TIMING OF ENZYME SYNTHESIS DURING SYNCHRONOUS DIVISION IN YEAST

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There is increasing evidence that in microbial cells, nucleic acid synthesis is ordered. In Escherichia coli Hfr both the synthesis of DNA (Cairns, 1963) and the replication of the genome (Nagata, 1962) is non-random and sequential. Similar findings have been reported for Bacillus subtilis (Yoshikowa and Sueoka, 1963; Wake, 1963). Taylor (1960) has also shown polarized sites of DNA replication in chromosomes of higher organisms. An ordered synthesis of mRNA mediated by phage DNA has been suggested since the mRNA is unstable, mRNA species from early and late infection differ (Kanko-Sueoka and Spiegelman, 1962) and phage proteins and specific enzymes involved in phage production are synthesized at different times during the latent period (Cohen et al., 1963).

These observations pose the question whether in vegetative cells the overall process of transcription and translation of genetic information is also ordered. If mRNA is unstable and is produced by ordered transcription of cistrons, then in synchronously dividing cells a step-wise production of a given enzyme would be expected. To test this possibility, we have investigated the production of several enzymes in synchronous cultures of yeast. The findings reported here are consistent with the findings of discontinuous enzyme synthesis in Escherichia coli K12 Hfr (Masters et al., 1964).

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The method of Williamson and Scopes (1962) was employed to induce the synchronous state with the exception that the centrifugation for sizing cells after 10 days starvation was omitted, and the cells were finally suspended in a 0.2% succinate and salts medium at 25°C. Fig. 1 shows that the synchronous division of a hybrid yeast (Saccharomyces fragilis x S. dobzhanskii) continued for 2 1/2 cell generations. The lag time was 240 min and the generation time 200 min. The total amounts of RNA and protein doubled within each generation and the rate of synthesis doubled at the beginning of each new cell cycle. DNA doubled during each generation; its synthesis was periodic, initiating at zero time of the generation cycle, but complete in 140 min. Nuclear division occurred at a time when buds appeared (McClary et al., 1962) and when DNA was not replicated. This may well reflect a period of karyokinesis analogous to that of higher organisms where DNA is replicated only during interphase.

If the synthesis of enzyme is periodic, then the rate of synthesis during some time interval must fall to zero. This is illustrated in Fig. 2A for β-glucosidase synthesis in the haploid S. dobzhanskii. Enzyme synthesis is step-wise; a single rise in enzyme occurs in each division cycle at approximately the same time after the rise in cell count. Non-random periodic production of enzyme could reflect the timing of unstable mRNA synthesis or of an unstable control governing messenger function. Yeast provide an opportunity to test the latter possibility since frequently they contain multiple, non-allelic structural genes for the same enzyme (Halvorson et al., 1963). For example the hybrid yeast (Fig. 1) is allotrophic, derived by mating the diploid S. dobzhanskii by the haploid S. fragilis. The hybrid contains two immunologically distinct species of β-glucosidase, each corresponding to the enzyme produced by the parent strains (Duerksen and Fleming, 1963). The similarity in the induction and repression behavior of β-glucosidase in the hybrid and parental strains suggest that the same regulatory system(s) govern both structural genes for β-glucosidase (MacQuillan, unpublished results). Therefore, if the timing of enzyme

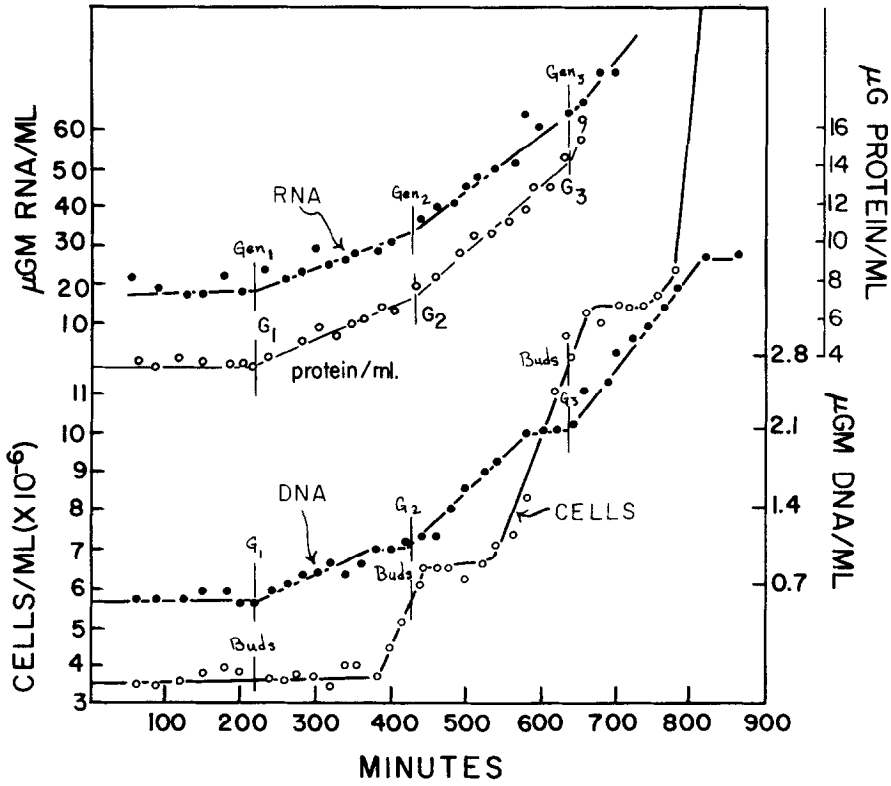

Fig. 1. Protein and nucleic acid synthesis in a synchronous culture of the yeast hybrid (S. dobzhanskii x S. fragilis). At intervals samples were removed for chemical determinations (25 ml) and cell counts (1 ml). Growth was arrested by the addition of formalin to a final concentration of 1%. Cell counts were determined in triplicate by means of a Levy hemocytometer employing the criterion of Williamson and Scopes (1962) to determine the completion of cytokinesis. Protein was determined in hot perchlorate precipitates by the method of Lowry et al. (1951). RNA was determined by the absorbance of the hot perchlorate soluble fraction at 260 mμ and DNA was determined in the same extract by the diphenylamine reaction (Burton, 1956).

synthesis is determined by the regulatory system, a single period of increase per generation would be expected in the hybrid. However, as can be seen in Fig. 2B, two marked periods of β-glucosidase synthesis occur in the hybrid: one was initiated 90 min after the onset of each generation

and stopped 50 min later, while the second was initiated approximately 30 min later depending on the generation. The total enzyme was doubled in each generation. The simplest explanation for these results is that the two structural genes for β-glucosidase are non-allelic and that the transcription of each is non-random and occurs at specific times in each cell generation.

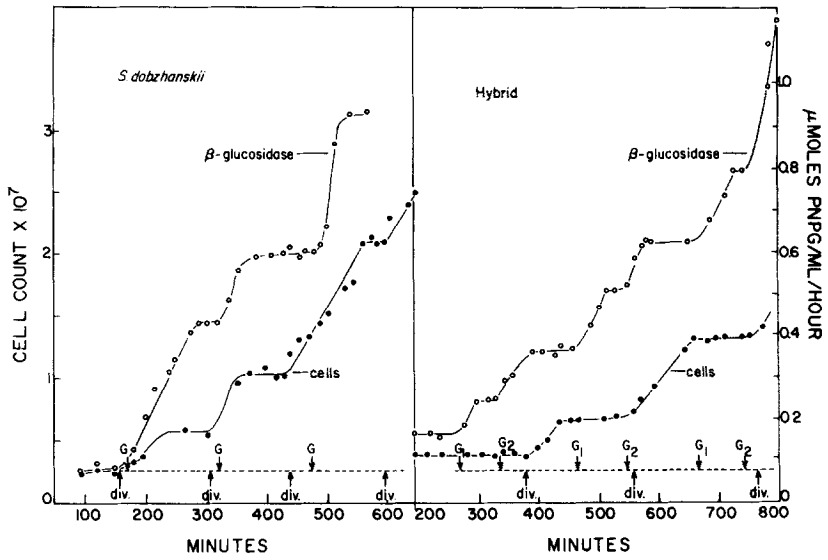

Fig 2. Synthesis of β-glucosidase in synchronous cultures of Saccharomyces dobzhanskii and a hybrid (S. dobzhanskii × S. fragilis). At intervals 5 ml aliquots were removed, washed twice by centrifugation and prepared and assayed for β-glucosidase by the method of Duerkson and Halvorson (1958). In the lower bar graph the time of initiation of cell division (div.) and of β-glucosidase synthesis (G) are indicated by arrows.

In order to test the generality of this phenomenon, the synthesis of several enzymes was examined in a diploid strain of S. cerevisiae (Winge H84) containing a single gene for α-glucosidase ($M_1M_1m_2m_2m_3m_3m_4m_4$) and known to contain several sucrose genes. The culture was synchronized as above and transferred to the synthetic medium containing 0.2% maltose. In Fig. 3, α-glucosidase, invertase and alkaline phosphatase synthesis are

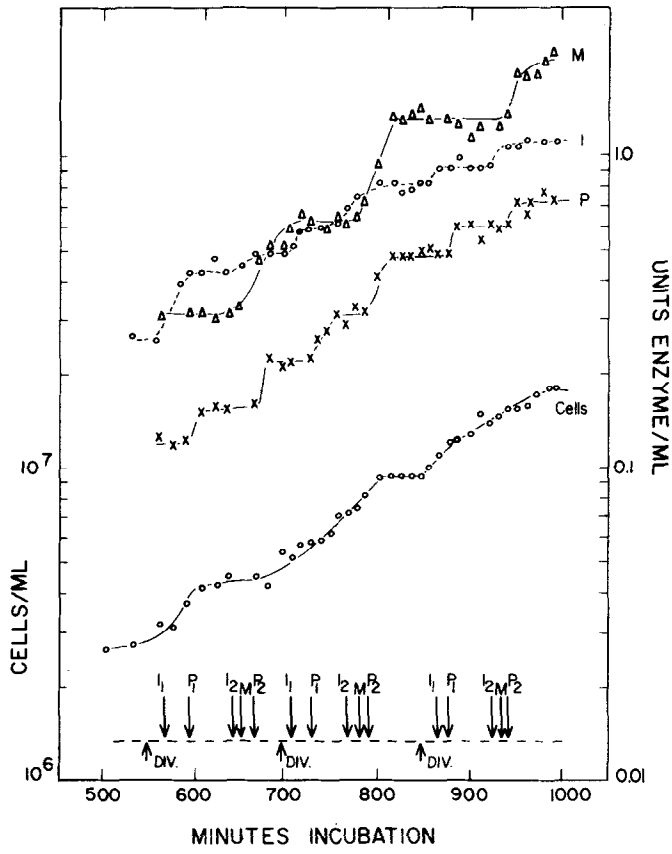

Fig. 3. Synthesis of α-glucosidase, invertase and alkaline phosphatase in a synchronous culture of Saccharomyces cerevisiae. Samples were prepared as above. α-Glucosidase (M) was assayed by the method of Halvorson and Ellias (1958), alkaline phosphatase (P) by the method of Torriani (1960) and invertase (I) by the method of Bacon (1955). Liberated glucose was measured with the Glucostat Reagent (Worthington Biochem. Corporation). The units of enzyme for M and P were 1 μmole substrate hydrolyzed/hr and for I, 0.1 m mole substrate hydrolyzed/hr. In the lower bar graph the time of initiation of cell division (div.) and of enzyme synthesis (M, P, I) are indicated by arrows.

all shown to be step-wise during growth of the synchronous culture. One period of α-glucosidase synthesis occurs in each cell division, being initiated approximately 95 min after cell division. In each division cycle there are two periods of initiation of synthesis of invertase (20

and 85 min) and of alkaline phosphatase (40 and 105 min). The phenotypic expression of α-glucosidase and invertase are consistent with the number of copies of each gene; the number of structural genes for alkaline phosphatase has not as yet been determined. Previously Sylven et al. (1959) observed in yeast that the highest levels of cathepsin and dipeptidase occurred immediately prior to division and declined during budding.

The simplest hypothesis that explains the linear synthesis of protein and RNA and a step-wise synthesis of specific enzymes is that transcription is ordered. Since RNA and protein synthesis continue during the period where no DNA synthesis is occurring (Fig. 1), transcription probably follows DNA replication. This might be expected if only certain morphological states of chromosomes (eg. uncoiled) serve as templates for mRNA synthesis. Experiments are now in progress to determine whether the order of timing of enzyme synthesis parallels the genetic map and whether the various chromosomes in yeast are transcribed sequentially or in parallel.

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