# Effect of Gene Position on the Timing of Enzyme Synthesis in Synchronous Cultures of Yeast

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## ABSTRACT

TAURO, PATRIC (The University of Wisconsin, Madison), AND HARLYN O. HALVORSON. Effect of gene position on the timing of enzyme synthesis in synchronous cultures of yeast. J. Bacteriol. 92:652–661.—In synchronously growing cultures of *Saccharomyces cerevisiae*, enzyme synthesis is periodic. The effect of various factors on the timing of $\alpha$-glucosidase synthesis has been investigated. The period of the cell cycle during which $\alpha$-glucosidase is synthesized is unaffected by the method employed to induce synchrony, as well as other environmental conditions. However, a definite relationship exists between the number of nonallelic structural genes present for $\alpha$-glucosidase and the number of periods of synthesis during the cell cycle. It is concluded that the periodic synthesis of enzymes observed in synchronously growing cultures of yeast is probably the result of an ordered process of transcription of the various structural genes.

In synchronously dividing cultures of yeast, deoxyribonucleic acid (DNA) synthesis is discontinuous (1, 7, 28), and the different classes of ribonucleic acids (RNA) and protein are synthesized throughout the cell cycle (5). In contrast to this, the synthesis of many enzymes is periodic (1, 5, 21, 24). The time during which a given enzyme is synthesized occupies only a fraction of the division time. This period is characteristic for each enzyme, and the same pattern occurs in subsequent cell cycles. These and similar observations made in bacteria (1a, 10–13, 19) have led to a renewed interest in the mechanism controlling the timing of enzyme synthesis during the cell division cycle.

Our initial findings in interspecific yeast hybrids, that the parental genes for a given enzyme are expressed at different periods of the cell cycle (5) and that addition of inducer does *not* change the time of induced enzyme synthesis (6a), led us to propose early (7) that the genome was transcribed in an ordered manner during the cell cycle.

The present paper provides further evidence for this conclusion and also illustrates some of the methodology and controls in experiments with synchronous cultures. In particular, these experiments were designed to provide answers to two

questions concerning the time of enzyme synthesis during the cell cycle. (i) Is the time of enzyme synthesis determined by the method employed to induce synchronous growth? (ii) For a given structural gene, what is the relationship between the gene position (centromere distance) and the time of its expression?

## MATERIALS AND METHODS

Table 1 summarizes the various strains of yeast employed.

*Materials.* Chemically pure grade maltose was obtained from Matheson, Coleman and Bell, East Rutherford, N.J., and contaminating glucose was removed by the method of Halvorson and Spiegelman (8). $\alpha$-*p*-Nitrophenyl-D-glucopyranoside ($\alpha$-PNPG) and $\beta$-*p*-nitrophenyl-D-glucopyranoside ($\beta$-PNPG) were obtained from General Biochemicals Corp., Chagrin Falls, Ohio. Disodium-*p*-nitrophenyl phosphate (PNPP) was a product of Calbiochem.

*Methods.* Two methods were employed to obtain synchronously growing cultures of yeast. Method A consisted of phasing a selected fraction of yeast cells by a modification of the method described by Sando (20). The procedure consisted of growing the yeast cells in 250 ml of Wickerham's medium (26) and harvesting the cells in the late logarithmic phase of growth. The larger cells, without buds, were selected from the heterogenous population by first allowing the cells to settle at 25 C for 2 hr and then repeatedly centrifuging at 100 × *g*. The cells were washed and suspended in 100 ml of starvation medium (27) at $10^8$ to $10^9$ cells per milliliter and aerated for 10 to 12

TABLE 1. *Yeast employed in this study*

| Strain | Source | Culture no. | Genotype[a] |
|---|---|---|---|
| Hybrid yeast (*S. fragilis* × *S. dobzhanskii* | L. J. Wickerham | Y-42 | — |
| *S. cerevisiae* | Ö. Winge | Y-55 | $M_1m_1 \ m_2m_2 \ m_3m_3 \ m_4m_4$ |
| *S. cerevisiae* | Ö. Winge | Y-62 | $M_1m_1 \ m_2m_2 \ M_3m_3 \ m_4m_4$ |
| *S. cerevisiae* | Ö. Winge | Y-70 | $M_1m_1 \ M_2m_2 \ M_3m_3 \ m_4m_4$ |

[a] The M genes govern the synthesis of $\alpha$-glucosidase.

hr at 25 C. The cells were then collected by slow centrifugation, washed, and resuspended at a high cell density ($10^9$ to $10^{10}$ per milliliter) in 10 ml of synthetic growth medium (5) and aerated for about 10 to 12 hr. The cell suspension was then diluted 500-fold with fresh synthetic growth medium containing 1% maltose, unless otherwise indicated, and was incubated at 25 C. The final suspension was uniform in size and morphology and contained over 95% of viable cells. This method takes about 2 to 3 days before the cells are ready for use.

Method B was essentially that described by Mitchison and Vincent (16). One-liter cultures of *Saccharomyces cerevisiae* grown in synthetic medium containing 1% maltose were harvested in the mid-logarithmic phase of growth and suspended in 5 to 6 ml of sterile water. A 2-ml amount of this suspension was layered on a 10 to 40% (w/v) linear sucrose gradient in a glass tube (19 by 170 mm) and centrifuged for 10 min at 500 × g in a swinging bucket rotor at 20 C. After centrifugation, the top 10 to 30% (v/v) of the gradient was collected, washed immediately, and suspended in fresh growth medium at 25 C. This fraction represents 3 to 4% of the population placed on the gradient. Microscopic examination and analysis of the size distribution (Fig. 1) showed that the selected fraction was homogenous.

*Determination of growth and division.* Samples (2 ml) were collected from synchronously growing cultures at intervals, and growth was arrested by adding 0.1 ml of 36% formaldehyde. Cell numbers were determined either with a hemocytometer, as described by Williamson and Scopes (27), or with an electronic cell counter (Coulter counter). For the latter, yeast samples were diluted 200-fold in 0.9% sodium chloride solution by use of an automatic diluter, and 0.5 ml of the diluted sample was counted, each time in triplicate, with the use of a 70-$\mu$ orifice tube. In experiments with strains which showed tendencies to aggregate, mild sonic treatment for about 10 sec with a 20-kc MSE ultrasonic probe (21) facilitated counting of the yeast cells without visible damage to the cells. For determination of the cell size distribution, the electronic particle size distribution plotter was set to count for a time period of 4 sec at each electronic window. Standard polyvinyl latex particles of uniform size were used to standardize the Coulter counter. Changes in optical density of the culture were measured with a Beckman DU spectrophotometer at 600 m$\mu$ with the use of a 1-cm cuvette.

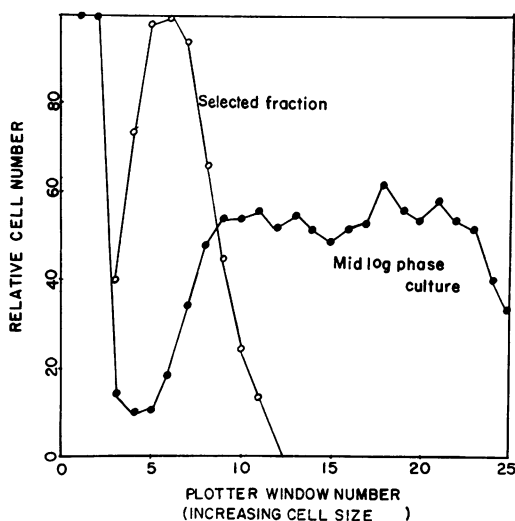*Dry-weight determination.* Samples (10 ml) of the



FIG. 1. *Cell size distribution of a culture in exponential growth and a fraction selected by sucrose density centrifugation.*

synchronously growing culture were withdrawn at intervals, and filtered rapidly through a tared 0.45-m$\mu$ membrane-filter disc (Millipore Filter Corp., Bedford, Mass.). This was washed with 20 ml of cold distilled water and dried at 85 C to a constant weight.

*Enzyme assays.* At intervals, samples were removed, washed twice by centrifugation, and prepared for enzyme assay as previously described (3). $\alpha$-Glucosidase was assayed by the method of Halvorson et al. (9). $\beta$-Glucosidase was measured by the method of Duerksen and Halvorson (3), and alkaline phosphatase was determined by the method of Torriani (25). In all cases, a unit of enzyme is defined as millimicromoles of substrate hydrolyzed per 100 sec. When enzyme levels are plotted as a function of the fraction of a generation (Fig. 9 and 12), the amount of enzyme present at the beginning of the cell cycle is normalized to unity.

## RESULTS

*Use of interspecific hybrids in synchronous growth experiments.* Interspecific hybrids, which one would expect to contain numerous non-

allelic genes, provide ideal experimental materials for examining the effect of gene position on the timing of specific enzyme synthesis during the cell cycle.

As previously noted (5, 7), cultures of such an interspecific hybrid (Y-42), synchronized by the phasing method of Williamson and Scopes (27), exhibit balanced growth (the amount of any component doubles in subsequent generations). Further characteristics of this system are illustrated in Fig. 2. Increases in the dry weight of cells during synchronous growth occurred at about the same time that the optical density began to increase and that the first signs of budding were observed microscopically. Once growth was initiated, the dry weight increased continuously, with the rate doubling with the beginning of each generation and the total amount essentially doubling during each generation. In similar experiments with this organism, the rate of protein synthesis was nearly linear over the generation cycle (7). In synchronous cultures of *S. cerevisiae* (21), as well as during the cell cycle of *S. cerevisiae* (15), *total* dry mass increases linearly, whereas protein and RNA increase in an exponential manner.

Inherent variables, such as a degree of asynchrony in the population and fluctuating pool levels, make precise determinations of the overall kinetics of macromolecular synthesis difficult. However, measurements such as those shown
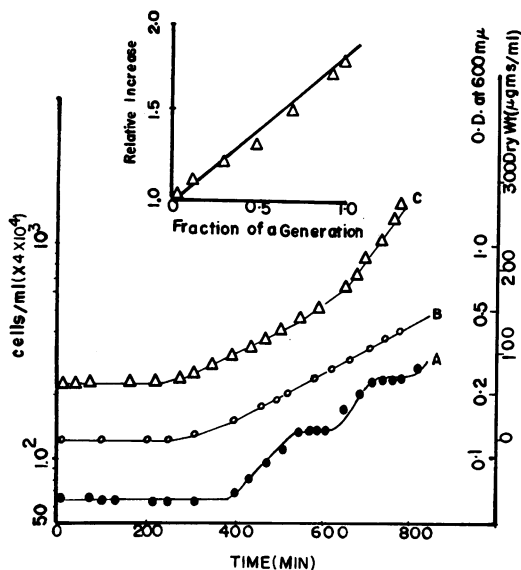
in Fig. 2 can be useful guides to monitor the extent to which a synchronous culture is under conditions of balanced growth.

In cultures of the hybrid yeast (Y-42), synchronized by the drastic phasing method of Williamson and Scopes (27), periodic enzyme synthesis has been observed (5). It was, therefore, of interest to re-examine the timing of enzyme synthesis in cultures synchronized by milder methods. Figure 3 illustrates such an experiment with the same hybrid yeast (Y-42) phased by method A. After a delay of 320 min, cell numbers increased periodically over two generations. In each generation, two periods of alkaline phosphatase and β-glucosidase synthesis and one period of α-glucosidase synthesis were observed. In these and parallel experiments, the fraction of the generation during which each of these enzymes was synthesized was closely approximated in subsequent generations and was in agreement with our earlier findings (5).

*Determination of the degree of synchrony in populations of S. cerevisiae.* The degree of synchrony was a function of the inherent characteristics of the cells and of the phasing method employed. Since the generation time of a synchronous culture did not represent only the period during which division of cells occurred, generation times were calculated from the theoretical growth curves, as shown by the dashed line T in Fig. 4. The growth curves, as determined either by use of the Coulter counter or by direct counting of the cells, showed similarity in pattern, although the cell numbers, as determined by the two methods, differed slightly. The bud ratio fluctuated in close agreement with the growth and
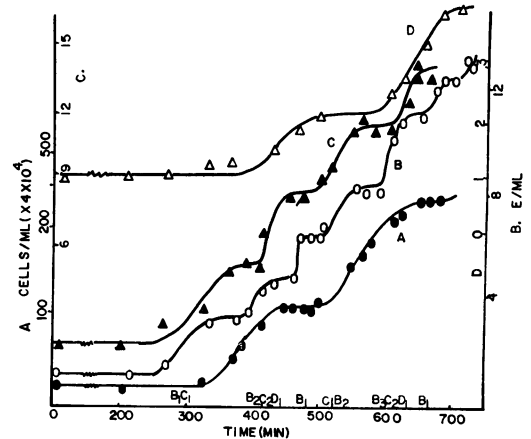


FIG. 2. *Dry-weight changes in a phased synchronous culture of a hybrid yeast (Y-42). (A) Cell number. (B) Optical density. (C) Dry weight.*



FIG. 3. *Enzyme synthesis in a phased culture of the hybrid yeast Y-42. (A) Cell number. (B) Alkaline phosphatase. (C) β-Glucosidase. (D) α-Glucosidase.*
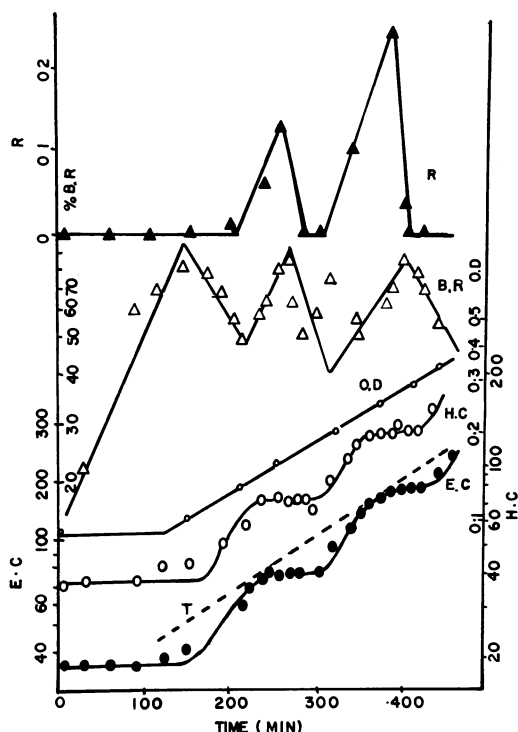
FIG. 4. *Determination of the degree of synchrony in a yeast population. Saccharomyces cerevisiae was grown synchronously in synthetic medium containing 1% maltose. E.C., Coulter Counter, total cells per milliliter times $4 \times 10^{-4}$; T, theoretical growth curve; H.C., microscopic, total cells per milliliter times $5 \times 10^{-4}$; O.D., optical density (600 m$\mu$); R, normalized rate of division per hour; and B.R., bud ratio.*

division of the yeast cells, reaching a level of 90% just before division, and this is consistent with a high degree of synchronization of the population. The degree of synchrony can also be followed by calculating the normalized rate of cell division (R) according to the equation of Engelberg (2):

$$R = (dn/dt)/N \text{ per unit of time}$$

where $n$ is the total number of cells dividing and $N$ is the total number of cells present at time $t$. The plot of $R$ against time (Fig. 3) shows the same periodicity as observed in the bud ratios.

The synchronization index (SI) for this experiment was calculated from the equation of Scherbaum (22):

$$SI = (N/N_0 - 1)(1 - t/g)$$

where $N_0$ and $N$ are the total number of cells before and after division, respectively, $t$ is the

time period during which division occurs, and $g$ is the generation time of the culture. An SI of 0.7 for the first division and 0.6 for the second indicates that the culture possesses a fair degree of synchrony; these values are in close agreement with the values reported by Scherbaum (22) for a number of synchronous microbial systems.

In practice, the individual division steps in synchronous growth have been monitored by following the frequency size distribution with the use of an electronic particle size distribution plotter. This procedure is illustrated in Fig. 5, in which a part of the spectrum representing different periods during a generation of synchronous growth of *S. cerevisiae* is presented. Initially, the population was composed mostly of single cells. During growth, the peak gradually dropped and the distribution shifted to the right as individual cells enlarged and produced buds. At the end of the generation, the curve showed a dramatic return to the original position and showed a near doubling of the relative cell number. These changes closely parallel the shifts in bud ratios (Fig. 4).

*Use of S. cerevisiae for synchronous growth experiments.* A clearer insight into the relationship between the position of a gene and its time of expression during the cell cycle would be afforded if the timing of the *same* structural gene could be examined at different positions along the chromosome. The M genes determining the synthesis of $\alpha$-glucosidase in *Saccharomyces* provide such an opportunity. Six non-allelic genes have been described; all are unlinked and two have been centromere-linked on different chromosomes (17). The same enzyme is coded by each of these genes (9), each functions independently (18), and all are induced in a similar manner. With the use of appropriate diploids, it should be possible to examine the *function* of each of these genes in the presence of a *common* regulatory system. Stepwise synthesis of $\alpha$-glucosidase in synchronous cultures has already been reported (6a, 7; and Fig. 3).

*Effect of the method of phasing on the timing of $\alpha$-glucosidase synthesis.* The diploid heterozygous strain of *S. cerevisiae* (Y-55) carrying one structural gene ($M_1m_1$) for the synthesis of $\alpha$-glucosidase was phased by the method A, and the synthesis of enzyme was examined during two generations of growth (Fig. 6). After a period of 200 min, the cell numbers increased periodically, and, in each of the two generations examined, only one period of $\alpha$-glucosidase synthesis was observed, which began at about the middle of the generation.
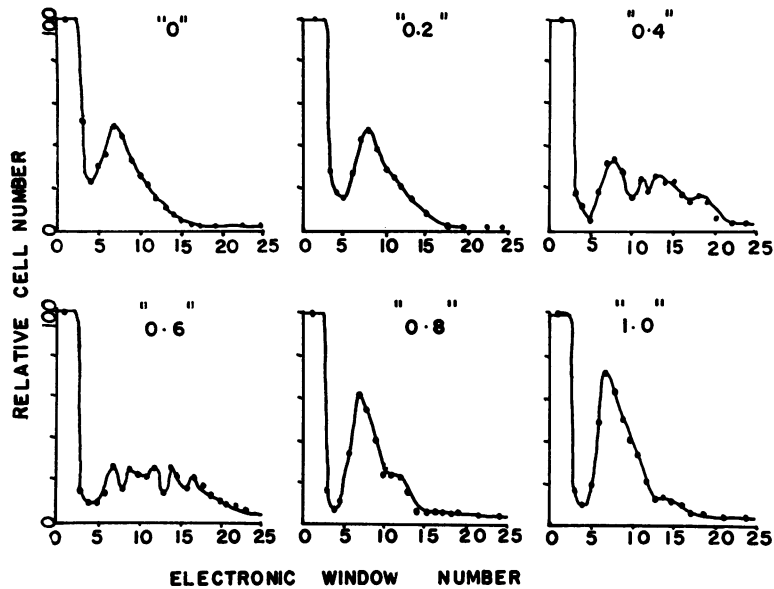
FIG. 5. *Cell size distribution in a synchronously dividing culture of Saccharomyces cerevisiae. The distribution spectrum was measured for cultures at 0, 0.2, 0.4, 0.6, 0.8, and 1.0 fraction of a generation.*
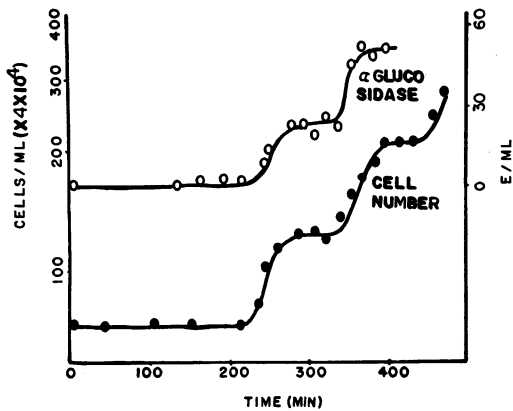


FIG. 6. *Enzyme synthesis in a phased, synchronously dividing culture of Saccharomyces cerevisiae (Y-55).*

To determine whether the periodicity was influenced by the method of inducing synchronous growth, a synchronous fraction from the same yeast was physically separated as in method B, and the synthesis of α-glucosidase was examined during two periods of synchronous growth (Fig. 7). After a very short lag, the cell number increased periodically, and, in each generation, a single period of enzyme synthesis was observed during the same fraction of the cell cycle observed in the experiment of Fig. 6.

As a further control to test the possibility that exposure and centrifugation introduced periodic enzyme synthesis in the population, the following
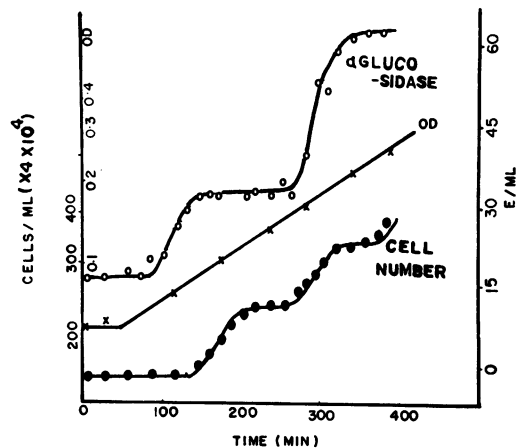


FIG. 7. *Enzyme synthesis in an unphased, synchronous culture of Saccharomyces cerevisiae (Y-55).*

experiment was conducted. A cell suspension of *S. cerevisiae* (Y-55) was centrifuged on a sucrose gradient in the same manner as described in Fig. 7. After centrifugation, the entire contents of the centrifuge tube were mixed, diluted into fresh growth medium, and allowed to grow. As shown in Fig. 8, the cell number in this asynchronous culture increased continuously. The synthesis of α-glucosidase was continuous and no bursts in synthesis were observed, further supporting the conclusion that periodic enzyme synthesis is not caused by the method employed to induce synchronous growth.
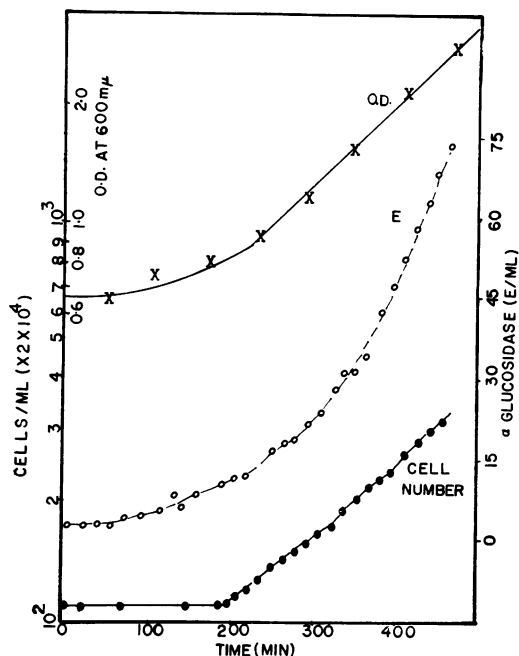
FIG. 8. *Enzyme synthesis in an asynchronous culture of Saccharomyces cerevisiae Y-55). The culture was first centrifuged in a sucrose density gradient. See text for details.*



FIG. 9. *Timing of enzyme synthesis in homozygous and heterozygous diploid strains of Saccharomyces cerevisiae. See Fig. 7 for data for $M_1m_1$ and Halvorson et al. (6a) for data for $M_1M_1$.*

*Comparisons of diploids homozygous and hetero- zygous for $M_1$.* One might expect that allelic copies of the same gene would be expressed during the same period of the cell cycle. This is confirmed by the results in Fig. 9, where increases in the level of $\alpha$-glucosidase as a function of the fraction of the generation time are compared in diploids of *S. cerevisiae* heterozygous ($M_1m_1$) and homozygous ($M_1M_1$) for the structural gene for $\alpha$-glucosidase. In each case, only one period of enzyme synthesis was observed, and this period was initiated at about the middle of the generation. Both genes were apparently expressed simultaneously.

*Effect of gene position on the timing of enzyme synthesis.* The various M genes function inde- pendently in exponential cultures, and the level of induced $\alpha$-glucosidase is strictly additive to the gene dosage (18). Further, in synchronous cul- tures, the times of basal and induced $\alpha$-glucosid- ase synthesis are identical; induction does not change the time of expression of the genome (6a). Therefore, since these various M genes are scattered over the yeast genome, examination of the time of $\alpha$-glucosidase synthesis in synchronous cultures enables one to test further whether the time of expression is determined by the position of the structural gene.
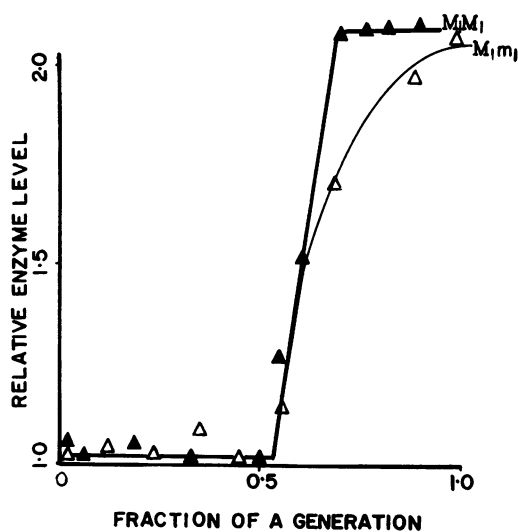
To examine this hypothesis, a series of experi- ments were carried out in which $\alpha$-glucosidase synthesis was followed in synchronous cultures carrying one or more of the various M genes. The following two typical experiments illustrate the methodology and determination of the time of expression of each gene. Two strains of *S. cere- visiae*, one carrying two structural genes, $M_1$ and $M_3$ (Y-62), and the other carrying three structural genes, $M_1$, $M_2$, and $M_3$ (Y-70), were synchronized by method A, and the synthesis of $\alpha$-glucosidase was followed. As shown in Fig. 10, the synthesis of enzyme in the heterozygous diploid with two different structural genes for $\alpha$-glucosidase occurred as two distinct steps which repeated in each generation. One step occurred shortly after the initiation of each cell cycle, whereas the second occurred approximately in the middle of the cell cycle. From the results of Fig. 6 and 7, it is clear that the second step occurs at the same time enzyme synthesis commences in cultures carrying only the $M_1$ gene. Presumably, the earlier step in enzyme synthesis is determined by the $M_3$ gene.

An example of enzyme synthesis in a synchro- nous culture with three structural genes for $\alpha$- glucosidase ($M_1m_1$, $M_2m_2$, $M_3m_3$) is shown in Fig. 11. As shown in this experiment, three periods of enzyme synthesis occurred in each cell cycle; these periods also recurred in subsequent generations. Two periods occurred during the same fraction of the generation cycle shown in
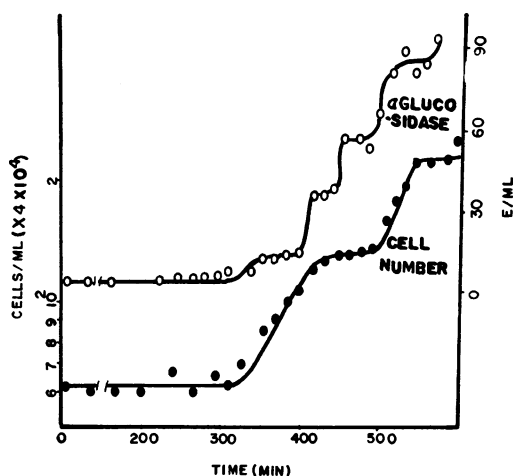
FIG. 10. *Enzyme synthesis in a synchronous culture of Saccharomyces cerevisiae (Y-62) having two structural genes for α-glucosidase ($M_1M_3$).*
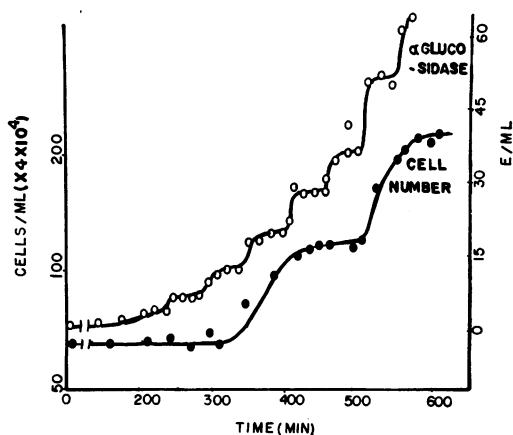


FIG. 11. *Enzyme synthesis in a synchronous culture of Saccharomyces cerevisiae (Y-70) having three structural genes for α-glucosidase synthesis ($M_1M_2M_3$).*

Fig. 10, and presumably correspond to the products of the $M_1$ and $M_3$ genes. The third period of enzyme synthesis began at about 0.75 fraction of the generation cycle, which is apparently the time of expression of the $M_2$ structural gene.

In parallel experiments, a burst in enzyme synthesis at 0.25 fraction of a generation was seen in cultures carrying the $M_4$ gene (6a). In Fig. 12 are summarized the times of expression of the four different M genes, as determined from the second generation of synchronous growth.

Our interpretation of these and similar experiments can be summarized as follows. (i) The number of periods of enzyme synthesis in the

cell cycle corresponds to the number of unlinked structural genes present. (ii) The time of expression of a given structural gene is unaffected by the presence of other structural genes. (iii) Each of the structural genes has a unique time of expression in the cell cycle.

These findings led us to conclude that the M genes are expressed independently of each other at different times of the cell cycle.

## DISCUSSION

An ordered transcription of the genome, which parallels the probable order of gene replication, has been explained by either of two distinct hypotheses. (i) The gene is continuously available to transcription; periodicity in enzyme synthesis is the result of end-product repression. (ii) Transcription is ordered or sequential, or both; each gene is available for transcription only during a specific period in each cell cycle.

As stated earlier (5, 7), evidence obtained with synchronous cultures of yeast makes the first hypothesis untenable. Addition of high concentrations of inducers, which one would expect to overcome the cyclic control by end-product inhibitors, has no effect on the time in which enzyme synthesis commences or ceases during the cell cycle (6a). Their only effect is to increase the differential rate of enzyme synthesis during a limited period of the cell cycle. These results may explain the well-known phenomenon in yeast where, under conditions of full induction or derepression, the maximal level of a given enzyme comprises only a fraction of a per cent of the total cell protein (6). This is in marked contrast to the situation found in bacterial cells.

If the periodicity of enzyme synthesis was regulated by repression (end-product or otherwise; 4), then one would expect the time of gene expression to be subject to alteration by drastic environmental changes. Such changes are, in fact, encountered in the methods of phasing cultures in which prolonged starvation is employed. As reported here, in experiments with a hybrid yeast, the timing of enzyme synthesis was the same in cultures phased either by the 13-day repeated starvation method of Williamson and Scopes (28) or by the short-cycle starvation method A. Further, in *S. cerevisiae*, α-glucosidase synthesis occurs at the same time of the cell cycle in cultures synchronized by starvation or by sucrose density centrifugation, a procedure which does not induce periodic synthesis in control experiments. Finally, attempts to interfere with levels of repressors by reducing the growth rate or following enzyme synthesis under conditions of glucose repression have not dissociated the time of enzyme synthesis from cell division.
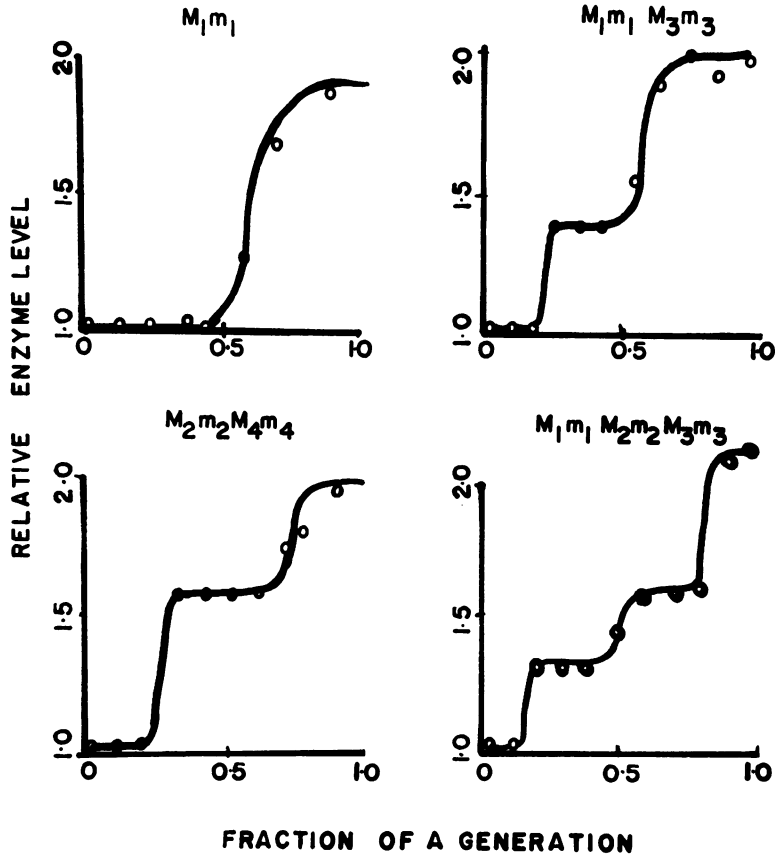
FIG. 12. *Timing of α-glucosidase synthesis in various M strains of Saccharomyces cerevisiae. The relative rate of enzyme synthesis is plotted as a function of the generation time. The data are derived from the second generation of synchronous growth (data from Fig. 7, 10, 11, and M.S. Thesis of P. Tauro, Univ. of Wisconsin, 1965).*

Our evidence is consistent with the second hypothesis. This conclusion is best summarized by recalling the predictions of this hypothesis which have been experimentally verified. (i) Closely linked genes are expressed at approximately the same time during the cell cycle (7). (ii) Genes which are located on the same chromosome, equidistant from the centromere (allelic), are expressed at exactly the same time (Fig. 9). (iii) The time of expression of genes, which *code for precisely the same enzyme* and *are located at different distances from the centromere*, varies.

One would anticipate that the third prediction is a common phenomenon in interspecific hybrids, since, in these strains during the course of evolution, one would expect not only the appearance of isozymes, but also considerable chromosomal rearrangement. This possibility was examined in the hybrid produced by crossing *S. dobzhanskii* × *S. fragilis* (5). Only one period of β-glucosidase synthesis occurs per generation in

each of the parental strains. In the hybrid, however, two periods of enzyme synthesis were observed per cell cycle, each corresponding to the time of enzyme synthesis in the parental strains. In this hybrid, several periods of alkaline phosphatase were also observed per cell cycle. The separation of two distinct alkaline phosphatases by chromatography (Nakao and Halvorson, *unpublished data*) may provide an explanation for these observations.

A more direct test has been afforded by the existence of different M genes in yeast, each responsible for the production of the enzyme α-glucosidase (9). These genes have been mapped (17). Of importance here is the fact that these genes are unlinked and are associated with different linkage groups. Each of these functions independently and, as shown here, is expressed at a different period of the cell cycle.

In cultures of *Shizosaccharomyces pombe*, synchronized by gradient sedimentation, single

steps of aspartic transcarbamylase and ornithine transcarbamylase were observed per cell cycle (1). However, sucrase, maltase, and alkaline phosphatase increased continuously over the division cycle. These findings led Bostock et al. to conclude "that similar modes of enzyme synthesis are found in both prokaryotic and eukaryotic cells." However, the lack of information on the genetics of *S. pombe* limits this conclusion. Since more than three steps of enzyme per cell cycle are difficult to determine, and numerous maltase ($\alpha$-glucosidase) and sucrase genes (17), as well as types of alkaline phosphatases, are known in yeast, it is not clear whether these are exceptions to the finding in *S. cereviseae* of one transcriptional period per structural gene during each cell cycle.

One possible explanation of sequential enzyme synthesis in yeast is that transcription is linked to replication of the genome. This possibility would seem unlikely, since DNA synthesis occupies only a portion of the cell cycle (1, 7, 28), whereas protein and enzyme synthesis are continuous throughout the cell cycle, both preceding and following the period of DNA synthesis. This possibility has been clearly eliminated in *Bacillus*. Periodic enzyme synthesis is observed both in a thymineless strain of *B. subtilis* in which DNA synthesis is inhibited by 5-fluorodeoxyuridine (12) and during outgrowth of spores of *B. cereus* (23) in which the few per cent increase of DNA observed is blocked by mitomycin (W. Steinberg, M.S. Thesis, Univ. of Wisconsin, Madison, 1965).

Findings such as these might lead one to suspect that similar mechanisms control transcription in both prokaryotic and eukaryotic cells. However, it is by now clear that some essential differences exist between these systems, making a unified hypothesis unlikely. The initial finding in yeast that transcription is ordered and related to gene position (5, 7) was later demonstrated for basal enzyme synthesis in bacteria (14). Despite these similarities, the two systems respond differently to the presence of inducers. Contrary to the findings in yeast (6a), in *Escherichia coli* and *B. subtilis* enzymes can be induced or derepressed at any period of the cell cycle (10, 12, 13), suggesting that the genome is accessible to transcription at all times. In these organisms, periodicity of enzyme synthesis during the cell cycle is difficult to interpret, being subject to all those regulatory systems known to influence enzyme levels in exponential cultures.

In summary, the present evidence in yeast favors an ordered, sequential transcription of the genome, the time of expression of a given gene being determined by its spatial arrangement on the yeast chromosome. Experiments are in progress to determine whether this order is from the centromere to its extremities or the reverse.

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