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REVIEW

Current Methods for *Saccharomyces cerevisiae*[1]

I. Growth

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Interest in the study of yeast biology has increased dramatically in the past few years. Since these organisms are eukaryotic, some phenomena observed in yeast may provide a useful model for similar phenomena in multicellular organisms. Yeast has several advantages as an experimental organism and many methods used for bacteria can be adapted to them. Yeast is simple to grow, cultures are easily maintained, and classical and molecular genetic techniques can be used. The ability to approach problems genetically and biochemically has lead to substantive progess with this group of organisms in areas such as cell biology (1) and gene expression (2). This review is intended to introduce investigators to practical techniques for the growth and radioactive labeling of yeast, primarily of *Saccharomyces cerevisiae.* For genetic techniques, readers are referred to a recent laboratory manual (3) and reviews (4,5).

STRAINS

The nature of the specific strain to be used is an important consideration. Here, only strains of *S. cerevisiae* are considered but *Schizosaccharomyces pombe* might be a wise choice for studies of the cell cycle (6) or of suppressor tRNAs. Strains with special fermentative or growth properties such as *Saccharomyces diastaticus* or *Schwanniomyces* sp. (e.g., *S. castellii*) for starch utilization may have special merit.

It is important to begin with pure cultures of well-characterized strains. Commercially available packaged yeast are contaminated with bacteria and are usually a mixture of strains. Mutants are necessary for many studies and isogenic or closely related strains should be used as controls.

One may choose to work with haploids or diploids. Haploids that stably maintain the genetic locus for mating type (*MAT*) as either *MAT***a** or *MAT*α are called heterothallic. *MAT***a** cells will mate with *MAT*α cells by fusion to produce *MAT***a**/*MAT*α diploids that can be propagated in the same way as haploids. Haploids that switch mating types are designated as homothallic. Cultures of the latter cells are maintained as diploids (for a review of mating type see (7)). Most commonly used strains are available from the American Type Culture Collection (Rockville, MD) or the Yeast Genetics Stock Center (Donner Labs, University of California, Berkeley, CA). Among the widely available haploid strains are D273-10B (ATCC 24657 used for many studies on mitochondria) and S288C (*MAT*α, *mal, gal2*). For sporulation studies, diploids SK-1 ((8); homothallic) and AP-1 ((9); heterothallic; iso-

[1] A group of reviews on yeast organized by Sanford J. Silverman.

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genic strains homozygous at *MAT* available) and Y55 ((10); homothallic) can be used. Strains lacking several major proteinases are also available (11).

When strains bearing an autonomously replicating plasmid expressing a gene of interest are to be used, steps must be taken to avoid plasmid loss. Attention should be given to copy number per cell and selection media that allow retention of the plasmid. Copy number of some yeast plasmids is affected by the presence of the 2μ plasmid, an episomal genetic element that most laboratory strains of *S. cerevisiae* harbor (12). Nutritional selection against plasmid loss can be achieved by using a strain deficient in a gene that is contained on the plasmid. The strain is then grown in a medium lacking the nutrient whose synthesis is dependent on the plasmid gene. Plasmids that carry genes required for resistance to a drug will also be maintained (13).

Many strains carry the "killer" plasmid, a double-stranded RNA in a virus-like particle that confers on its host the ability to secrete a protein toxic to other yeast. A large number of genes control the "killer" character (14).

MITOTIC CYCLE

The stages of the mitotic cell cycle of diploid or haploid cells are similar and are defined by morphological and molecular events. The precise timing of "landmark" events is still an area of research interest (15) but the general picture is clear. The initiation of a new cycle ("Start") is followed by an S (DNA synthesis) phase and the emergence of a new bud. A growth (G2) phase and nuclear migration precede mitosis and nuclear division. Cell separation (cytokinesis) occurs and the G1 phase begins, during which the new cell enlarges.

Stored yeast cells are usually arrested in the G1 phase and there is generally a lag before exponential growth is attained in a fresh medium. Mutants in the cell division cycle (*cdc* mutants)[2] exhibit stage-specific defects and result in uniform terminal phenotypes (16). These mutants, along with specific inhibitors of cell growth (see *Synchrony*), can be used to study the physiological events of the cell cycle. Starvation for many different nutrients (e.g., carbon, nitrogen, sulfur, phosphorus, biotin) results in a cessation of development in the G1 phase.

GROWTH

Glycolysis

S. cerevisiae will grow on a wide variety of fermentable carbon sources including glucose, fructose, and mannose. The best growth occurs in glucose-rich (>2%) media. Under such conditions many mitochondrial and other functions, e.g., other sugar-degrading or transporting enzymes, are repressed (carbon catabolite repression), and glycolytic enzymes comprise a high percentage of soluble protein (for a review see (17)).

The most common rich medium for the growth of yeast is YEPD (1% yeast extract, 2% peptone, 2% glucose (dextrose)) in liquid or solid (2% agar) form. The optimal growth temperature is usually 30°C. Generation times of about 90 min and yields of up to 20 g wet wt of cells (about 3.7 g dry wt; 2×10^{11} cells) per liter of aerated culture can be expected from haploid cells at stationary phase (usually achieved by overnight growth). Diploids are larger and their yields per cell will be roughly 80% higher than those of haploids. Many mutants grow more slowly than prototrophs and will take a longer time to achieve a high density. Yields of macromolecules vary, but approximate values are on the order of the following, cited per gram (dry

[2] Abbreviations used: *cdc* mutants, mutants in the cell division cycle; YEPD, yeast growth medium consisting of 1% yeast extract, 2% peptone, and 2% glucose (dextrose); SD, synthetic dextrose; YNB, yeast nitrogen base; Mes, 2-(*N*-morpholino)ethanesulfonic acid; DAPI, 4',6-diamidino-2-phenylindole; SDS, sodium dodecyl sulfate.

wt) of stationary phase cells: DNA, 1 mg (18); RNA, 100 mg (19); protein, 400 mg (19). Synthetic media, necessary for most labeling and metabolic studies, provide lower overall yields. One common minimal medium (synthetic dextrose medium or SD) contains 2% glucose and 0.67% Difco yeast nitrogen base (YNB). The latter is supplied in more than one form. YNB "without amino acids" does not contain the small amount of histidine, methionine, and tryptophan present in YNB. These small quantities can complicate labeling experiments, but such low concentrations may not allow the growth of strains (auxotrophs) that require any one of these amino acids. Auxotrophic strains require additions of nutrients such as amino acids or nucleic acid precursors to at least 20 μg/ml.

A semisynthetic medium, used for studies with mitochondria, contains galactose (0.3%) as carbon source (minimizing carbon catabolite repression), but is supplemented with 0.3% yeast extract (which may contain a small amount of glucose), 0.04% $CaCl_2$, 0.05% NaCl, 0.07% $MgSO_4 \cdot 7H_2O$, 0.1% KH_2PO_4, 0.12% $(NH_4)_2SO_4$, and 0.0005% $FeCl_3$. The generation time in this last medium is typically 150 min with yields of 5 to 6 g/liter (20). A medium similar to synthetic dextrose but with galactose instead of glucose may have advantages in radiolabeling experiments, but slower growth rates can be expected. Vigorous aeration of cultures is important for maintenance of mitochondrial function (20). The isolation of mitochondria and mitochondrial subfractions such as matrix and inner and outer membranes may be achieved by standard procedures (21). Monitoring the efficiency of the separation of mitochondria from other organelles can be achieved by a variety of enzyme assays (22).

Gluconeogenesis

For many metabolic studies it is desirable to grow yeast under gluconeogenic conditions (23). Carbon sources such as pyruvate, acetate, ethanol, or dihydroxyacetone are commonly used at 100 to 200 mM. Yeast extract (1%) and peptone (2%) may serve as a nitrogen source. Alternatively, cells can be supplemented with 0.67% YNB.

The growth of yeast will cause acidification of the medium. Where the pH of the medium may be a factor in carbon source availability, buffers such as 2-(*N*-morpholino)ethanesulfonic acid (Mes, e.g., 0.1 M at pH 6.0), potassium phthalate (e.g., 0.05 M at pH 5.0), or sodium succinate (0.08 M at pH 5.5; not metabolized; E. Cabib, personal communication) can be added.

Sporulation of *S. cerevisiae* requires adaptation to gluconeogenesis. To obtain spores, it is sufficient to transfer sporulation-competent cells growing in a rich (glucose) medium to a medium lacking a nitrogen source, such as 1% potassium acetate. Supplementation of the latter with 0.1% yeast extract and 0.05% glucose may increase the efficiency of sporulation. The media for sporulation may be solid (agar, 2%) or liquid. For a review of sporulation methods, see the accompanying article (24).

Anaerobiosis

S. cerevisiae is a facultative anaerobe. Thus, cells with defective mitochondria are viable, allowing a genetic dissection of mitochondrial functions. There is also substantial interest in the expression of oxygen- and heme-regulated genes under aerobic and anaerobic conditions. Yeast can be grown under strict anaerobic conditions that lead to the production of nonrespiring mitochondria. However, supplements of oleic acid and ergosterol are needed (Tween 80 (polyoxyethylene sorbitan mono-oleate), 2.6 g/liter, and ergosterol, 12 mg/liter). Anaerobic growth of *S. cerevisiae* can be achieved in fermentors (25). Alternatively, cells may be inoculated into YEPD or the above-mentioned galactose synthetic media, for example, in a flask that can be closed to form an air-tight seal. Nitrogen is vigorously bubbled

through the suspension for 15 min. The flask is sealed and shaken for the desired time (26).

Synchrony

The spectrum of metabolic events that are tied to the cell cycle can often be studied best with synchronous cultures. Such cultures are obtainable by the physical separation of cells at different stages in the cell cycle or by arresting whole cultures at a specific stage and shifting back to permissive conditions. For example, cultures may be arrested in the S phase by the addition of inhibitors of DNA synthesis such as hydroxyurea (27). The addition of mating pheromone to responsive haploids will arrest cells late in G1 near Start (see (28) for a review of mating pheromones). Certain *cdc* mutants, when arrested briefly at the nonpermissive temperature, will resume relatively synchronous growth from one point in the cell cycle (e.g., *cdc4* for synchronous DNA synthesis).

A variety of techniques can be used for the separation of cells of different size, i.e., presumably at different stages of the cell cycle. Among such methods are gradient centrifugation (29,30) and elutriation centrifugation (31). Physical separation methods and physiological arrest methods exert different types of stress on cells so that comparison between experiments using different synchronization procedures should be approached with caution. Unfortunately, good synchrony cannot be maintained for much longer than one or two generations.

Monitoring Growth

Growth of yeast, like that of any microorganism, can be followed by such conventional methods as light scattering in a spectrophotometer, by direct cell counting in a hemocytometer, or with a Coulter counter. Scattering can vary greatly with cell number because the cells may form clusters (often alleviated by vortexing) and because cell volumes differ; e.g., haploids are smaller than diploids. In addition, the geometry of the cell suspension vessel (e.g., test tube versus spectrophotometer cuvette) will affect the optical density determination.

The percentage of budded cells is often a good indication of the state of a growing culture. During the exponential growth of a nonsynchronized culture only part of a population is budded. The percentage of budded cells depends on the growth rate and is generally the highest in a glucose-containing medium. At stationary phase nearly all cells are unbudded. A good phase-contrast microscope will allow examination of the relatively large structures that constitute buds and vacuoles. Live cells are phase bright and lysed cells are phase dark (see Methods of Analysis). In addition to examining the morphology of growing cells by phase microscopy, it is useful to stain cells and their organelles. Trypan blue and methylene blue (32) are useful for monitoring viable cell count. For trypan blue staining, cells are collected by centrifugation and resuspended in 0.2 M KH_2PO_4 containing 0.1 μg/ml of the dye (33).

The most common fluorescent stain for nuclei is DAPI (4′,6-diamidino-2-phenylindole). DAPI may simply be added to a concentration of 1 μg/ml to a small aliquot of cells in growth medium. Alternatively, cells may be fixed in 70% ethanol for 30 min, washed in water, and suspended in water containing DAPI at 0.1 to 0.5 μg/ml. A fluorescence microscope with a standard ultraviolet excitation filter (e.g., 365 nm) should be used to detect the blue fluorescence of the DAPI/DNA complex. Mitochondria are also detected with this dye ((34); see also (35) for other fixation and fluorescent staining procedures). Mithramycin is a second useful fluorescent indicator for nuclei (36).

STORAGE

Most strains of *S. cerevisiae* remain viable on plates or in liquid medium for a few weeks at 4°C. Cells will retain viability for several months on slants of YEPD–agar me-

dium and can be stored for extended periods at −70°C in 15 to 50% glycerol in water or YEPD. Some mitochondrial mutants give rise to *rho*$^-$ cells (mitochondrial DNA deletions (37)) during storage. The latter strains can be stored for 2 to 3 years at −70°C if 0.2 ml of a fresh culture is added to 0.6 ml of a solution containing 2% glucose, 25% glycerol, 1% peptone, and 1% yeast extract.

RADIOACTIVE LABELING

Proteins

The transport and compartmentation of metabolites are primary concerns when designing an investigation involving growth or sporulation of yeast (for a review see (38)). The vacuoles contain pools of many metabolites, including most amino acids. Thus, in order to label macromolecules to a high specific radioactivity it may be necessary to grow cells in the presence of radioactive precursors for many generations. Auxotrophs can be starved for a specific nutrient that must be added in radioactive form to increase labeling efficiency. Although cell proteins can be uniformly labeled with most ^{14}C or ^3H-labeled amino acids, the rate of isotopic equilibration varies greatly among different amino acids, methionine being the fastest (about 30 s (39)). [4,5-^{3}H]Leucine at a concentration of 1 mCi/ml of semisynthetic medium will yield approximately 10^6 dpm/mg of protein (20). Higher specific activities can be achieved by labeling with $^{35}SO_4^{-2}$, although this will work efficiently only if the strain is not a methionine auxotroph and NH_4^+ and Mg^{+2} components of the growth medium are in the form of chloride salts.

Changes in the pH of the medium, one of many factors that affect the transport of small molecules, must be taken into account when sporulating cells are to be labeled (40,41). The inhibition of transport may be avoided by maintaining the pH above 6.5 through the addition of Mes or potassium phthalate buffer to the sporulation medium. Specific labeling of mitochondrial proteins can be achieved by using cycloheximide to inhibit cellular protein synthesis ((42); and for other effects of the drug see (43)).

Nucleic Acids

S. cerevisiae has far more RNA than DNA. Since these organisms lack thymidine kinase, thymidine cannot be used to specifically label DNA, but mutants that take up dTMP have been isolated (44,45). Isotopic equilibration of other nucleic acid base precursors into DNA is relatively slow and depends on the rate of transport and the size of sequestered pools. Amino acid starvation can decrease the uptake of nucleic acid precursors and of phosphate. Nevertheless, RNA has been pulse labeled with [^{3}H]adenine or [^{3}H]uridine during amino acid starvation (46). Total nucleic acids can be labeled with [^{32}P]orthophosphate added to a low phosphate medium. The latter is prepared by adding 10 ml of 1 M $MgSO_4$ and 10 ml of concentrated aqueous ammonia per liter of 1% yeast extract and 2% peptone. The phosphates that precipitate after 30 min are filtered through Whatman No. 1 paper and the pH of the filtrate is adjusted to 5.8 with HCl. The solution is autoclaved, after which glucose or another carbon source is added (47).

RNA can be labeled at the 5′ terminal cap with [*methyl*-^{3}H]methionine in the presence of excess guanine and adenine so as to prevent purine ring labeling.

METHODS OF ANALYSIS

For most purposes cells are collected by centrifugation; at least 500*g* for 10 min is adequate. When very rapid isolation of relatively large culture volumes is demanded, suction filtration through, e.g., nitrocellulose membranes (0.45 μm) is the method of choice (48).

Most investigations require that the integrity of membranes and organelles be maintained or that macromolecules such as enzymes, DNA and RNA are not denatured or-

destroyed. Under a number of conditions yeast will autolyse and the proteolysis that ensues is generally undesirable. In all of the following procedures, inclusion of proteinase inhibitors (e.g., phenylmethylsulfonyl fluoride, pepstatin, leupeptin, antipain) may be necessary.

In addition, consideration must be given to the tough cell wall. The cell wall of yeast can be enzymatically digested, resulting in spheroplasts (or protoplasts) that require isotonic media to prevent lysis. Such cells remain viable and can be used in studies of, for example, cell wall synthesis or to facilitate transport of metabolites. One of two enzymes is generally used to make spheroplasts. A crude preparation from the gut of the snail *Helix pomatia* (Glusulase, available from Endo Labs, Garden City, NY) contains a glucanase and a sulfatase, among other hydrolytic enzymes. Zymolyase, which contains a glucanase and a protease, is available from Miles Pharmaceuticals. The speed and extent of cell wall digestion depend on both the physiological state of the cell and the specific yeast strain and should be determined for each experiment. For example, 5×10^8 cells of strain DBY1043 in 1 M sorbitol–0.1 M sodium citrate, pH 8–0.6% 2-mercaptoethanol with 0.2 mg/ml Zymolyase will form spheroplasts in 20 to 30 min at 37°C (18). To test the extent of spheroplast formation, a small amount of cell suspension is mixed with an equal volume of 10% sodium dodecyl sulfate (SDS). Those cells that have formed spheroplasts lyse and appear dark under phase illumination unless the cell wall has been completely eliminated, in which case no cells will be detected after lysis.

Cells may also be broken by disruption with glass beads (0.5 mm in diameter). Large quantities may require such equipment as a Braun homogenizer or a Dyno-mill (20). For small quantities of cells disruption with glass beads can be done manually or with a Bead Beater (Biospec Products). Cells are collected in a small, sturdy test tube and are suspended in about one-half volume of buffer. Glass beads are added to the meniscus of the cell-buffer slurry and the suspension is vortexed vigorously. Intermittent cooling is advisable and the extent of breakage should be monitored by microscopic examination for residual phase-bright (unlysed) cells.

For the determination of intracellular metabolites, chemical methods of membrane disruption and protein precipitation are necessary to quickly stop metabolic activity. Filtered cells are resuspended in a cold acid solution (48) and the cellular extract is concentrated and analyzed by appropriate methods (49). If intracellular concentrations of metabolites or the number of molecules per cell are needed, the intracellular fluid volume can be determined using [^{14}C]inulin (49).

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