JOURNAL OF BACTERIOLOGY, Nov. 1973, p. 925-930
Vol. 116, No. 2

Printed in U.S.A.

Sporulation Synchrony of *Saccharomyces cerevisiae* Grown in Various Carbon Sources

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Received for publication 20 August 1973

Sporulation of several strains of *Saccharomyces cerevisiae* grown in a variety of carbon sources that do not repress the tricarboxylic acid cycle enzymes was more synchronous than the sporulation of cells grown in medium containing dextrose which does repress those enzymes. Dextrose-grown cells showed optimal sporulation synchrony when inoculated into sporulation medium from early stationary phase when the dextrose in the medium is exhausted. Logarithmic-phase cells grown in either non-fermentable carbon sources (acetate and glycerol) or a fermentable carbon source that does not repress tricarboxylic acid cycle enzymes (galactose) sporulated more synchronously than the early stationary-phase dextrose cells. Attempts were made to sporulate cells taken from both complex and semidefined media. The semidefined acetate medium failed to support the growth of a number of strains. However, cells grown in the complex acetate medium, as well as both complex and semidefined glycerol and galactose media, sporulated with better synchrony than did the dextrose-grown cells.

The yeast *Saccharomyces cerevisiae* is a unicellular organism which is capable of sporulation, a process in which diploid cells undergo meiosis and the subsequent four spores form within an ascus. The process provides a model system in which meiosis, as well as other developmental processes involved in spore formation, can be studied in a simple eukaryote.

Sporulation has been examined from a variety of biochemical, genetic, and cytological perspectives (reviewed by Fowell, reference 6). Many of these studies have been aimed at developing and characterizing a series of events during the sporulation process. In an asynchronous population, it is often difficult to determine precisely the time of initiation and duration of such events. Thus, it is of value to develop and utilize methods that achieve a high degree of synchrony in the sporulating population.

A common procedure employed in sporulation studies is the pregrowth of cells in a complex medium with dextrose as a carbon source (YPD) and subsequent inoculation of cells from the early stationary phase of growth into 2% acetate. This regimen results in the sporulation of a high proportion of the cells, although the sporulation of the population is not highly synchronous. Logarithmic-phase cells pregrown in a synthetic acetate medium (SA) and inoculated into sporulation medium, sporulate with a much higher degree of synchrony than do the YPD-grown cells (10).

One of the approaches to the study of sporulation has been through the isolation and characterization of sporulation-deficient (*spo*) mutants (3, 5; and Moens, Esposito, and Esposito, in press). By using the YPD pregrowth procedure, information has been obtained concerning the effects of these mutations on macromolecular synthesis, genetic recombination, and fine structural development during sporulation. To examine these and other parameters in a more synchronous sporulation system, an attempt was made in the following study to utilize the SA presporulation growth procedure (10) with a variety of *spo* mutants and the parental wild-type strain. It was found that these strains grew very slowly or not at all in the SA medium.

A procedure involving pregrowth in a complex medium with acetate as a carbon source was developed to provide an alternative method to achieve good synchrony and a high percentage of sporulation. Furthermore, it was shown that improved sporulation synchrony also occurs after pregrowth in a variety of carbon sources that do not repress trichloroacetic acid cycle enzymes.

MATERIALS AND METHODS

Yeast strains. Three homothallic diploid strains were used in this study: S41, Z190-8B, and W66. The

genotype of S41 is *a/α*, *HO$_a$/HO$_a$*, *HM/HM*, *arg* 4-1/*arg* 4-1, and *cyh* 1/*cyh* 1. The genotype of Z190-8B is *a/α*, *HO$_a$/HO$_a$*, *HM/HM*, *ade* 2/*ade* 2, *ade* 6/*ade* 6, *lys* 2-2/*lys* 2-2, *trp* 5-R/*trp* 5-R, *leu* 1/*leu* 1, and *his* 7-1/*his* 7-1. The genotype of W66 is *a/α*, *HO$_a$/HO$_a$*, *HM/HM*, *ade* 2-1/*ade* 2-1, *trp* 5-48/*trp* 5-2, *LEU* 1/*leu* 1-12 *lys* 2-1/*lys* 2-1, *can* 1-100/*CAM* 1, *met* 4/*met* 4, *gal* 2/*GAL* 2, *ura* 3-1/*URA* 3, and *SUC*/*suc*.

The symbols are as follows: *a* and *α*, mating-type alleles; *ade*, adenine auxotroph; *arg*, arginine auxotroph; *can*, canavinine resistance; *cyh*, cyclohexamide resistance; *gal*, galactose fermentation; *his*, histidine auxotroph; *HO$_a$*, and *HM*, genes controlling homothallism (12); *leu*, leucine auxotroph; *lys*, lysine auxotroph; *met*, methionine auxotroph; *SUC*, sucrose fermentation; *trp*, tryptophan auxotroph; and *ura*, uracil auxotroph.

Media. For presporulation growth, four complex media (YPD, YPA, YPG, and YPGal), and four semidefined media (SA, SD, SG, and SGal) were used. YPD contains 10 g of yeast extract (Difco), 20 g of peptone (Difco), and 20 g of dextrose per liter of medium (pH 6.5). YPA, YPG, and YPGal are similar except 10 g of potassium acetate, 30 ml of glycerol, or 20 g of galactose is substituted for the dextrose, respectively. SA presporulation medium (8) contains 6.7 g of yeast nitrogen base without amino acids (Difco), 1 g of yeast extract, and 10 g of potassium acetate per liter of 0.05 M potassium phthalate buffer (pH 5.0). SD, SG, and SGal are similar except 20 g of dextrose, 30 ml of glycerol, or 20 g of galactose, is substituted for the acetate, respectively. A 20-mg amount of tetracycline (Squibb) per liter was added to each of the growth media to prevent bacterial contamination. Cells were sporulated in a medium containing 20 g of potassium acetate per liter (pH 7.0). A 75-mg amount of each of the following was added to each of the growth and sporulation media: adenine, arginine, histidine, leucine, lysine, methionine, and tryptophan. For solid culture, 15 g of agar (Difco) was added to a liter of the above media.

Growth conditions. Three- to five-day-old colonies grown on YPD or YPG plates were inoculated into the growth media at 10^5 cells/ml. At 12 to 18 h the logarithmic-phase cells were reinoculated at 10^5 cells/ml into 100 ml of the growth medium and grown at 30 C in 500-ml flasks on a New Brunswick model G-86 rotary shaker at 300 rpm.

Sporulation conditions. Cells were collected from the growth culture by centrifugation, washed with sterile water, and suspended at 5×10^7 cells/ml in 10 ml of sporulation medium in a 50-ml flask on a rotary shaker as above.

Counting procedures. Cell number was determined by hemocytometer counts of 300 to 500 cells. The percent ascospore formation was determined by hemocytometer counts of cells containing refractile spores. Cell number includes buds and cells irrespective of size. This represents an overestimate of the number of complete cells since some of the buds do not yet contain nuclei. Thus, the percentage of asci stated is a minimal estimate. Samples that could not be counted immediately were fixed in 4% Formalin and refrigerated.

RESULTS

Cells grown in YPD are optimally adapted to sporulation in early stationary phase. This stage of growth corresponds to the time of transition from fermentation to respiration as the dextrose in the medium is exhausted, and suggests that actively respiring cells are required for sporulation (1, 4, 8).

Cells grown in a semidefined medium containing acetate as a carbon source sporulate optimally when transferred to sporulation medium from logarithmic phase (10, 11). Since acetate is not fermentable, the cells are respiring during logarithmic phase growth. This method results in a high degree of sporulation synchrony as well as a high percentage of asci.

Growth in SA presporulation medium. In a survey of 19 respiratory-sufficient (grande) diploid strains, including 4 wild-type strains capable of sporulation and 15 temperature-sensitive sporulation-deficient (*spo*) mutant strains, 9 showed virtually no growth over a 5-day period in SA presporulation growth medium. The remaining 10 showed cell generation times of 4 to 10 h in logarithmic phase growth. Though the strains are not petites, the inability to grow appears to be related to acetate utilization in synthetic medium since they grow in SD medium, which is similar to SA medium but contains dextrose as a carbon source. Therefore, we sought an alternative presporulation growth medium to improve the sporulation synchrony.

Carbon sources. The wild-type parental strain of the *spo* mutants, S41, was cultured in a complex medium with different carbon sources. The resulting growth in dextrose, acetate, and glycerol media with logarithmic-phase cell generation times of 90, 135, and 150 min, respectively, is shown in Fig. 1.

Samples were taken in logarithmic phase, early stationary phase, and stationary phase from each of the growth media and inoculated into sporulation medium. The kinetics of the appearance of asci are shown in Fig. 2. The acetate- and glycerol-grown cells sporulated maximally, and most sporulated synchronously when taken from logarithmic-phase cultures. In contrast, the dextrose-grown cells did not sporulate at all from the logarithmic phase and showed maximal sporulation when transferred to sporulation medium from the early stationary phase of growth, in agreement with earlier studies (1, 4, 8).

When the percentage of three- and four-spored asci is examined separately from the total percentage of asci, additional information about the kinetics of sporulation becomes ap-

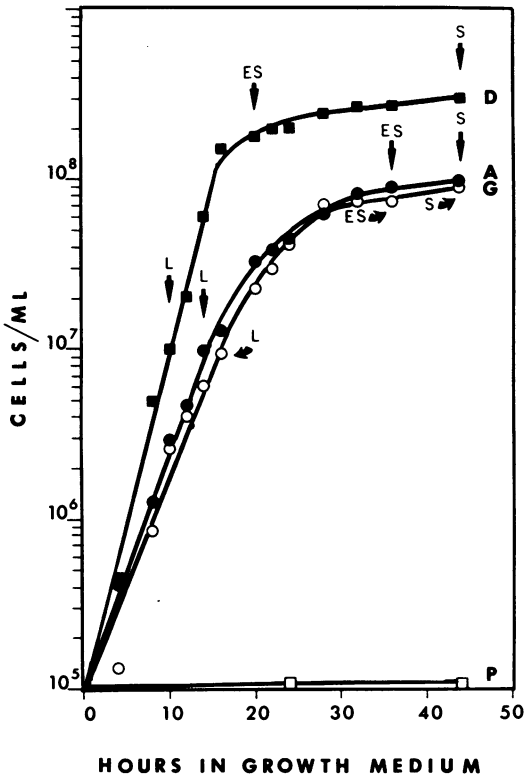

FIG. 1. *Effect of carbon source on growth of strain S41. Logarithmically growing cultures were inoculated into the growth media at 10^6 cells/ml. The growth media differ in respect to the carbon source used, dextrose in YPD (■), acetate in YPA (●), and glycerol in YPG (○). Samples were taken in logarithmic phase (L), early stationary phase (ES), and stationary phase (S) for inoculation into sporulation medium. No growth was observed in SA presporulation medium (□).*

parent (Fig. 3). For acetate-grown cells, all asci that appear after 20 h are one- and two-spored asci. (There are always many more two-spored asci than one-spored asci.) The three- and four-spored asci appear during one discrete period of time. In contrast, three- and four-spored ascus production by glycerol- and dextrose-grown cells occurs in two phases. After an initial rapid production of asci, further ascus formation is protracted over a long period.

Growth phase. Since logarithmic-phase cultures of YPA-grown cells give better sporulation synchrony than the previously used YPD pregrowth procedure, several parameters of YPA presporulation growth were analyzed. To determine the growth phase at which a culture is optimally adapted for sporulation induction, samples were taken at several points of logarithmic phase and early stationary phase and inoculated into sporulation medium. The results (Fig. 4) indicate that, when the cell

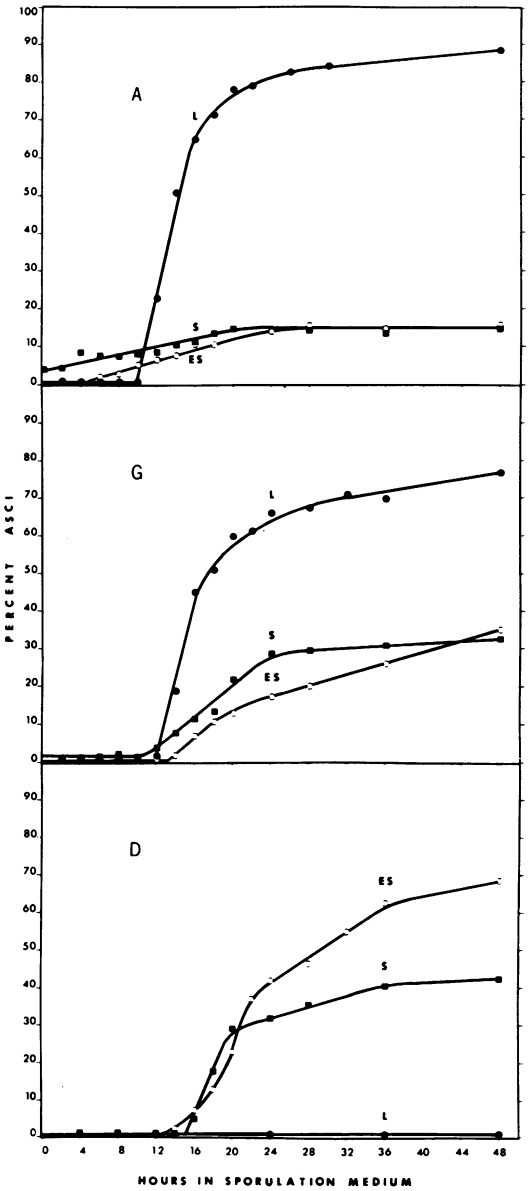

FIG. 2. *Effect of presporulation growth conditions on percentage of ascus formation. Cultures of S41 grown in YPA (A), YPG (G), and YPD (D) media, were sampled at three times during growth as indicated in Fig. 1. The samples from logarithmic phase (●), early stationary phase (○), and stationary phase (■) were inoculated into sporulation medium, and at intervals, samples were removed and examined for the presence of asci.*

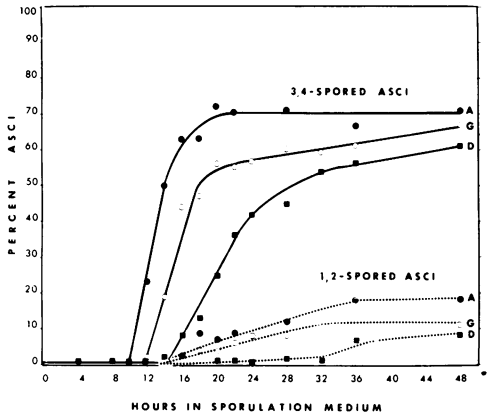

FIG. 3. *Effect of presporulation growth conditions on the percentage of three- and four-spored asci and one- and two-spored asci. Cultures taken from logarithmic-phase acetate-grown cells (●), logarithmic-phase glycerol-grown cells (○), and early stationary-phase dextrose-grown cells (■) were inoculated into sporulation medium. The one-and two-spored asci as a percentage of the total number of cells are indicated by a dotted line, and three- and four-spored asci are indicated by a solid line.*

number is no longer increasing exponentially, synchronous sporulative ability decreases.

Acetate concentration. In cultures containing 0.5, 1, 2, and 5% potassium acetate, optimal synchrony and maximal sporulation were obtained from the 1% acetate pregrowth.

Other strains. Two other strains were cultured in the four growth media (YPA, YPG, YPD, and SA). Cells were transferred to sporulation medium from the phase of growth that in S41 gave optimal sporulation: logarithmic phase cells for the non-fermentable media and early stationary-phase cells for the dextrose medium (Fig. 5). Z190-8B, like S41, does not grow in SA medium. Although final sporulation for cells from YPA, YPG, and YPD media is the same, cells from the YPA medium give the most synchronous sporulation. W66 grows in SA medium, and the resulting sporulation is more synchronous than from YPD medium but not as synchronous as from YPA or YPG media.

Galactose presporulation media. Galactose, though a fermentable carbon source, does not repress the tricarboxylic acid cycle enzymes as does dextrose (9). S41 grows in YPGal, SGal, and SG, with cell generation times of 165, 210, and 225 min, respectively (Fig. 6). When inoculated into sporulation medium from logarithmic cells from these media, the kinetics of the appearance of asci are similar to that of YPA- or YPG-grown cells.

DISCUSSION

A variety of experiments shows that the ability to respire is necessary for sporulation. Cells pregrown in dextrose medium are optimally adapted for sporulation in early stationary phase, the time when the dextrose is exhausted (1, 4, 8). If dextrose is present, it represses the enzymes of the tricarboxylic acid cycle and thus represses respiration (9). The final percentage of spores formed is directly proportional to the ability of cells to respire when introduced into sporulation medium (1). Diploid petites, incapable of respiration, cannot sporulate (2).

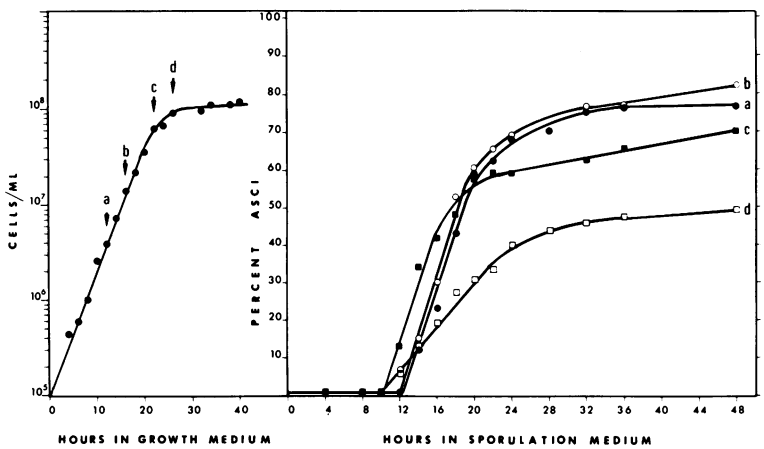

FIG. 4. *Effect of growth phase in YPA medium on the ability to sporulate. Samples were removed from a culture of S41 in YPA medium with 75 mg of arginine per liter at the times indicated by the arrows and inoculated into sporulation medium containing 75 mg of arginine per liter. At intervals, samples were removed and counted for percent sporulation.*

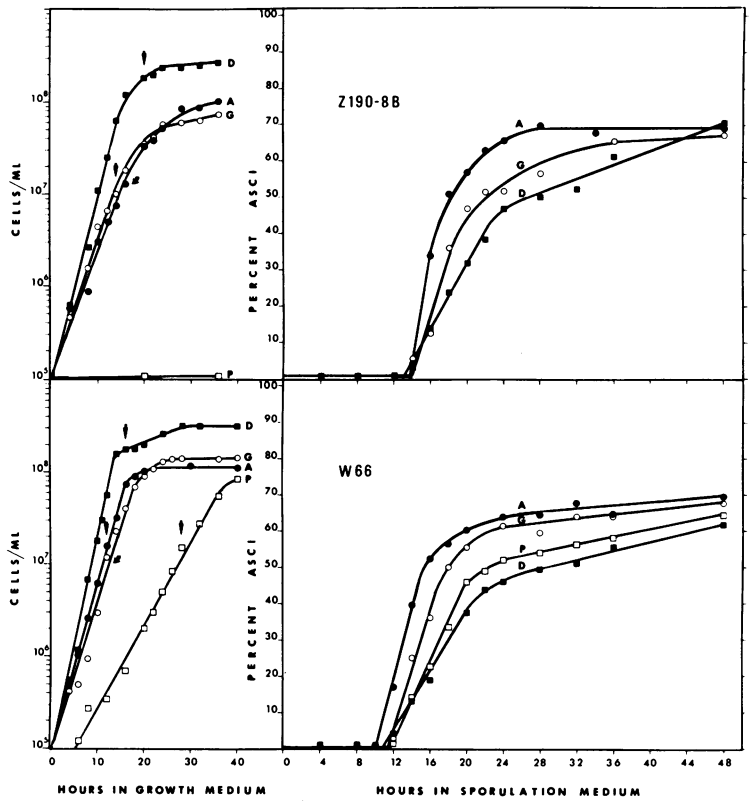

FIG. 5. *Growth of strains Z190-8B and W66 in various media and subsequent sporulation. Logarithmically growing cultures were inoculated into YPD (■), YPA (●), YPG (○), and SA presporulation media (□). At the time indicated by the arrow, a sample was inoculated into sporulation medium. Samples were removed from the sporulation medium at intervals and examined for the presence of spores.*

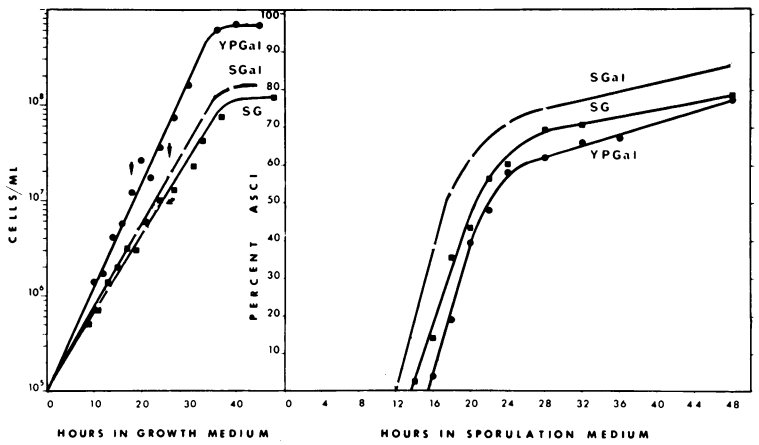

FIG. 6. *Growth of strain S41 in various media and subsequent sporulation. Logarithmically growing cultures were inoculated into YPGal (●), SGal (○), and SG (■) media. At the time indicated by the arrow, a sample was inoculated into sporulation medium. Samples were removed from the sporulation medium at intervals and examined for the presence of spores.*

Roth and Halvorson (10) found that logarithmic-phase cells grown in a semidefined presporulation medium with acetate, a non-fermentable carbon source, sporulate more synchronously than dextrose-grown cells. However, the medium used in their experiments does not

support the growth of a number of other strains of genetic and biochemical interest. The present study has shown that a complex medium containing acetate as a carbon source supports the growth of strains that do not grow in the semidefined acetate medium, and that logarithmic-phase cells from the complex acetate medium sporulate with improved synchrony.

This study has shown further that growth in a variety of carbon sources produces cells capable of sporulating synchronously. Logarithmic-phase cells grown in glycerol and galactose media, like those grown in acetate medium, sporulate more synchronously than early stationary-phase dextrose-grown cells. In glycerol and acetate media, the cells are respiring and require tricarboxylic acid cycle enzymes. Galactose, though a fermentable carbon source, does not repress the tricarboxylic acid cycle enzymes. Thus, optimal sporulation synchrony can be achieved by utilizing logarithmic-phase cells grown in carbon sources that do not repress respiratory enzymes.

Even under these conditions, however, there is still residual asynchrony which is in part due to the effect of the cell division cycle on sporulation. Haber and Halvorson (7) have shown that the ability of a cell to sporulate and the sporulation synchrony depend on the cell's position in the division cycle. The percentage of sporulation is proportional to the percentage of cells with buds attached; cells without buds usually do not form asci and most cells with buds form one ascus per cell plus bud. Thus, the cell most capable of sporulating, the mother cell, is physically linked to one that will probably not sporulate, the bud. This suggests that there is an optimal point in the cell division cycle to initiate sporulation and that a cell that is not at that point may need to complete certain events of the growth cycle before it can begin the sporulation process. Small single cells and buds may represent cells unable to complete those events and thus unable to sporulate.

A random logarithmic-phase cell population should include (i) cells that are at the point in the cycle that is optimum for initiating sporulation, (ii) cells that require a certain period of time to reach that point, and (iii) cells that are unable to reach that point. The period of time necessary for the second class of cells to reach the optimal point will probably be reflected in the asynchrony present in the appearance of spores. Well-nourished cells that have a short cell division cycle may be able to reach that point faster than starved cells that are dividing slowly. This could explain why the optimal sporulation synchrony is obtained from logarithmically dividing cells. As a population reaches stationary phase, two changes may occur. If the cell is depleted of nutrients, it may take longer to reach the optimal point to begin sporulation, thus increasing the asynchrony of the appearance of spores. Furthermore, some cells may collect at a point which does not allow them to complete the events leading up to the optimal time to begin sporulation, thus reducing the total percent sporulation. Thus, the optimal conditions for sporulation seem to include pregrowth in a medium that supports a high rate of growth and contains a carbon source that does not repress respiratory enzymes.

ACKNOWLEDGMENTS

The author expresses his appreciation to Michael Esposito, Rochelle Esposito, and Rodney Rothstein for many helpful discussions concerning these experiments and for the strains which were used.

This work was supported by a Public Health Service training grant 2 TO1 HD-00174-06 from the National Institute of Child Health and Human Development, and by National Science Foundation grants GB 8564 and GB 27688.

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