NONIDENTICAL TIMES OF GENE EXPRESSION IN TWO STRAINS OF *SACCHAROMYCES CEREVISIAE* WITH MAPPING DIFFERENCES[1]

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SUMMARY

α-aminoadipic acid reductase activity and galactokinase activity were both found to be periodically synthesized in synchronized cultures of baker's yeast. In strain S288C (wild type map of Chromosome II) the synthetic period of the reductase began at 0.25-.35 fraction of a generation while that for the kinase began at 0.45-.55 fraction of a generation. However, in strain 61009 where gal_1 (the gene for galactokinase) and lys_2 (the gene for α-aminoadipic acid reductase) map in entirely different positions, the respective enzyme activities begin to accumulate at 0.15-0.25 and 0.65-0.75 fraction of a generation. These results indicate that transcriptional control of the timing of periods of enzyme synthesis in at least one eucaryote is influenced by the position of the structural gene on the chromosome.

INTRODUCTION

Limited testing of the effect of a gene's position on a chromosome on the time of periodic synthesis of that genes product in a synchronized culture of baker's yeast has been accomplished (1). However, more rigorous appraisal is available when one considers the reported genetic maps of Chromosome II of two strains of yeast (Fig. 1) (2,3). Several of the genes are reported in substantially different positions, e.g., gal_1 and lys_2 in the two strains probably as a result of a double inversion. If then products of these two genes can be shown to "burst" in cell synchronization studies, it becomes possible to ascertain whether map position has a bearing on the presumed time of synthesis of a given genes specific protein.

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METHODS AND MATERIALS

Saccharomyces cerevisiae stocks were kindly provided as follows: Strain S288C (α wild type) from Dr. R.K. Mortimer, University of California, Berkeley, and strain 61009 from Drs. Carl and Gertrude Lindegren, Southern Illinois University, Carbondale. The latter strain was lys_2^+ and was from the same tetrad as the lys_2^- mutant mapped in Fig. 1 (3).

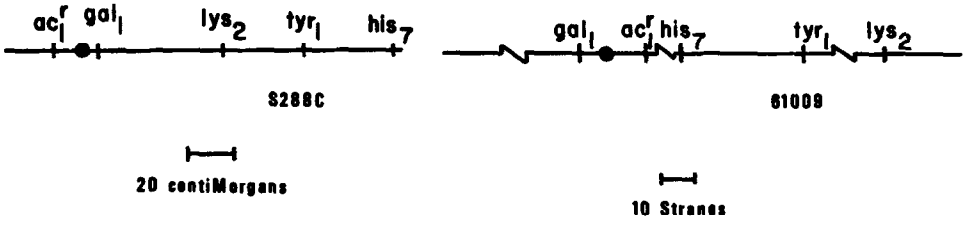

Fig. 1. Genetic maps of chromosome II in Saccharomyces cerevisiae strains S288C (2) and 61009 (3).

Strain S288C was grown in a minimal liquid medium (4). To this was added one ml. of a vitamin mixture (5) per 100 ml of minimal medium and 1 ml of a mineral salt solution per liter of minimal medium (6). D (+)-galactose or d-glucose was used as the carbon source in experiments depending on whether galactokinase or α-aminoadipic acid reductase, respectively, was to be subsequently determined. Either carbon source was added to a concentration of 1% of the medium. Strain 61009 was grown in a liquid medium (7) containing per liter of water: 20 g. of glucose or galactose, 2 g. $(NH_4)_2SO_4$, 2 g. KH_2PO_4 and 5 g. of yeast extract.

Synchronous cultures of yeast cells were prepared by density gradient centrifugation (8). In a 50 ml centrifuge tube, approximately 40 ml of an 8 to 35% linear sucrose or galactose gradient was formed. This was in turn layered with newly harvested cells. Such tubes were then centrifuged in a swinging bucket International Model CM using head no. 240 at a speed of 300 g. for 15 minutes. The top of the cell layer by then had traversed approximately one-half the distance to the bottom of the tube. 7 ml. of

the top of the cell layer was removed and suspended in 350 ml of fresh medium to start the synchronous culture.

At indicated times, in general, 12 ml samples were removed from the synchronized cultures: 1 ml was mixed with 0.1 ml 36% formaldehyde, for cell number determination; 10 ml was centrifuged, the pellet resuspended in water and recentrifuged, and the cells were then frozen until enzyme assay; 1 ml was centrifuged, the pellet was resuspended in water, recentrifuged and the pellet resuspended in water such that its final volume was 1 ml. This last sample was used for nitrogen determination by a colorimetric micromethod (9) employing $CuSeO_3$ as oxidant.

Counts of cell number and of the number of buds utilized a Levy hemocytometer. The method and the criterion for differentiation between cells and buds was that of Williamson and Scopes (10). Generations were considered as beginning with the appearance of buds and ending approximately with cell division. The cell synchrony achieved was determined using the Synchronization Index (10,11). The Index values were, in general, (equivalent degree of synchrony in parenthesis), 0.75-0.60 (97-90%) for the first generation, 0.65-0.50 (90-85%) for the second generation.

The yeast cells were disrupted for enzyme assay by grinding in a mortar and pestle with glass powder for 10 minutes at 5°C. Appropriate buffer was added and the extract centrifuged at 29,000 g at 2° for 20 min. The supernate was used for enzyme determinations. The protein content of the supernate was determined by the Lowry method (12).

The α-aminoadipic acid reductase (lys_2 gene product) was assayed by colorimetric determination of the ρ-dimethylaminobenzaldehyde adduct of α-aminoadipic-δ-semialdehyde (13,14). The galactokinase (gal_1 gene product) activity was determined at 25° according to Kalckar et. al. (15) except that the acid-labile phosphate (galactose-1-phosphate) was determined using ammonium molybdate according to Gomori (16).

Sources of certain materials used were as follows: vitamins in growth media, Nutritional Biochemicals Corp., Cleveland; Isocitric dehydrogenase,

DL-α-aminoadipic acid and triphosphopyridine nucleotide, Sigma Chemical Corp., St. Louis.

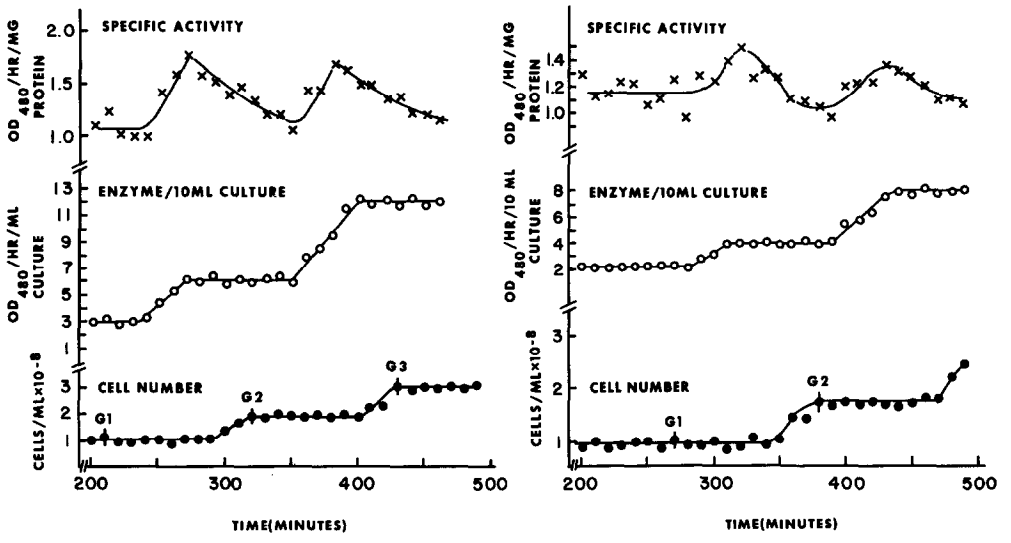

Fig. 2. Synthesis of α-aminoadipic acid reductase in Saccharomyces cerevisiae strain S288C (left) and strain 61009 (right). 0 time (not shown) represents time of innoculation and beginning incubation of the sized (synchronized) cells into the culture medium. G1, G2, G3 designate beginning of first, second, third generation respectively.

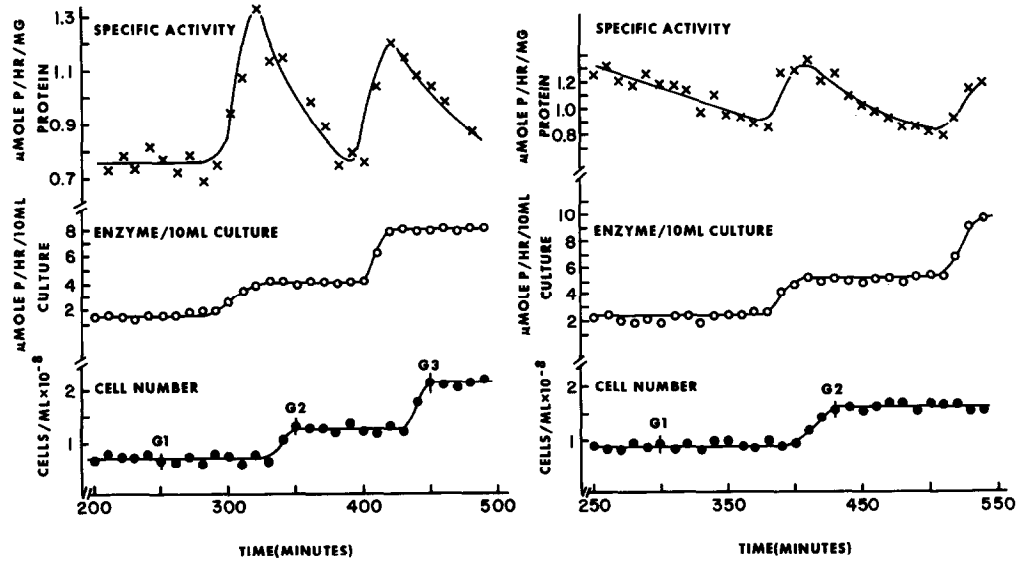

Fig. 3. Synthesis of galactokinase in Saccharomyces cerevisiae strain S288C (left) and strain 61009 (right). Notation identical to that in Fig. 2.

RESULT

The specific activity of each enzyme was plotted as a function of time, (Figs. 2 and 3). The increases of specific activity indicate periods of enzyme synthesis; decreases, periods of enzyme dilution or breakdown. Also plotted against time were cell number and the quantity "enzyme/10 ml culture."

The synthesis of α-aminoadipic acid reductase activity (specific activity curve, Fig. 2) and the synthesis of galactokinase (specific activity curve, Fig. 3) were observed to be periodic in synchronized cultures of wild type S288C. Furthermore, only one episode of synthesis occurred per cell cycle. This finding was also obtained for strain 61009 (Figs. 2 and 3).

The time of initiation of protein synthesis was next ascertained using a replot of the "enzyme/10 ml culture" curve (Fig. 4). Using this plot the value was determined for both the first and the second generation of a given synchronized culture. The values obtained for the beginning of the synthetic

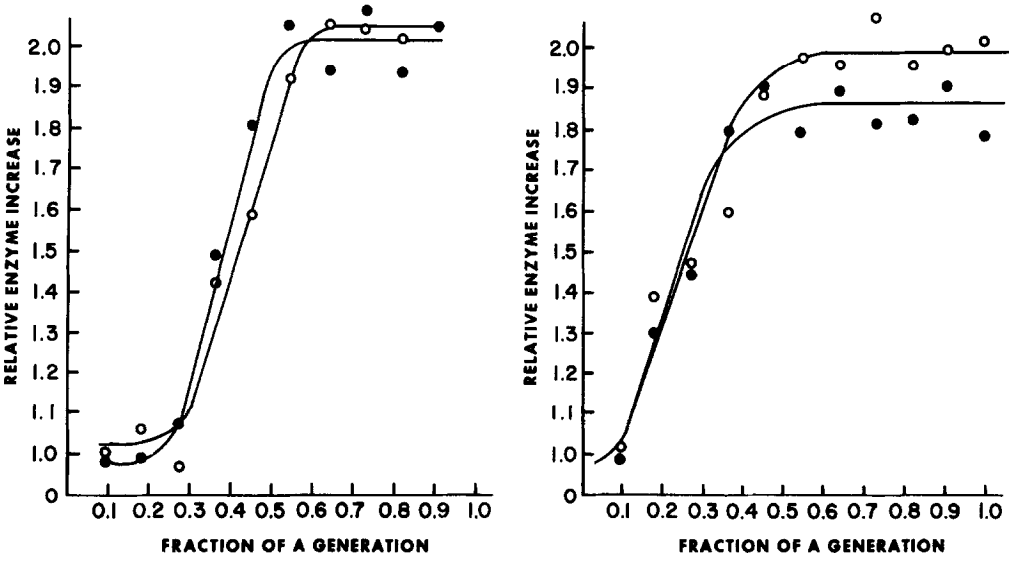

Fig. 4. Timing of synthesis of α-aminoadipic acid reductase during the cell cycle in strain S288C (left) and strain 61009 (right). (●) first generation, (○) second generation of a synchronous culture.

period were: _____ for α-aminoadipic acid reductase; in strain S288C, 0.25-0.35; in strain 61009, 0.15-0.25; _____ for galactokinase: in strain S288C, 0.45-0.55; in strain 61009, 0.65-0.75 fraction of a generation. The specific values have been plotted in Fig. 5. In each strain a given enzyme's synthesis begins at a characteristic and reproducible time.

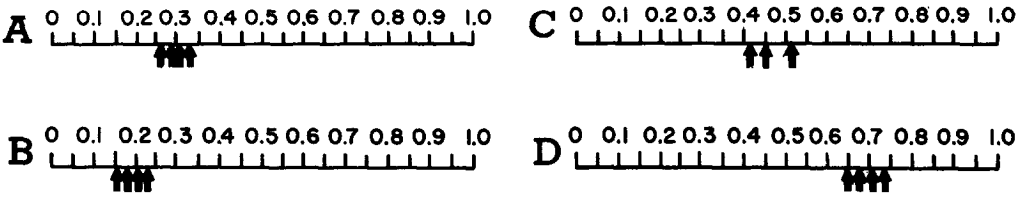

Fig. 5. Linear representation of the specific values obtained for the time of the beginning of the synthetic period of 1) α-aminoadipic acid reductase in strain S288C (A), in strain 61009 (B); and 2) galactokinase in strain S288C (C), in strain 61009 (D). Each arrow represents a single determination obtained from one generation of a synchronized culture.

DISCUSSION

If, as is thought to be the case in some procaryotes, cyclic variations in the regulatory system of the organism are important factors in controlling the time of synthesis of a particular enzyme, then the times of synthesis of α-aminoadipic acid reductase and galactokinase should not vary in these two strains. That is, when the genome is continuously open to transcription then the periodicity of enzyme synthesis would result from oscillations in repressors, etc. If, however, the order of the genes on the chromosomes, regulates to a great measure the order of transcription, then changing the position of a genetic locus on the chromosome should vary the time of its expression. Indeed, the change in time of enzyme synthesis might be proportional to the distance between the two identical loci along the specific chromosome in the two differing strains.

As indicated (Fig. 5), the times of the expression of the synthetic period for each of the two enzymes selected does differ when compared in the two strains. It, therefore, may be stated that changing the position of a genetic locus does not have an effect on the timing of the synthetic period for the gene product of that locus in the cell cycle. This evidence supports the hypothesis of an ordered transcription dependent on the linear geometry of the DNA functioning in periodic enzyme synthesis in eucaryotes.

The reported map of chromosome II, identical to that in strain S288C, (Fig. 1) shows absolute genetic distances. However, in strain 61009 certain of the distances between genes are unknown as indicated by the broken line. So the results above can only be interpreted in a qualitative sense since certain loci span such breaks.

While the data of this paper are too scanty to permit generalization, it may be noted in passing that in strain 61009 (Fig. 1) the lys_2 locus is located closer to the end of the chromosome arm than it is in strain S288C. Furthermore, in strain 61009 the distance between lys_2 and gal_1 is much greater than in strain S288C. Taking the timing data (Fig. 4) together with the map information just described it is possible to state that these findings are compatable with a unidirectional linear transcription mechanism - one which begins at the lys_2 end of the chromosome and proceeds through the centromere. [These results do not rule out a regional (on a chromosome) method of transcription].

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