AN ENRICHMENT FOR TEMPERATURE-SENSITIVE MUTANTS IN TETRAHYMENA THERMOPHILA

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ABSTRACT

The parameters for the killing of Tetrahymena by 5-bromodeoxyuridine (BUdR) and near-ultraviolet light have been determined. Significant preferential killing by UV* of cells that have incorporated BUdR was obtained when the cells were irradiated in a nonnutrient buffer. UV alone was found to be toxic to cells irradiated in growth medium. Mutants defective in division at a restrictive temperature were isolated from mutagenized cultures that had been treated with BUdR and UV and from mutagenized cultures that had no such treatment. Results indicate that the number of temperature sensitive (ts) growth mutants can be increased five to six times using the BUdR/UV treatment. Data are presented that indicate differences in the frequency of occurrence of various types of ts mutants, with and without enrichment. A mutant that immediately stopped macromolecular synthesis and cell division upon being placed at the restrictive temperature was more resistant to BUdR/UV treatment than wild type by 1000-fold. Using the above techniques, BUdR-resistant mutants altered in the phosphorylation of thymidine have been isolated.

THE unicellular protozoan Tetrahymena has been used as a model system for many biochemical and cell cycle studies (Hill 1972; Elliot 1973). Recently, genetic analysis has become routine (Orias and Bruns 1976) and mutants affecting the cell cycle (Frankel et al. 1976), oral development (Orias and Rasmussen 1976) and RNA and protein synthesis (Hallberg, Gritz and Sylvan 1978) have been obtained and used to investigate the processes in which they are defective.

With two independently controlled nuclei of the same genetic origin in the same cytoplasm (macronucleus and micronucleus) and with extrachromosomal rDNA genes capable of replication at a time when the bulk nuclear DNA is not replicating (Engberg, Mowat and Pearlman 1972; Anderson and Engberg 1975), Tetrahymena thermophila offers interesting and unique opportunities for the study of DNA replication and its control. In order to isolate mutants of this process, an enrichment giving an advantage in survival over wild-type cells to DNA replication mutants is desirable since mutants specifically defective in DNA

* Throughout this paper, “UV” refers to near-ultraviolet wavelengths of peak intensity at 365 nm, often referred to as “black light”.

replication have been found to be rare [e.g. 1 to 2% of ts mutants in *Saccharomyces cerevisiae* and Ustilago (Hartwell 1967; Unrau and Holliday 1970)]. The most successful enrichment for DNA synthesis mutants is based on the increased sensitivity to near ultra-violet (black light) conferred on cells by the incorporation of the thymidine analog 5-bromodeoxyuridine (BUdR). Cells defective in DNA replication do not incorporate BUdR and remain resistant to UV treatment. This enrichment has been used for isolating temperature-sensitive DNA synthesis mutants in *Escherichia coli* (Carl 1970), as well as auxotrophic or temperature-sensitive mutants in *Physarum polycephalum* (Haugli and Dove 1972), *Dictyostelium discoideum* (Kessin, Williams and Newell 1974), *Paramecium tetraurelia* (Peterson and Berger 1976) and mammalian cell lines (Chu, Sun and Chang 1972; Farber and Unrau 1975; Roufa and Reed 1975). BUdR-resistant mutants have also been isolated using this technique (Lunn, Cook and Haugli 1977).

We have determined the parameters for BUdR/UV enrichment using *Tetrahymena*. Mutants defective in division at a restrictive temperature were isolated with and without BUdR/UV enrichment. A method for the mass screening of temperature-sensitive mutants to isolate those specifically affected in DNA or RNA synthesis was developed. BUdR-resistant mutants were also isolated.

**MATERIALS AND METHODS**

**Strains:** The strains of *T. thermophila* (formerly *T. pyriformis*, syngen 1 [Nanney and McCoy 1976]) used were C*, a strain that induces genomic exclusion (Allen 1967), and the derivatives of inbred strain B, CU329 (Chz-2/Chz-2[cy s,II]) and CU324 (Mpr-1/Mpr-1[6mp s,IV]). These strains were obtained from Peter Bruns, Cornell University.

**Media:** The cells were cultured at 29° with shaking in a complex proteose peptone and yeast extract medium (PPY) (Leick, Engberg and Emmersen 1970) diluted to one-third of the original concentration. Defined medium (Rasmussen and Modeweg-Hansen 1973) with one-half the normal concentration of pyrimidines and 1% proteose peptone containing 250 μg per ml streptomycin sulfate (PP) were also used where noted.

**BUdR/UV treatment:** The cells were grown in defined medium at 29° without shaking for at least 12 hr before BUdR (Sigma) was added. The defined medium, which contains uridine as the pyrimidine source, has a low concentration of folinic acid (10^{-6} g per ml). The endogenous supply of thymidine compounds is thus limited and incorporation of exogenous BUdR into DNA facilitated (Lykkesfeldt 1975). After BUdR was added, the early log-phase culture was immediately shifted to 38°. After 12 hr in BUdR at 38°, the culture was washed twice by low-speed centrifugation into 10mM Tris-HCl, pH 7.4. Five ml of the cells were then placed in one of four compartments in a covered X-plastic petri dish (Falcon Plastics) underneath two Sylvania S40-BL fluorescent black lights and left for nine hr during enrichment. The lights were 5 cm above the black surface of the table and the dose rate was 2400 J/cm² as measured with a Blak-ray long-wave UV meter (model no. J221). The temperature under the UV apparatus was 32 ± 1°. To determine the fraction of cells surviving this or any other treatment, the cells were spread on agar plates and allowed to recover at 29° (Gardonio, Crerar and Pearlman 1975).

**Mutant isolation:** After growing CU324 or CU329 to about 10^6 cells per ml in PP at 29°, the culture was treated for three hr with 10 μg per ml N-methyl-N'-nitro-N-nitrosoguanidine (NG) (Aldrich Chemical Company) (Orias and Bruns 1976). The cells were washed into fresh PP without NG and allowed to recover for 24 hr at 29°. Mating and short-circuited
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Genomic exclusion were carried out to allow expression of micronuclear recessive mutations (Bruns, Brussard and Kavka 1976). We had more success with CU329 than CU324 when doing short-circuited genomic exclusion. Cycloheximide (25 μg per ml final concentration) or 6-methylpurine (15 μg per ml final concentration) was added to a culture of cells allowing only those that had successfully short-circuited to survive. The drugs were added to the bulk culture, so that a large number of mutated cells would be available for enrichment. After four days at 29°, the cells were washed out of the drug-containing medium and allowed to recover in defined medium. Nonenriched cells were picked at this time and tested for temperature sensitivity, as described below for cells that had undergone BUdR/UV treatment. BUdR/UV enrichment was carried out on the remaining cells and surviving cells were picked singly into drops of PPY on petri plates and placed at 38° for 16 to 20 hr. Clones with cells that did not divide at the restrictive temperature were noted and allowed to recover at 29°. Only mutants that could recover from the restrictive temperature would be enriched by BUdR/UV treatment. Therefore, these were the only type examined. Clones that recovered were transferred with a sterile toothpick into flat-bottomed microtiter plates (Falcon Plastics) and re-tested for temperature sensitivity. Only those clones with cells that failed to divide the second time at the restrictive temperature were examined further.

DNA, RNA and protein accumulation: DNA accumulation was monitored by adding 1 μCi per ml 3H-thymidine (50 Ci per mmole, New England Nuclear) to a 10 to 20 ml culture growing at 29° for 10 to 12 hr before sampling began. The culture was in early log phase when the first sample was taken. To measure acid precipitable radioactivity, duplicate 50 μl samples of culture were added to 50 μl of 1% sodium dodecyl sulfate (SDS) and 2 μl of 10 mg per ml Proteinase K (Merck). Samples were frozen until all time points were collected, then thawed and incubated overnight at room temperature. Seventy-five μl from each sample were spotted on Whatman 3MM filter discs, washed four times with 10 ml per disc of 1 N HCl containing 0.01 M sodium pyrophosphate, followed by two washes in 95% ethanol and drying. Acid-precipitable radioactivity was determined using a toluene-based scintillation solution in a liquid scintillation counter. Combined monitoring of DNA and RNA accumulation used the same procedure except that 0.1 μCi per ml 14C-thymidine (49.2 mCi per mmole, NEN) and 1 μCi per ml 3H-uridine (24.2 Ci per mmole, NEN) were used. To follow protein accumulation, 1 μCi per ml 3H-leucine (54.6 Ci per mmole, NEN) was used in the above procedure, but without proteinase digestion.

DNA/RNA ratio: The temperature-sensitive mutants were grown in test tubes to stationary phase. One drop of the culture was then placed into 1 ml of PPY (one to 20 dilution). After growth for eight to twelve hr at 29°, 0.2 ml of PPY containing 0.1 μCi 14C-thymidine and 1.0 μCi 3H-uridine was added to the culture, which was shifted to 38° for six hr. Acid precipitable radioactivity was determined and the ratio of 14C counts to 3H counts calculated. All ratios were normalized by first dividing by the ratio obtained for wild-type cells. A value of one was subtracted if the resulting ratio was equal to or greater than one. If the ratio was less than one, the inverse of the ratio was calculated and subtracted from one. Thus, ratios smaller than wild type are negative and on the same scale as those larger.

Thymine nucleoside to nucleotide conversion: Strains were grown to 80,000 cells per ml. Ten μCi per ml 3H-thymidine was added to 20 ml of culture, which was divided so that 10 ml was placed at 38° and 10 ml at 29°. After four hr, the cells were harvested by centrifugation and washed once with cold distilled water. To each pellet, 0.5 ml of 5% TCA was added, and the suspension was frozen in an acetone/dry-ice slurry, thawed and put on ice for 30 min. The precipitate was removed by centrifugation and the supernatant extracted three times with equal volumes of ether. The final extract was dried overnight and 20 μl of distilled water was added. The samples were spotted on PEI cellulose thin-layer strips (British Drug House) and developed with 1.6 M LiCl. The chromatographs were cut into 1.5 × 1.0 cm rectangles, placed in plastic counting vials, treated with 200 μl of 2 M ammonium hydroxide for 20 min and counted in a 1:1 PCS (Amersham):xylene scintillation cocktail.
RESULTS

Parameters of BUdR/UV enrichment: The parameters of the BUdR/UV enrichment were adjusted so that maximum killing was obtained with combined BUdR and UV treatment within a reasonable amount of time without obtaining a significant amount of killing with BUdR or UV alone.

High concentrations of BUdR were toxic to Tetrahymena grown in defined medium at 38° for more than nine or ten hours. The fraction of cells surviving BUdR treatment fell drastically between concentrations of 20 and 50 µg per ml BUdR. A concentration bordering on toxicity was tested to determine whether it would sensitize the cells to UV. The time in BUdR at 38° was varied and survival monitored. With four hr of UV treatment following 12 hr in the pres-

![Graph showing survival vs. hours of exposure to UV(BL).](image)

**Figure 1.**—Survival vs. hours of exposure to UV(BL). Two cultures of CU329 were grown in defined medium for 12 hr at 38°, washed into 10 mM Tris-HCl (pH 7.4) and exposed to UV. The fraction of cells surviving was determined using agar plates containing defined medium, as described in the text. The plating efficiency was 15 to 20%. (Δ—Δ), no BUdR; (○—○), 25 µg per ml BUdR.
ence of 25 μg per ml BUdR, a 100- to 1000-fold difference in survival can be obtained, compared with cultures that had been treated with either BUdR or UV alone. Higher concentrations of BUdR did not increase this difference. The time in BUdR at 38° must not be too long or temperature-sensitive mutants will not recover.

Tetrahymena were found to be resistant to more than nine hr of UV treatment when they were washed out of defined medium and into 10 mM Tris-HCl (pH 7.4) before irradiation. However, when irradiated in defined medium without BUdR, survival dropped rapidly, so that by two hr of UV treatment, the drop in survival was 1000-fold. The survival curve of cells that incorporated BUdR and were then subjected to increasing times of UV treatment in 10 mM Tris-HCl (pH 7.4) approximates a curve with one-hit kinetics (Figure 1). Although the slopes of the survival curves were constant between experiments, 25 μg per ml BUdR alone sometimes caused a drop in survival, so that up to a log more killing of cultures with BUdR and UV than shown in Figure 1 could be obtained. An attempt to increase the rate of killing was made by increasing the intensity of the UV by placing tinfoil underneath the petri plates. It was found that the higher intensity of UV increased the rate of killing of cells that had not incorporated BUdR more than that of cells that had incorporated BUdR. It is likely that the increased intensity of the UV saturates the repair capabilities of Tetrahymena.

Mutant isolation: A total of 305 temperature-sensitive mutants have been isolated, 139 of which did not undergo BUdR/UV enrichment and 166 of which did (Table 1). The method of isolation and screening used selects for mutants that stop growth quickly at the restrictive temperature and that are able to recover from the restrictive temperature. However, the frequency of induction of a particular mutation by a given mutagen cannot be accurately determined using this method since the mutagenized culture is allowed to grow before cloning, and a difference in growth rate may exist between the mutant and wild-type strain. Growth before cloning also increases the chance that sister mutants will be isolated (see Discussion).

The ratio between the cell concentration after six hr at 38° and the cell concentration just before the shift to 38° was determined for mutants from the third and fourth isolations (see Table 1). As can be seen in Figure 2, there is a considerable difference between the ratios for BUdR/UV enriched mutants and the nonenriched mutants. This indicates that the BUdR/UV treatment is enrich-

<table>
<thead>
<tr>
<th>Experiment number</th>
<th>Nonenriched</th>
<th>BUdR/UV enriched</th>
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<tbody>
<tr>
<td>1</td>
<td>34/3631 = 0.9%</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>35/889 = 3.9%</td>
<td>45/234 = 19.2%</td>
</tr>
<tr>
<td>3</td>
<td>26/951 = 2.7%</td>
<td>30/897 = 3.3%</td>
</tr>
<tr>
<td>4</td>
<td>44/942 = 4.7%</td>
<td>91/295 = 30.8%</td>
</tr>
<tr>
<td>Total</td>
<td>139/6423 = 2.2%</td>
<td>166/1426 = 11.6%</td>
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Figure 2.—Cell concentration ratios. Cells growing at 29° were shifted to 38°. Cell concentration was determined just before the temperature was shifted to 38° and after six hr at 38°, and the ratio of these values was calculated. (Note: DM114, a non-ts BUdR-resistant strain with the same growth kinetics at 29° and 38° as CU329 was used to determine the wild-type ratio in this experiment.)

ing for either very slow-growing cells or mutants that stop division rapidly (within a cell cycle). Detailed growth curves on ten mutants with various ratios indicated that the latter was the case; mutants with low ratios grew with an approximately normal generation time at 29°, but stopped growth immediately upon shift-up to the restrictive temperature.

A technique was developed for screening for mutants that are unable to synthesize DNA at the restrictive temperature, while retaining their ability to synthesize RNA. Using the ratio of 14C-thymidine to 3H-uridine accumulation at 38° as a measure of the relative rate of DNA and RNA accumulation at the restrictive temperature, mutants likely to be specifically defective in DNA (or RNA) synthesis can be selected relatively easily from a large number of cells. We were able to screen at least 135 mutants at a time using this technique.

The DNA/RNA ratio at the restrictive temperature of the ts mutants was determined. As can be seen in Figure 3, there seemed to be a greater deviation
FIGURE 3.—DNA/RNA ratios of mutants. $^{14}$C-TdR and $^3$H-Uri were added to cultures growing exponentially at $29^\circ$, which were immediately shifted to $38^\circ$. After six hr, counts in acid precipitable material were determined, and normalized DNA/RNA was calculated as described in the text. This figure contains data using 170 mutants obtained from three independent isolations. The labelled arrows indicate the ratios of the mutants examined in Figure 4.

from the mean in mutants isolated after BUDr/UV treatment than in those isolated without enrichment. The variation seen in the nonenriched mutants was probably not significant. A mutant that stopped RNA or DNA synthesis preferentially would likely have a ratio outside the range of ± 1. Five mutants isolated after BUDr/UV enrichment were chosen for further examination as representatives of the various ratio (Figure 3 and 4a-e). The one with the lowest DNA/RNA ratio, DM331, did not incorporate thymidine into acid precipitable material at $29^\circ$ or $38^\circ$ and would be expected to be resistant to BUDr. Cell division slowed down immediately upon temperature shift-up (Figure 4a). We had previously isolated BUDr-resistant mutants that were not temperature sensitive in cell division. The low DNA/RNA ratio of DM155 made it the most likely candidate for a DNA replication mutant, whereas DM252 with its high ratio might be expected to be specifically defective in RNA synthesis. Figure 4b shows that DM155 indeed had the expected phenotype of a DNA replication mutant. Figure 4e shows that RNA accumulation decreased with time in DM252, whereas all other
Figure 4.—Label incorporation into macromolecules in selected mutants. Exponentially growing cultures were incubated for 16 to 20 hr at 29° in \(^3\)H-Leu or \(^{14}\)C TdR and \(^3\)H-Uri. At zero hours, the cultures were divided into two, with one being placed at 38° and the other at 29°. Cell concentration (cells per ml) and counts per minute (CPM) were determined at intervals for nine hr after the temperature shift. \(^3\)H-Leu incorporation was determined in a separate experiment from the growth curves and \(^{14}\)C-TdR and \(^3\)H-Uri incorporation. The scale is such that one division on the ordinate indicates a doubling in CPM or cells per ml. Note: There is no incorporation of \(^{14}\)C-TdR in DM331. \(^3\)H-Uri, (○—○) 29°, (●—●) 38°; \(^{14}\)C-TdR, (△—△) 29°, (▲—▲) 38°; \(^3\)H-Leu (□—□) 29°, (■—■) 38°; cell concentration, (△—△) 29°, (▼—▼) 38°. Cell types (a) DM331, (b) DM155, (c) DM260, (d) DM188, (e) DM252, (f) CU329.
macromolecular accumulation stopped and remained constant at the restrictive temperature. Two mutants, DM188 and DM260, which had DNA/RNA ratios similar to wild type, were also examined. Figures 4c and 4d show that although the DNA/RNA ratio for these cells were approximately the same, DM188 stopped all macromolecular accumulation immediately upon temperature shift-up, whereas in DM260 there was only a very gradual shutoff.

DM155 was examined in more detail. Using the same conditions as in the original enrichment (nine hr UV), a BUdR/UV enrichment of about 10,000-fold over the wild-type strain was seen. When the cells were examined microscopically, it was found that DM155 greatly increases in size at the restrictive temperature, and the micronuclei keep dividing even though the macronucleus and cell have stopped division. After five hr at 38°, as many as eight micronuclei were seen in single cells.

An F1 cross and a backcross were carried out. In the F1 cross, DM155 [ts/ts \(Mpr^+/Mpr^+\) (tm s, 6 mp s)] was crossed with CU324, a functional heterokaryon (BRUNS and BRUSSARD 1974) [ts+/ts+ \(Mpr^-/Mpr^-\) (tm r, 6 mp s)]; 61% (147/240) of the pairs survived, but only 1.4% (2/146) of these completed mating, as determined by the acquisition of resistance to 6-methylpurine. The F1 progeny were found to be temperature resistant in growth (tm r). DM155 therefore carries a recessive temperature-sensitive (ts) gene for division.

An F1 that had phenotypically assorted (NANNEN and DUBERT 1960; SONNEBORN 1974) to 6-methylpurine sensitivity was crossed to DM155 and pairs picked. Those that were resistant to 6-methylpurine were chosen, since they would be true conjugants. In this cross 68% (163/240) of the pairs survived. Since 55 of the pairs were resistant to 6-methylpurine, about 67% (110/163) of the surviving pairs completed mating. An approximately 1:1 ratio of progeny ts in division to non-ts in division was found (30 ts:25 non-ts). To determine whether the temperature sensitivity in division and in thymidine accumulation was due to the same gene, ten backcross progeny ts in division and ten not ts in division were tested for their ability to incorporate thymidine at the restrictive temperature. It was found that five of the ten progeny ts in division and four of the ten progeny not ts in division were also defective in thymidine incorporation into DNA. Therefore, the phenotype of DM155 must be due to two independently assorting genes, one ts in cell division only (div) and one ts in thymidine incorporation (tdr).

The inability of DM155 to incorporate thymidine into DNA at 38° could be due to a lack of thymidine transport into the cell or to the inability of the cell to phosphorylate thymidine. To answer this question, labelled thymidine was added to cultures of DM155 at 29° and 38° and left for four hr. The cells were washed free of medium containing label and the acid soluble cell extracts chromatographed on PEI cellulose. Thymidine was found to enter the cell at both 29° and 38°, but it was phosphorylated only at 29°. A mutant previously isolated as being BUdR resistant (DM114) also allowed thymidine to enter the cell, but did not phosphorylate it at either 29° or 38°.

A reconstruction experiment was done to determine whether a mutant that stops DNA synthesis upon temperature shift-up would be enriched for by
B UdR/UV treatment. DM307, a mutant isolated after BUdR/UV enrichment in experiment 4 (Table 1) was used. Upon temperature shift-up, this mutant stops all macromolecular synthesis and degrades RNA in the same manner that DM252 does (see Figure 4e). Using the same conditions for the original enrichment of DM307 (12 hr in 25 μg per ml BUdR at 38° followed by nine hr of UV), a 1000-fold difference in survival between CU329 and DM307 is seen when the slopes are extrapolated to nine hr UV (Figure 5). Clearly, DM307 is strongly enriched for by BUdR/UV treatment.

DISCUSSION

We have determined the parameters for obtaining significant preferential killing by UV of cells that have incorporated BUdR. A concentration of BUdR was used that is significantly toxic to the cells only when they are irradiated

![Figure 5](image-url)

**Figure 5.**—B UdR/UV treatment of DM307: Separate cultures of DM307 and CU329 growing in defined medium were placed at 38° for 12 hr after addition of 25 μg per ml BUdR. The fraction of cells surviving UV treatment was determined by plating as described in the text. (O—O) DM307, (●—●) CU329.
with UV. This was done because the toxicity of BUdR alone might be due to factors unrelated to its substitution into DNA (Bick and Davidson 1974). We found that UV alone is very toxic to cells that are irradiated in growth medium. This observation has also been made with mammalian cells and has been attributed to the photodynamic effect of compounds such as riboflavin and tryptophan in the medium (Wang, Stoien and Landa 1974). Cells irradiated in a non-nutritive buffer are resistant to the doses of UV used in these experiments, except when they have incorporated BUdR.

A method of isolating temperature-sensitive mutants has been used that selects cells that stop division rapidly at the restrictive temperature and that are able to recover on return to the permissive temperature. Although the method does not eliminate the possibility of picking sister mutants, this problem is minimized by screening only a portion of the survivors of mutagenesis and short-circuited genomic exclusion. BUdR/UV enrichment greatly increases the chances of sister-mutant isolation, but since we are looking for a particular type of rare mutation, the advantage offered by a simple isolation procedure outweighs the drawback of possible sister-mutant isolation.

Isolation of 305 temperature-sensitive strains with and without BUdR/UV enrichment indicated that the percentage of temperature-sensitive cells can be increased five- to six-fold with BUdR/UV treatment. The percentage of these ts mutants that stopped division within a cell cycle after temperature shift-up was higher in the BUdR/UV enriched group (see Figure 2). A mutant that immediately stopped DNA synthesis, as well as RNA and protein synthesis upon temperature shift-up, was found to be resistant to BUdR/UV treatment. In a reconstruction experiment using the same conditions as when it was originally isolated, DM307 was found to be approximately 1000-fold more resistant than a wild-type strain to BUdR/UV treatment. Also the differences found in the DNA/RNA incorporation ratios at 38° between enriched and nonenriched mutants imply differences in the frequency of occurrence of various types of mutants. The ability of the ratio method to pick out a mutant that mimics the expected phenotype of a DNA replication mutant is encouraging.

The finding that DM155 was a double mutant, temperature sensitive in cell division and in thymidine phosphorylation, emphasizes the advisability of carrying out a genetic analysis on interesting mutants. This type of double mutant is strongly enriched for by the procedures used, since we examine only ts growth mutants, and mutants resistant to BUdR are very strongly enriched for by BUdR/UV treatment. As can be seen in Figure 3, nine of the 75 BUdR/UV-enriched ts growth mutants tested did not incorporate thymidine into DNA at 29° or 38° and are likely double mutants, with one mutation being responsible for their ts growth and the other responsible for the lack of thymidine and BUdR phosphorylation. When examining potential DNA replication mutants, the possibility of the loss of thymidine incorporation into DNA due to such things as a block in thymidine phosphorylation must be accounted for. Measuring DNA synthesis by a method other than thymidine incorporation into acid precipitable material should routinely be done on these mutants.
Mutants conditionally and nonconditionally defective in the phosphorylation of thymidine have been isolated with the aid of BUdR/UV treatment or with toxic amounts of BUdR alone. In DM155, thymidine phosphorylation was found to be recoverable after a return to the permissive temperature. Mutants deficient in thymidine phosphorylation are of interest because they are the first isolated in Tetrahymena that are defective in a DNA replication pathway. They will be useful in the isolation and study of the thymidine kinase or nucleoside phosphotransferase (BOLS and ZIMMERMAN 1977) in this organism.

We have looked for mutants that are unable to synthesize DNA at the restrictive temperature, while retaining their ability to synthesize RNA. This was done because this phenotype, if found, would be unambiguously implicated in DNA synthesis. However, other phenotypes for DNA replication mutants probably exist. For example, JOHNSTON and GAME (1978) found that the mutants with defects in DNA synthesis isolated in S. cerevisiae also stopped RNA synthesis, but not protein synthesis. We have isolated mutants that immediately stop DNA, as well as RNA and protein, synthesis upon shift-up to the restrictive temperature, and they are strongly enriched for by BUdR/UV treatment. The search is continuing for ts mutants that stop only DNA synthesis upon temperature shift-up and also for mutants that stop DNA and RNA synthesis, but not protein synthesis, at the restrictive temperature. If these types of mutants exist, it is hoped that they will be useful in studies in our laboratory on the replication of rDNA in Tetrahymena (GANZ, CHENG and PEARLMAN, in preparation).

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