ENZYME SYNTHESIS IN SYNCHRONOUS CULTURES OF BACTERIA

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THE RATE OF SYNTHESIS OF AN ENZYME HAS BEEN SHOWN TO VARY WITH THE NUMBER OF COPIES OF THE GENE DETERMINING ITS STRUCTURE WHICH ARE PRESENT (JACOB ET AL, 1960; DONACHIE, 1964). THE QUANTITATIVE STUDIES OF DONACHIE (1964) SHOW A DIRECT PROPORTIONALITY BETWEEN NUMBER OF GENES AND AMOUNT OF ENZYME SYNTHESIS IN NEUROSPORA CRASSA UNDER SOME CONDITIONS. IN BOTH BACILLUS SUBTILIS (YOSHIKAWA AND SUEOKA, 1963A) AND ESCHERICHIA COLI (NAGATA, 1963; CAIRNS, 1963) THE GENOME IS REPLICATED IN A SEQUENTIAL NON-RANDOM FASHION. THERE WILL THUS BE A SPECIFIC TIME IN THE DIVISION CYCLE OF EACH CELL OF THESE ORGANISMS WHEN THE NUMBER OF COPIES OF A GIVEN GENE WILL DOUBLE IN CONCENTRATION.

THIS LEADS US TO ASK HOW THE CELLULAR CONCENTRATION OF THE ENZYMES CONTROLLED BY THESE GENES WILL VARY WITH TIME. IF CELLULAR CONTROL MECHANISMS DO NOT INTERVENE TO OBSCURE THE EFFECT OF GENE DOSAGE, THE CAPACITY TO SYNTHESIZE EACH ENZYME WOULD BE EXPECTED TO INCREASE IN THE SAME STEP-WISE MANNER AS THE GENE CONCENTRATION. AN EARLY ATTEMPT TO FIND SUCH DISCONTINUOUS ENZYME SYNTHESIS IN SYNCHRONOUS CULTURES OF E. COLI B (ABBO AND PARDEE, 1960) PROVED NEGATIVE. SINCE IT IS LIKELY THAT THE E. COLI B GENOME IS REPLICATED IN THE SAME MANNER AS THAT OF E. COLI K12 F^-, IT WOULD NOT BE EXPECTED TO SHOW A GENE DOSAGE EFFECT.

WE THEREFORE DECIDED TO RETEST THIS HYPOTHESIS USING SYNCHRONOUSLY DIVIDING POPULATIONS OF E. COLI K12 HFR AND B. SUBTILIS. THE RESULTS REPORTED HERE ARE CORROBORATED BY THE EXPERIMENTS OF GORMAN ET AL (1964) WITH YEAST. E. COLI K12 HFR CS101 WAS SYNCHRONIZED BY THE PROCEDURE OF NAGATA (1963). FIGURE 1 SHOWS THE DIVISION SYNCHRONY OF THE CELLS AND THEIR CAPACITY TO SYNTHESIZE ASPARTATE TRANSCARBAMYLASE (ATC-ASE) AND B-GALACTOSIDASE. THE DEREPRESSED (PRODUCED BY ADDITION OF 6-AZAURACIL)

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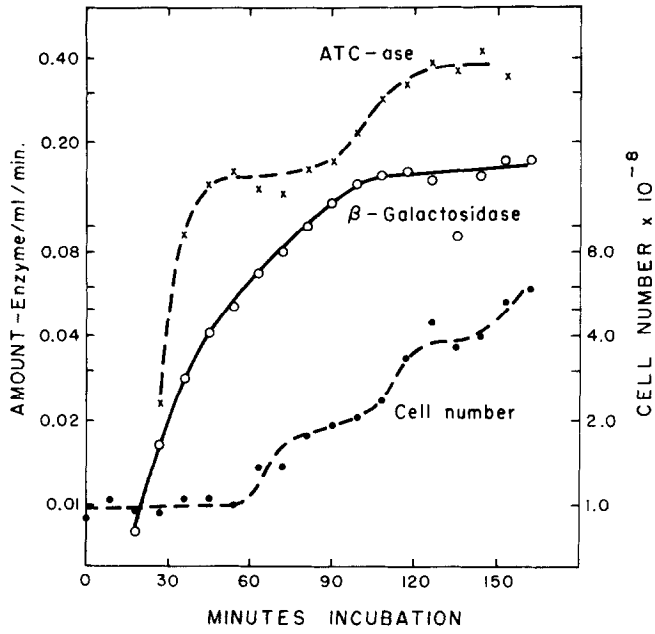

FIG. 1. ABILITY OF A SYNCHRONOUS CULTURE OF E. COLI K12 HFR CS101 TO SYNTHESIZE ATC-ASE AND β-GALACTOSIDASE. SAMPLES WERE REMOVED AT 9 MINUTE INTERVALS AND DE-REPRESSED FOR ATC-ASE WITH 1 MICROGRAM OF 6-AZAURACIL/ML. AND INDUCED FOR β-GALACTOSIDASE WITH 5×10^{-4} M ISOPROPYL-THIO-β-D-GALACTOSIDE. THE RATES OF SYNTHESIS WERE DETERMINED FROM THE SLOPES OF THE CURVES FOR DE-REPRESSION AND INDUCTION IN THE SEPARATE SAMPLES. ATC-ASE WAS ASSAYED BY THE PROCEDURE OF GERHART AND PARDEE (1962): ONE UNIT OF ENZYMIC ACTIVITY PRODUCES 0.1 MICROMOLE OF CARBAMYL ASPARTATE/HR. β-GALACTOSIDASE WAS ASSAYED BY THE HYDROLYSIS OF O-NITROPHENYL-β-D-GALACTOSIDE (ABBO AND PARDEE, 1960); ONE UNIT OF ENZYMIC ACTIVITY HYDROLYZED 20 MILLIMICROMOLES OF THIS SUBSTRATE/MIN. THE INCUBATION TIME IS IN MINUTES AFTER THE STATIONARY PHASE AND FILTERING PROCEDURES USED TO OBTAIN SYNCHRONY (NAGATA, 1963).

RATE OF ATC-ASE SYNTHESIS INCREASES STEP-WISE; THE INDUCED RATE OF β-GALACTOSIDASE SYNTHESIS DOES NOT INCREASE IN THIS MANNER. THIS CONTINUOUS INCREASE IN THE INDUCIBILITY OF β-GALACTOSIDASE INDICATES THAT ALL ENZYMES DO NOT SHOW A SPECIFIC DOUBLING TIME. POSSIBLY THE STATIONARY PHASE TREATMENT AND THE GLUCOSE MEDIUM ACT TO OBSCURE ANY GENE DOSAGE EFFECT FOR β-GALACTOSIDASE.

THE DISCOVERY THAT STATIONARY PHASE CULTURES OF WILD TYPE B. SUBTILIS, W23 SHOWED SOME SYCHRONY OF CHROMOSOMAL REPLICATION ON REDILUTION (YOSHIKAWA AND SUEOKA, 1963B) LED US TO LOOK FOR SYNCHRONY OF CELL DIVISION AND ENZYME FORMATION IN SUCH CULTURES. ON RICHER MEDIA, INCLUDING MINIMAL MEDIUM SUPPLEMENTED WITH GLUCOSE OR GLYCEROL AS CARBON SOURCE, W23 GROWS AS CHAINS WHICH LATER FRAGMENT. THIS

RENDERS ENUMERATION DIFFICULT. WHEN THE BACTERIA WERE GROWN ON SPIZIZEN (1958) MINIMAL MEDIUM USING HISTIDINE AS A CARBON SOURCE, WE FOUND THAT THEY GREW AS SINGLE CELLS, MAKING IT POSSIBLE TO DETERMINE THE DEGREE OF SYNCHRONY. TO OBTAIN SYNCHRONOUS CULTURES THE BACTERIA WERE ALLOWED TO GROW TO STATIONARY PHASE IN MINIMAL MEDIUM CONTAINING HISTIDINE. ALIQUOTS OF THESE CULTURES, CENTRIFUGED AND RESUSPENDED INTO FRESH MEDIUM OF THE SAME KIND, WERE FOUND TO DIVIDE SYNCHRONOUSLY. THE LOWER CURVE IN FIGURE 2 SHOWS THE WAY CELL DIVISION PROCEEDS IN SUCH A SYNCHRONIZED CULTURE. THE FACTORS DISCUSSED BY YANAGITA AND KANEKO (1961) MAY BE OPERATIVE HERE.

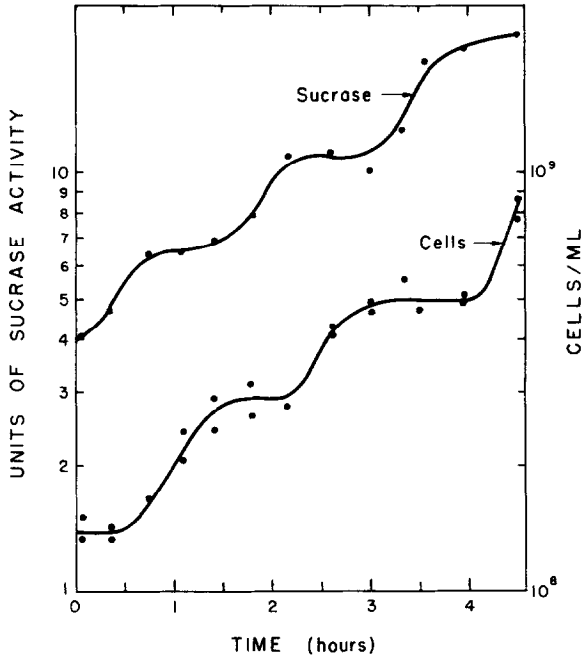

FIG. 2. GROWTH AND SUCRASE INDUCIBILITY IN A SYNCHRONOUS CULTURE OF B. SUBTILIS. CELL NUMBER WAS DETERMINED BY DILUTION AND PLATING OF THE CULTURE ON PENNASSAY AGAR. SUCRASE INDUCIBILITY WAS DETERMINED BY MIXING 5 CC. ALIQUOTS OF THE CULTURE WITH 0.2% SUCROSE. INDUCTION WAS STOPPED AFTER TEN MINUTES BY THE ADDITION OF 50 µGMS. OF CHLORAMPHENICOL/ML. ALIQUOTS WERE LYSED WITH LYSOZYME AND INCUBATED WITH 5% SUCROSE FOR ONE HOUR. THE AMOUNT OF GLUCOSE LIBERATED WAS MEASURED WITH THE GLUCOSTAT REAGENT (PURCHASED FROM THE WORTHINGTON BIOCHEMICAL CORPORATION; FREEHOLD, N.J.). A UNIT OF SUCRASE ACTIVITY HYDROLYSES 0.2 µMOLES OF SUCROSE/HR.

WE THEN LOOKED AT THE WAY IN WHICH SEVERAL ENZYMES ARE SYNTHESIZED IN THESE SYNCHRONOUS CULTURES. THE UPPER CURVE IN FIGURE 2 SHOWS HOW SUCRASE INDUCIBILITY VARIES IN THE CULTURE WHOSE DIVISION IS ILLUSTRATED IN THE LOWER CURVE. IT CAN BE SEEN THAT THE ABILITY OF THE CULTURE TO PRODUCE THE ENZYME DOUBLES ONCE DURING EACH DIVISION CYCLE,

AND THAT THE TIME OF DOUBLING OCCURS AT APPROXIMATELY THE SAME TIME DURING EACH CYCLE. IN FIGURE 3 SUCRASE INDUCIBILITY AND THE ACTIVITIES OF HISTIDASE AND ATC-ASE ARE SHOWN AT VARIOUS TIMES DURING THE GROWTH OF THE INDICATED CULTURE. NOT ONLY DO THE ENZYMES INCREASE IN A STEP-WISE MANNER, BUT THEIR TIMES OF DOUBLING FALL AT DIFFERENT TIMES IN THE CELL CYCLE. THIS IS MORE CLEARLY SEEN (FIGURE 4) WHEN THE MID-POINTS OF THE ENZYME AND CELL DOUBLING TIMES ARE PLOTTED ON A LINEAR TIME SCALE. DURING THE FIRST TWO DIVISION CYCLES (THE IRREGULARITY IN THE THIRD IS PROBABLY CONNECTED WITH THE APPROACH OF STATIONARY PHASE) HISTIDASE INCREASES ABOUT TEN MINUTES BEFORE CELL DIVISION, WHILE ATC-ASE INCREASE ABOUT FIFTEEN AND SUCRASE ABOUT FIFTY MINUTES AFTER DIVISION.

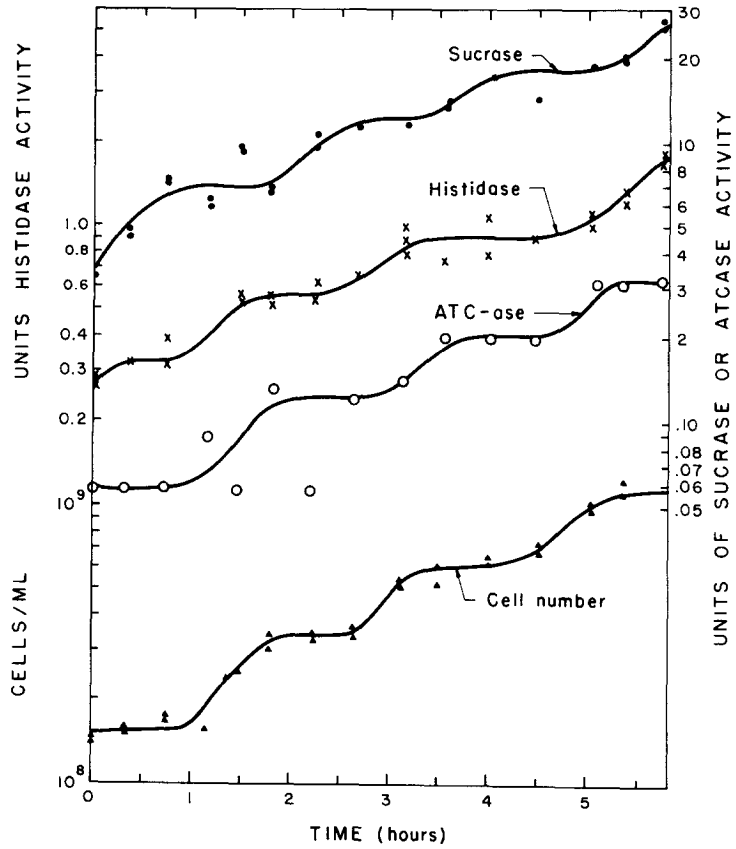

FIG. 3. VARIATION IN ACTIVITIES OF ATC-ASE, HISTIDASE, AND SUCRASE INDUCIBILITY IN A SYNCHRONOUSLY GROWING CULTURE. ATC-ASE ACTIVITY IS MEASURED AS ABOVE. A UNIT OF ATC-ASE ACTIVITY IS HERE DEFINED AS THE FORMATION OF 0.1 MICROMOLE OF CARBAMYL ASPARTIC ACID/HOUR. HISTIDASE ACTIVITY IS MEASURED BY THE METHOD OF TABOR AND MEHLER (1955) IN WHOLE CELLS, USING A BECKMAN DB DOUBLE BEAM SPECTROPHOTOMETER TO MEASURE OPTICAL DENSITY CHANGE AT 277 Mμ. A UNIT OF HISTIDASE ACTIVITY PRODUCES ONE MILLIMOLE/HOUR OF UROCANIC ACID. A UNIT OF SUCRASE ACTIVITY, DETERMINED AS ABOVE, HERE HYDROLYZES 0.1 MICROMOLE OF SUCROSE/HOUR.

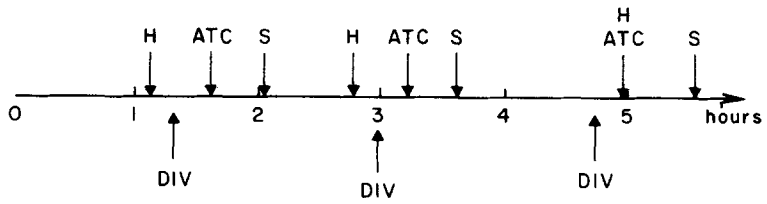

FIG. 4. POINTS OF INFLECTION IN THE DOUBLINGS OF EACH OF THE ENZYMES AND OF CELL DIVISION PLOTTED ON A LINEAR TIME SCALE. HISTIDASE IS REPRESENTED BY H, ASPARTATE TRANSCARBAMYLASE BY ATC, AND SUCRASE BY S.

OUR TENTATIVE EXPLANATION OF THE SPECIFIC DOUBLING TIMES IS THAT THEY OCCUR SOON AFTER THE CORRESPONDING GENE IS REPLICATED. EXPERIMENTS ARE NOW IN PROGRESS TO TEST THIS HYPOTHESIS. FIGURE 4, IT MAY BE NOTED, RESEMBLES A LINEAR GENETIC MAP. IF THE ENZYME DOUBLING TIMES DO TURN OUT TO BE CORRELATED WITH THE REPLICATION TIMES OF THE CORRESPONDING STRUCTURAL GENES, ENZYME STUDIES COULD BE USED TO INDEPENDENTLY MAP SUCH GENES. ATTEMPTS ARE IN PROGRESS TO MAP CERTAIN GENES WHOSE MUTATIONS ARE USUALLY LETHAL AND WHICH THEREFORE CANNOT BE MAPPED BY THE USUAL PROCEDURES.

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