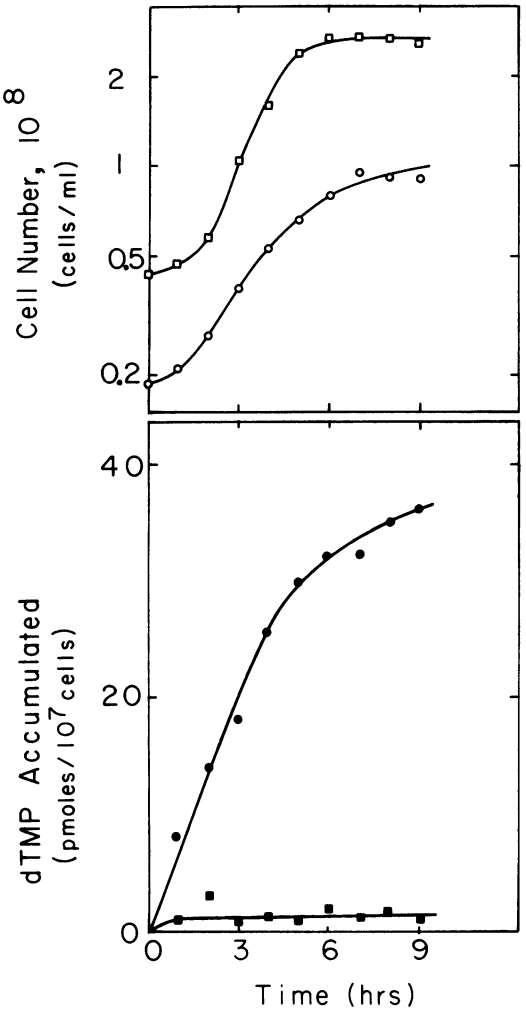

FIG. 1. Accumulation of dTMP during growth of yeast strains. Cells of strains BT27C (*tup7*) (circles) and X2180-1B (*TUP*$^+$) (squares) were grown in medium A (pH 4.8) to mid-exponential phase (2×10^7 to 4×10^7 cells per ml) and then transferred to flasks containing [*methyl*-^{3}H]dTMP (5,000 cpm/nmol) at a final concentration of 0.2 mM. Samples were removed, the amount of label accumulated by the cells (closed symbols) was measured, and cell counts (open symbols) were determined at each of the time points.

The parent strain showed no apparent uptake of dTMP. The effect of pH on dTMP uptake was examined in SD medium that contained succinate (50 mM) as an additional buffering species. Previously it had been reported only that utilization of dTMP required pH values below 6 (15, 34). For strains carrying the *tup7* mutation, uptake of dTMP was dramatically increased when the pH value of the medium was lowered (Table 3). At 1 mM [*methyl*-^{3}H]dTMP (25,000 cpm/nmol) and pH 3.8, it was possible to demonstrate dTMP uptake by exponential-phase cells of the *tup7* mutant even over a short time course (35 pmol accumulated/10 min per 10^7 cells), but none by stationary-phase cells over the same period (data not shown). Normal *TUP* cells at either growth stage did not take up dTMP detectably under these conditions.

dTMP concentration affects uptake. The amount of dTMP taken up by the cells at different extracellular concentrations of the compound was measured. A double-reciprocal plot of the amount of dTMP taken up by the cells versus the concentration of dTMP yielded an apparent K_m for entry of 2 mM (data not shown). Over the 100-fold concentration range tested (0.02 to 2 mM), dTMP uptake did not appear to reach saturation; however, measurement of dTMP uptake at higher concentrations (>0.5 mM) of the nucleotide, which would presumably be in the saturating range, was difficult because such high concentrations were somewhat inhibitory to growth (data not shown). Equivalent concentrations of other pyrimidine 5'-mononucleotides, namely dUMP and dCMP, were not at all inhibitory to growth of the *tup7* strain. Growth of the wild-type strain X2180-1B was completely unaffected by the presence of any of these pyrimidine 5'-mononucleotides. This inhibitory effect of high external dTMP concentration on the growth of dTMP-permeable strains had been previously reported for certain *typ* mutants (22, 24).

The effect on dTMP permeability of other structurally related compounds was examined. The presence of a fivefold molar excess of thymidine did not detectably block dTMP uptake. Similarly, the presence of another 5'-mononucleotide, dUMP, did not prevent dTMP uptake at all (data not shown).

Since studies reported elsewhere (Bisson and Thorner, Genetics, in press) showed that the *tup7* mutation is a regulatory locus involved in the control of the cellular response to phosphate limitation, the effect of P_i on dTMP accumulation was examined. Phosphate had a pronounced inhibitory effect on dTMP uptake (Fig. 2). This effect was due specifically to the pres-

TABLE 3. Effect of pH on dTMP uptake by the *tup7* strain[a]

pH	Accumulation of dTMP (pmol/h per 10^7 cells)
5.8	86
4.8	220
3.8	390

[a] Cells of strain BT27C were grown in SD medium buffered with 50 mM succinate at the indicated pH value. Uptake was measured, using 1 mM [^{3}H]dTMP (3,000 cpm/nmol), over the course of 2 h.

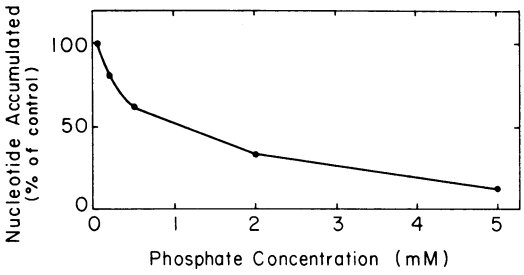

FIG. 2. Effect of extracellular phosphate concentration on dTMP uptake by *tup7* cells. Cells of strain BT27C were grown as before on medium A (pH 4.8), collected by centrifugation, washed three times with phosphate-depleted SD medium (30 μM PO_4^{-3}), and suspended in the same low-phosphate medium. P_i was added from a concentrated stock at pH 4.8 to the final concentration indicated along with [^{3}H]dTMP (0.2 mM; 5,000 cpm/nmol), and uptake was monitored over the course of 2 h.

ence of P_i in the medium and not to the presence of potassium or increased ionic strength generally, because KCl at equivalent or higher concentrations had no effect on dTMP accumulation. Although cells carrying the *tup7* or the other *tup* mutations are not more sensitive than their parent strains to growth inhibition by a wide variety of metabolic inhibitors (including cycloheximide, nystatin, and cordycepin), the *tup7* mutant was at least two to three times more sensitive to the phosphate analog arsenate, as determined by an agar diffusion assay (Bisson, Ph.D. thesis).

Nature of labeled dTMP inside of cells. To determine whether the ring-labeled dTMP was taken up as the monophosphate or as some other form derived from the monophosphate, cells of a *tup7* strain were grown in the presence of [^{3}H]dTMP, and soluble metabolites were extracted with formic acid and analyzed by thin-layer chromatography. Greater than 95% of the label in such extracts could be accounted for in species which comigrated with dTTP, dTDP, dTMP, and thymidine (Fig. 3). In every experiment, the majority of the label (40 to 70%) was present as dTDP, and a significant fraction (~10%) was present as dTTP, indicating that the incoming dTMP was efficiently converted to its more highly phosphorylated forms. Since the *tup7* strain does not taken up thymidine and does not have detectable TK activity (Bisson, Ph.D. thesis), the low proportion of the intracellular label present as thymidine (~6%) suggested that degradation of the internal pool of dTMP to the nucleoside is negligible (or at least much slower than the rate of dTMP phosphorylation). In this same regard, unlike what has been reported for several other dTMP-permeable mutants (9, 29), no detectable degradation of the [^{3}H]dTMP in the medium occurred even after 8 h of incubation with the *tup7* strain (data not shown).

Labeled dTMP is efficiently incorporated into DNA by the *tup7* strain. The incorporation of exogenous [*methyl*-^{3}H]dTMP into alkali-stable, acid-precipitable material was measured in the *tup7* cells (Table 4). The net increase in the total DNA content of the cells also was measured, and the amount of thymidylate residues needed to synthesize this amount of DNA was calculated, assuming an adenine-thymine content of 60% for yeast DNA (27). Under the various conditions used, 40 to >80% of the thymidylate residues in the newly synthesized DNA were derived from the exogenously provided dTMP. Although approximately 10 times more dTMP was apparently accumulated by the cells at the highest concentration of dTMP tested than at the lowest dTMP concentration, the net increase in efficiency of labeling of DNA was only approximately 25%. This finding suggests that the cellular pool of dTMP derived from de novo biosyn-

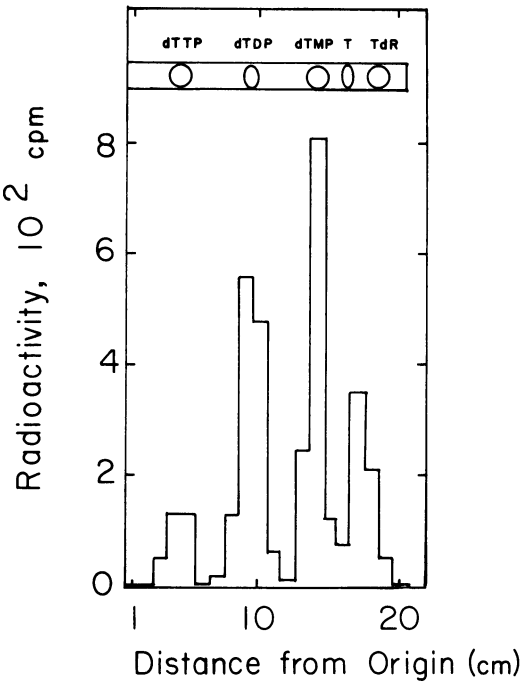

FIG. 3. Thymidine nucleotide pool of the *tup7* strain labeled with [^{3}H]dTMP. Cells of strain BT27C were grown for 3 h at 30°C in medium A (pH 4.8) containing 0.5 mM [*methyl*-^{3}H]dTMP (5,000 cpm/nmol) and harvested, and the nucleotides were extracted. Sample lane insert shows the approximate relative positions of authentic markers run simultaneously, as determined by UV absorbance. T, Thymine; TdR, thymidine.

TABLE 4. Uptake and incorporation of dTMP into DNA by the *tup7* strain[a]

dTMP (mM)	Total uptake (pmol/h per 10^7 cells)	Incorporation into DNA (pmol/h per 10^7 cells)	Thymidylate residues in new DNA derived from labeled dTMP (%)
0.1	30	11	60
0.2	75	12	70
0.5	160	13	70
0.7	300	12	70
1.0	360	15	85

[a] Cells were grown in medium A (pH 4.8) at 30°C. The specific activity of the dTMP (5,000 cpm/nmol) was held constant. Uptake of [^{3}H]dTMP, increase in cell number, incorporation of radioactivity into DNA, and synthesis of total cellular DNA were monitored over a 2-h time period.

thesis is very low and rapidly equilibrates with the dTMP taken up by the cells.

A double mutant carrying the *tup7* mutation and a mutation in the structural gene for thymidylate synthetase (*tmp1*) (2) was constructed. As expected, in the double mutant, essentially 100% of the thymidylate residues in newly synthesized DNA were derived from the exogenously provided dTMP (Table 5). This result supports our previous conclusion (2) that the thymidylate synthetase reaction is normally the sole source of dTMP in yeast cells.

Because the *tup7 tmp1* double mutant was a respiratory-deficient strain, the effect of the petite character on dTMP uptake was also measured (Table 5). A spontaneously arising petite derivative (B527C) was obtained directly from the respiratory-competent strain BT27C. The amount of dTMP taken up by the petite derivative was considerably lower than that taken up by BT27C. However, the amount of label incorporated into DNA in the two strains was approximately the same, again indicating that the cellular pool of dTMP from de novo biosynthesis is low. Respiratory capacity thus affects the level of uptake of dTMP by the strain, but not the efficiency of its subsequent utilization for DNA synthesis.

Strain BT27C was dramatically sensitive to growth inhibition by FdUMP (Bisson and Thorner, Mol. Gen. Genet., in press). Work in other systems (12, 20) has indicated that the mechanism of growth inhibition by FdUMP is due to inhibition of thymidylate synthetase, which depletes the cell of its dTMP pool. If this is indeed the case, then *tup7* cells should grow in the presence of FdUMP if dTMP is also provided, and, furthermore, incorporation of [^{3}H]dTMP into DNA should be enhanced. As anticipated, in the presence of FdUMP, essentially all of the thymidylate residues in newly synthesized DNA were derived from the exogenously supplied dTMP (Table 6).

DISCUSSION

Strains carrying the *tup7* mutation showed the highest level of permeability to 5'-mononucleotides of all of the *tup* mutants examined. The accumulation of dTMP occurred continuously during exponential growth, but stopped rather abruptly as the cells entered stationary phase. Jannsen et al. (21) and Brendel and Haynes (8) reported that a prolonged period was required before their dTMP-permeable strains accumulated detectable dTMP, which they ascribed to "medium conditioning." No such lag was observed before dTMP uptake by the *tup7* mutant occurred. However, in the studies presented here, dTMP uptake was examined in a minimal medium rather than in the rich medium used by these other workers. Uptake of dTMP by the *tup7* mutant was best at low pH values, as has also been reported for strains bearing the *typ1* mutation (15). This result suggests that the ionization state of the dTMP molecule is an impor-

TABLE 5. Effect of the *tmp1* mutation and petite character on dTMP accumulation and incorportion in a *tup7* mutant[a]

Strain	Total uptake (pmol/h per 10^7 cells)	Incorporation into DNA (pmol/h per 10^7 cells)	Thymidylate residues in new DNA derived from labeled dTMP (%)
BT27C (*tup7 rho*$^+$)	420	10	70
B510C (*tup7 tmp1 rho*$^-$)	60	16	105
B527C (*tup7 rho*$^-$)	20	6	80

[a] Conditions were as described in the footnote to Table 4, at a dTMP concentration of 1 mM.

TABLE 6. Effect of growth in the presence of FdUMP on dTMP uptake and incorporation into DNA in a *tup7* strain[a]

FdUMP	Total uptake (pmol/h per 10^7 cells)	Incorporation into DNA (pmol/h per 10^7 cells)	Thymidylate residues in new DNA derived from labeled dTMP (%)
None	350	10	80
10 μg/ml	320	15	120

[a] Before uptake was measured, the cells were grown in medium A with dTMP (50 μg/ml) in the presence or absence of FdUMP (10 μg/ml). Under these conditions, the extracellular dTMP concentration was sufficient to permit a normal growth rate. Cells were centrifuged, washed with fresh medium, and suspended in fresh medium containing 1 mM [^{3}H]dTMP (5,000 cpm/nmol), again with or without FdUMP (10 μg/ml).

tant factor in its transport, as suggested previously by others (15, 16). On the other hand, because transport of P_i in yeast occurs optimally at pH values above 6.0 (5), there is an alternative explanation: dTMP is transported via a phosphate permease, and at pH values below 6 competition between phosphate and dTMP for the transport system is reduced. Consistent with this interpretation, P_i was shown to be an effective inhibitor of the uptake of dTMP by the *tup7* strain. The *tup7* mutation has been identified as an allele of the *pho80* locus (Bisson and Thorner, Genetics, in press), which is a regulatory gene involved in the negative control of the synthesis of acid and alkaline phosphatase. Thus, it is unlikely that the *tup7* mutation creates directly a system for the transport of nucleoside monophosphates, but rather the *tup7* mutation results in derepression of a phosphate permease(s) with cross-specificity for certain classes of phosphate-containing molecules, including dTMP. We have been unable to demonstrate dTMP inhibition of transport of radioactive P_i, so it is possible that dTMP and phosphate enter by different mechanisms. On the other hand, the lack of detectable competitive inhibition by dTMP could be due to its poor affinity (K_m, ~2 mM) for the permease compared with that of phosphate. Indeed, yeast cells have been reported to have at least two phosphate-transporting systems: one with a K_m of about 30 μM and another sodium-dependent system with a K_m of 1 to 10 μM (30). Moreover, the uptake of phosphate is elevated in cells carrying the *tup7* mutation (Bisson and Thorner, Genetics, in press) just as it is in phosphate-starved cells (32), so both the *tup7* mutation and physiological phosphate limitation may result in the derepression of an additional phosphate-scavenging transport system(s) other than those previously studied.

The dTMP accumulated by the *tup7* strains was readily phosphorylated and incorporated into DNA. There was no apparent degradation of the dTMP in the medium by either the mutant strain or its wild-type parent. At the lowest concentration of dTMP tested (0.1 mM), about 60% of the thymidylate residues in newly synthesized DNA in a strain carrying the *tup7* mutation were derived from the dTMP provided in the medium. When the concentration of exogenous dTMP was raised to 2 mM, about 80% of the thymidylate residues in new DNA came from the medium. However, concentrations of dTMP above 0.5 mM were somewhat inhibitory to growth. This effect may represent limitation for one or more of the other deoxyribonucleotides due to allosteric inhibition of ribonucleotide reductase by the high internal pool of dTTP accumulated in *tup7* cells. Indeed, over this same concentration range there was a 20-fold increase in the total amount of dTMP taken up by the cells; hence, it appears that the endogenous pool of dTMP supplied by de novo biosynthesis is indeed low and is easily saturated by relatively low external concentrations of dTMP. A corollary to this finding is that the uptake of dTMP is apparently not regulated by the demand for DNA synthesis. All of the thymidylate residues in DNA were derived from the [^{3}H]dTMP provided in the medium when thymidylate synthetase activity was eliminated either by mutation (*tmp1*) or through inhibition by FdUMP.

McNeil and Friesen (25) have reported that the TK of herpes simplex virus (type 1) can be expressed in yeast when transcription of the gene on a plasmid is initiated from a yeast promoter. Although detectable TK activity is present in the cells, a rather high concentration of thymidine (~1 mM) is required to support the growth of cells in which de novo synthesis of dTMP has been blocked (by a *tmp1* mutation or by the addition of sulfanilamide plus methotrexate). Consequently, incorporation of [^{3}H]thymidine into DNA in such TK plasmid-containing yeast cells is very inefficient (~5% of the potential thymidylate residues) and requires long-term labeling (25). This situation is not surprising considering the known relative impermeability of yeast strains to this pyrimidine nucleoside (18). Thus, it has yet to be demonstrated that the introduction of a foreign TK gene provides any

advantage in studies requiring radiolabeling of yeast DNA.

In contrast, the *tup7* mutation seems to be a very useful tool for studies involving the specific labeling of DNA. There is little waste since [^{3}H]dTMP is not appreciably degraded either extra- or intracellularly. If a high specific activity of labeled dTMP is utilized, it is possible to measure readily rates of incorporation of label over very short time periods at modest external dTMP concentrations. DNA replication studies thus can be done in the absence of antifolate drugs. Strains carrying the *tup7* mutation have been recently used successfully for studies of nuclear and plasmid DNA replication (V. Zakian, personal communication), repair of radiation-induced chromosome breaks (4), and in density shift experiments substituting 5-bromo-dUMP for dTMP (W. Fangman, personal communication). In addition, the ability of *tup7* strains to take up high levels of [^{3}H]dTMP and 5-bromo-dUMP should allow the development of "suicide" techniques for the enrichment of auxotrophs and for the selection of mutants conditionally defective in DNA replication and other macromolecular syntheses.

Strains carrying the *tup7-1* allele may be obtained from the Yeast Genetic Stock Center, University of California, Berkeley, CA 94720.

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