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Selection of Yeast Auxotrophs by Thymidylate Starvation

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A rapid procedure for the recovery of *Saccharomyces cerevisiae* auxotrophs was developed by exploiting the protection of these mutants from thymineless death when a required metabolite was withheld. The method can be used for thymidine 5'-monophosphate-requiring auxotrophs or wild-type strains blocked in de novo synthesis of thymidylate by folate antagonists.

Nutritional auxotrophs of *Saccharomyces cerevisiae* can be readily obtained by using chemical or physical treatments that increase mutation frequencies to conveniently detectable levels. One disadvantage of this approach is that the mutagenic treatments can induce mutations at several loci within a single cell. Consequently, they are of limited use in the construction of strains differing in genotype at a single locus but remaining otherwise isogenic. A procedure for selecting spontaneous auxotrophs by using inositol-less death has been employed with success in some laboratories but requires that the inositol markers be first crossed into an appropriate background (1).

Thymineless enrichment of auxotrophic mutants depends upon the same general strategy as that used in the inositol-less procedure. Growing yeast cells in which deoxythymidine 5'-monophosphate (dTMP) biosynthesis is abolished either by mutation (*tmp* mutants) or by folate antagonists (aminopterin and sulfanilamide) undergo thymineless death (J. G. Little and R. H. Haynes, manuscript in preparation). Cells harboring mutations that result in a block in ribonucleic acid or protein synthesis are relatively immune. One or more cycles of thymineless killing can therefore be used as an intense enrichment for such mutants. Since *S. cerevisiae* strains appear to be generally sensitive to the combination aminopterin plus sulfanilamide, we suppose that this method may be of general utility.

The selection principle is illustrated in Fig. 1, which shows the thymineless death kinetics of a methionine-requiring dTMP auxotroph deprived of dTMP in minimal medium in the presence or absence of exogenous methionine. After 24 h in the presence of methionine, approximately 0.5% of the cell population remained viable. In the absence of methionine no cell death occurred.

Depletion of cellular tetrahydrofolates occurs when wild-type yeast cells are treated with aminopterin plus sulfanilamide. This results in growth arrest. The biosynthetic pathways in yeast of adenine, histidine, methionine, and dTMP are tetrahydrofolate dependent. In strains permeable to dTMP, "rescue" of cells from folate antagonists is effected when these four metabolites are supplied exogenously (Little and Haynes, manuscript in preparation). If dTMP is withheld or if the strain is impermeable to dTMP, provision of adenine, histidine, and methionine to the drug-treated culture induces thymineless death (Fig. 2). Methionine withdrawal again confers substantial protection against killing under these conditions. Spontaneously occurring mutants in the population that are auxotrophic for unsupplied metabolites are at a selective advantage. Table 1 shows that a high proportion of the cells surviving one or two cycles of dTMP starvation, effected either by dTMP withdrawal or by folate antagonists, were auxotrophic. Randomly selected auxotrophs were scored for nutritional requirements. Mutants auxotrophic for either methionine, lysine, leucine, histidine, tyrosine, or uracil were recovered.

The experimental procedure for wild-type cells was as follows. Exponential cells grown in minimal medium at 30°C were harvested by filtration, washed in 0.1 M phosphate buffer, and suspended at approximately 10^6/ml in fresh medium containing aminopterin (100 μg/ml) and sulfanilamide (5 mg/ml) and supplemented with adenine sulfate (30 μg/ml), L-methionine (20 μg/ml), and L-histidine (20 μg/ml). Aminopterin, which is poorly soluble in water, can be dissolved by adding drops of 1 N sodium hydroxide followed by reneutralization to pH 7.0 by 1 N hydrochloric acid. (Since LL5 cells are permeable to dTMP, they were pregrown in minimal medium containing the drugs plus the four required metabolites.) The cells were starved for thymidylate in this medium for 24 h. They were then collected by filtration, washed in 0.1 M phosphate buffer, and transferred to complete

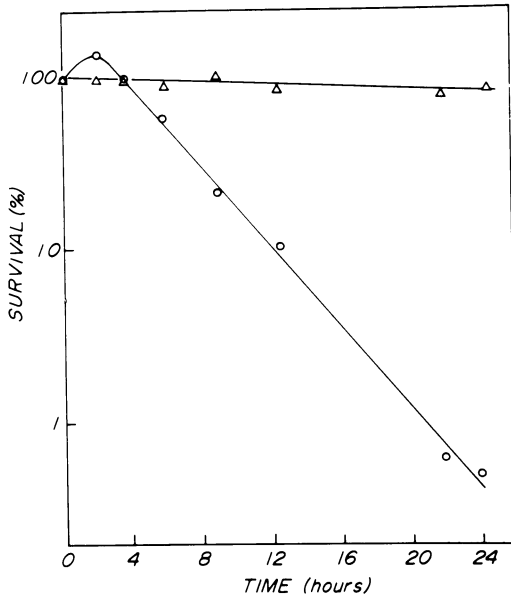

FIG. 1. *Effect of amino acid starvation on thymineless death in yeast. Exponential cells of a methionine auxotroph isolated from strain ML 1-4 by thymineless enrichment were starved for dTMP at 34°C in the presence (○) or absence (△) of L-methionine (20 μg/ml).*

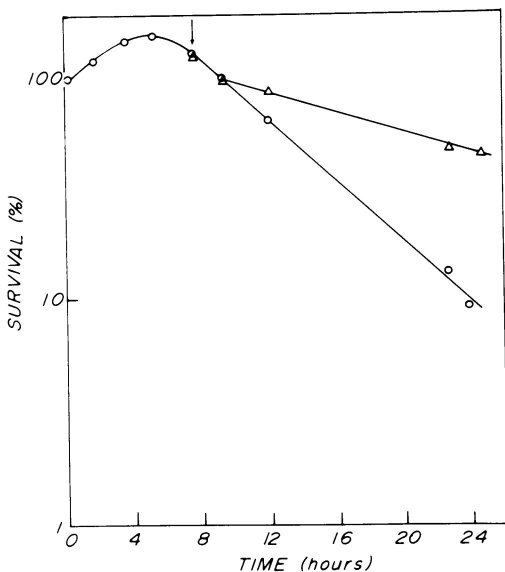

FIG. 2. *Effect of amino acid starvation on the viability of wild-type yeast cells during tetrahydrofolate depletion. Exponential LL5 cells were incubated at 30°C in minimal medium containing aminopterin (100 μg/ml), sulfanilamide (5 mg/ml), L-histidine (20 μg/ml), and adenine sulfate (30 μg/ml), with (○) or without (△) L-methionine (20 μg/ml). Methionine was withdrawn at the time indicated by the arrow.*

TABLE 1. *Recovery of auxotrophs*

Strain	Genotype[a]	Cycles	Auxotrophs (%)
ML 1-4	*α ρ⁻ tmp1-4 tup*	2[b]	1.1
308/6C	**a** *ρ⁺ tmp1-6 tup his1,7 ilv1-92 lys1 trp5*	2[b]	12.5
LL5	*α ρ⁺ tup*	1[c]	0.4

[a] *tmp1* designates dTMP auxotrophy. *tup* confers dTMP permeability. Other markers are represented by the standard notation (3).

[b] Thymineless death induced by dTMP withdrawal in a dTMP auxotroph.

[c] Thymineless death induced by aminopterin and sulfanilamide in a dTMP prototroph.

medium. After growth to stationary phase, the cells were collected, washed, and diluted to 10^6/ml in minimal medium containing the drugs plus adenine, histidine, and methionine, and a second cycle of dTMP starvation was completed. After growth to stationary phase in complete medium, cells were spread on YPD agar plates after appropriate serial dilution, and emergent colonies were replica-plated on minimal and complete agar plates. Putative auxotrophs were streaked on supplemented minimal agar plates to identify their nutritional requirements, by the method of Holliday (2). The thymineless enrichment procedure was similar when dTMP auxotrophs were used. Exponential cells were starved for dTMP in minimal medium, followed by a growth period in complete medium supplemented with dTMP (100 μg/ml). Complete medium (YPD) contained (per liter): yeast extract, 10 g; peptone (Difco), 20 g; and glucose, 20 g.

By this procedure it is possible to isolate rapidly large numbers of auxotrophs and identify their nutritional requirements. The mutants arise spontaneously, since dTMP starvation does not appear to be mutagenic for nuclear genes in yeast (Barclay and Little, manuscript in preparation). Although we have not attempted to isolate temperature-sensitive mutants defective in macromolecular synthesis, it may be possible to do so by starving cells for thymidylate at an elevated temperature, followed by growth under permissive conditions.

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