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Protein Turnover in the Cell Cycle of *Escherichia coli*

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ABSTRACT

NISHI, ARASUKE (University of Tokyo, Tokyo, Japan), AND TOKIO KOGOMA. Protein turnover in the cell cycle of *Escherichia coli*. J. Bacteriol. **90**:884-890. 1965.—Protein metabolism and enzyme formation throughout the cell cycle were investigated in synchronized cultures of *Escherichia coli*. The cells showed a temporary cessation of the net increase of bulk protein and of constitutive β-galactosidase activity during the division period. By contrast, when tested by short-term experiments performed with cells at different growth stages, the bacteria displayed a constant incorporation of labeled protein precursors into the protein fraction, even during the fission period. Similar results were obtained with respect to the capacities for induced enzyme formation. On the other hand, when the cells were previously labeled and then subjected to synchronization in a nonradioactive medium, the radioactivity of the protein fraction decreased temporarily by nearly 10% during the fission period and then regained its previous level at the beginning of the ensuing phase of growth. This indicates that the products of partial degradation of protein were again utilized for protein synthesis in the next cell cycle. It was concluded that the temporary lagging of net increase of bulk protein may be due to the partial breakdown of protein occurring during the fission period.

It is known that in some species of protozoa protein synthesis is suppressed prior to or during the division period (Prescott, 1955; Hamburger and Zeuthen, 1960; Plesner, 1963). With yeast cells, on the other hand, it was reported that protein synthesis occurs during the whole course of the cell cycle (Williamson and Scopes, 1961). Observations made with synchronized bacteria are diverse; Maruyama (1956) showed that in *Escherichia coli* the increase of protein ceased during the fission period, whereas Abbo and Pardee (1960), using the same organism, reported a steady increase of protein.

The present paper deals with the mode of protein metabolism during the cell cycle of *E. coli*. At successive stages of the cell cycle, the process of protein metabolism was followed by measuring (i) the net increase of bulk protein, (ii) the activities of induced or constitutive enzymes, (iii) the rate of incorporation of labeled protein precursors into protein, and (iv) the fate of previously labeled protein of cells synchronized in nonradioactive ("cold") medium. The results obtained show that the apparent cessation of protein increase, as observed by Maruyama (1956), during the fission period is due to a partial degradation of protein occurring temporarily during that period.

MATERIALS AND METHODS

Organisms and cultivation. The organisms used were two strains of *Escherichia coli*, ML 30 and 308, in which β-galactosidase is inducible and constitutive, respectively. Both of these strains were inducible for tryptophanase and D-serine deaminase. These strains were kindly supplied by I. Zabin of the University of California. Cells were grown at 37 C in a vigorously aerated synthetic medium (*p*H 7.0) of the following composition: KH_2PO_4, 13.6 g; $(NH_4)_2SO_4$, 2 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $FeSO_4 \cdot 7H_2O$, 0.005 g; succinic acid, 4 g; and water to 1 liter (Herzenberg, 1959).

Synchronization of culture. The synchrony of cell division was achieved by the filtration method of Maruyama and Yanagita (1956). A 1-liter culture of each strain was grown to an optical density at 550 mμ of about 0.15 (approximately 10^8 cells per milliliter). Cells were collected by centrifugation, and a thick suspension of the cells was absorbed onto the top layer of a wet filter-paper pile consisting of 20 sheets of Toyo no. 126 paper (5.5 cm in diameter). Cells were then rapidly washed through the pile with 70 ml of medium. The cells remaining in the third to sixth paper sheets from the top were eluted with the medium, and the eluate was used as starting material for the synchronous culture. The temperature was maintained at 37 C throughout the treatment. The concentration of bacteria in the starting suspension was 10^8 to 1.5×10^8 cells per milliliter of culture.

Enzyme induction and assay of the activities. β-Galactosidase was induced with 5×10^{-4} M isopropyl-thio-β-D-galactoside (IPTG). The enzyme was assayed by measuring the rate of hydrolysis of o-nitrophenyl-β-D-galactoside (Herzenberg, 1959). Tryptophanase was induced by 2.5×10^{-3} M L-tryptophan, and its activity was determined by the colorimetric estimation of indole with the Ehrlich reagent (Pardee and Prestidge, 1961). D-Serine deaminase was induced by 3×10^{-3} M D-serine, and activity was determined by the estimation of pyruvate formed (Wood and Gunsalus, 1949). All enzyme assays were made with cell suspensions previously subjected to repeated freezing and thawing.

Labeling of protein fraction. The capacity for de novo synthesis of protein was followed by determining the rate of incorporation of S^{35}-sulfate or tritiated leucine (Daiichi Pure Chemicals Co., Tokyo, Japan) into protein. To a 4-ml sample of bacterial suspension were added 5 μliters of the solution of S^{35}-sulfate or tritiated L-leucine, and the mixture was incubated for a short definite period. In some cases, acid-killed cells were added to the sample as carrier to facilitate the centrifugation and fractionation. The incorporation was stopped by the addition of 1 ml of 10% trichloroacetic acid, and the mixture was heated at 90 C for 15 min. The trichloroacetic acid-insoluble fraction was collected by centrifugation, washed three times with cold trichloroacetic acid, dissolved in a small amount of 1% NH_4OH, placed on a planchette, and dried.

When 1 μc of C^{14}-labeled *Chlorella* protein hydrolysate (The Radiochemical Centre, Amersham, England; specific activity, 200 μc/mg) was added to a 1-liter culture of exponentially growing cells, it was found that, after 10 min of incubation at 37 C, most of the C^{14}-amino acids added were consumed. To estimate the rate of protein turnover, the cells which had been incubated for 30 min under the above conditions were grown synchronously in a "cold" medium after being subjected to filtration and elution with a "cold" medium.

The radioactivity was measured with a windowless gas-flow counter (Tracerlab Inc., Waltham, Mass.) or in a liquid scintillation counter (Packard Instrument Co., Inc., La Grange, Ill.).

Paper chromatography of protein degradation products. To identify the products formed by the temporary, partial degradation of protein occurring during the fission period, the bacterial cells were previously labeled with C^{14}-phenylalanine as described above. After synchronization in the "cold" medium, 20-ml samples were taken from the culture, and the cells collected on a membrane filter were extracted with cold 5% trichloroacetic acid solution. The extract was concentrated after the removal of trichloroacetic acid by repeated shakings with ether and was subjected to paper chromatography with a solvent system of phenol–water (4:1). Each paper strip was then cut into pieces 1 cm long, which were extracted with water for the measurement of radioactivity.

Measurement of protein content. The content of bulk protein in bacterial cells was determined by the method of Lowry et al. (1951). Crystalline bovine albumin was used as the standard.

RESULTS

Courses of increase in bulk protein and constitutive enzyme during cell cycle. In Fig. 1 are shown the levels of total protein and constitutive β-galactosidase as they changed during the cell cycles of ML 308. The rates of net increase of protein and of the enzyme were reduced markedly during the fission period. In both cases, the courses of increase during the interdivision phase were linear rather than exponential.

Rates of formation of inducible enzymes during cell cycle. Samples of synchronized cells were removed at intervals, and their ability to form D-serine deaminase, β-galactosidase, and tryptophanase upon addition of their respective inducers was followed. The results presented in Fig. 2 show clearly that, in contrast to the course of net increase of the bulk protein, the formation of these induced enzymes occurred throughout

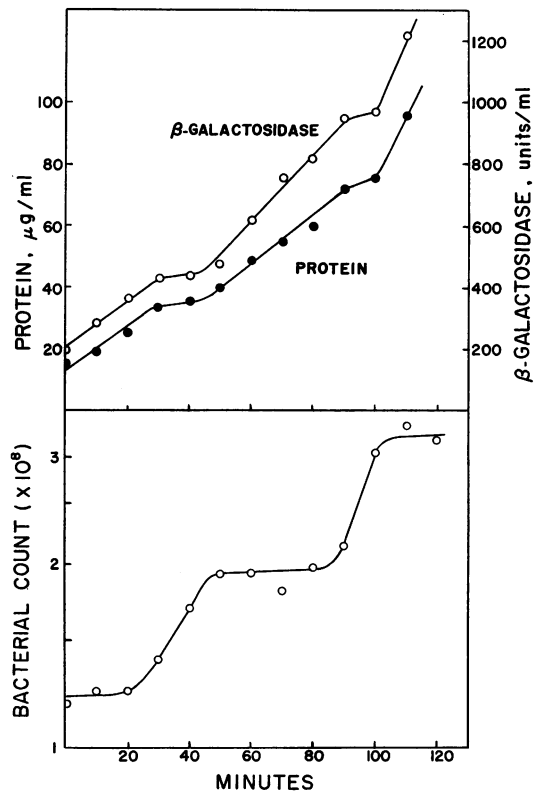

FIG. 1. *Rate of increase in protein and constitutive β-galactosidase activity in a synchronized culture of Escherichia coli ML 308.*

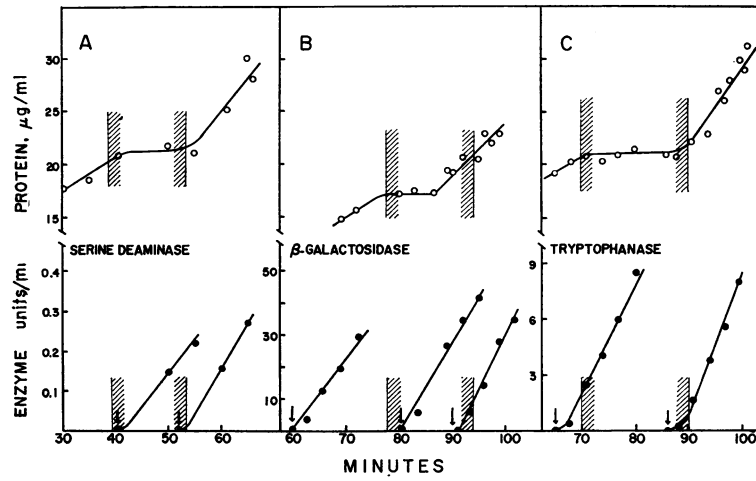

FIG. 2. *Change in total protein content and inducible enzyme formation in a synchronized culture of Escherichia coli ML 30. Arrows indicate the time of addition of the respective inducers. Shaded portions indicate the periods in which cell fission took place.*

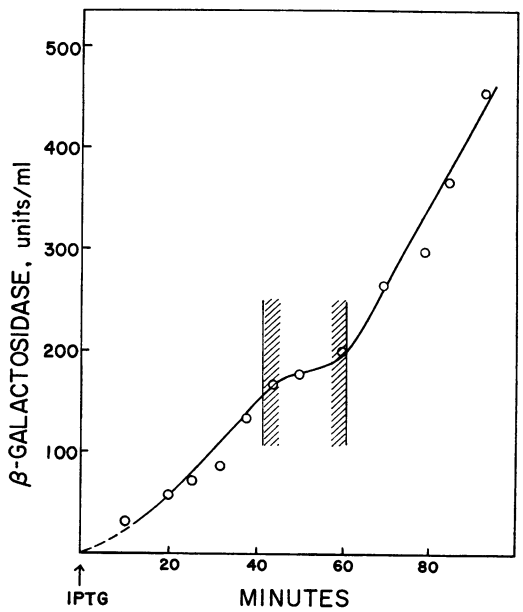

FIG. 3. *Inducible β-galactosidase formation in a synchronized culture of Escherichia coli ML 30. IPTG was added to the culture at the beginning of synchronization of the culture (arrow).*

the entire cycle of growth. However, when IPTG was added to cultures of strain ML 30 at the beginning of synchronization, β-galactosidase formation increased in a stepwise fashion (Fig. 3).

Rate of incorporation of labeled protein precursor during cell cycle. Samples were removed at intervals from a synchronized culture and tested for their ability to incorporate S^{35}-sulfate or tritiated leucine into protein. As already demonstrated by Abbo and Pardee (1960), the rate of incorporation of these labeled substances increased exponentially throughout the cell cycle (Fig. 4A). By contrast, when a sufficient amount of tritiated leucine was added to the culture at the beginning of the synchronized culture, the radioactivity of the protein fraction ceased to increase at the fission periods (Fig. 4B).

Partial degradation of protein occurring during the fission period. The observations described above led us to consider that a partial breakdown of cellular protein occurred during the fission period. This was confirmed by the experiments shown in Fig. 5. The cells, previously labeled with C^{14}-amino acids, were grown synchronously in a "cold" medium, and the radioactivity of the hot trichloroacetic acid-insoluble fraction (protein fraction) during division cycles was followed. As shown in Fig. 5A, the radioactivity of the protein fraction temporarily decreased during the fission period. It is important to note that the radioactivity converted to an acid-soluble form is reincorporated into protein at the beginning of the interdivision phase following the division.

In a parallel experiment, the cells taken at intervals of the cell cycle were collected on membrane filters after being treated or not treated with cold 3% trichloroacetic acid, and the radioactivities of the insoluble fraction and whole cells, respectively, were measured (Fig. 5B). In the cold trichloroacetic acid-insoluble fraction, the labeling was found mostly in the protein fraction. The radioactivity of whole cells re-

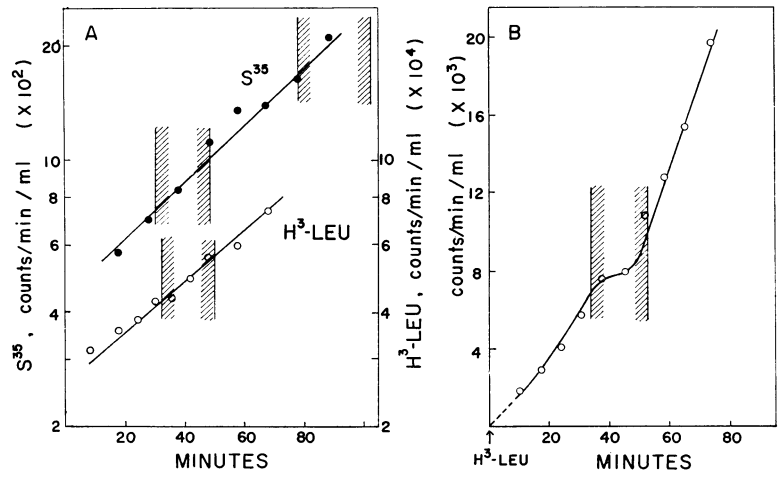

FIG. 4. *Incorporation of S^{35}-sulfate and H^3-leucine into the protein fraction of Escherichia coli ML 30 during the course of synchronized culture. (A) Samples (4 ml) were taken at intervals and exposed to either 100 μc of $S^{35}O_4^{-2}$ for 4 min or 12.5 μc of H^3-leucine for 1 min. Specific activity of S^{35} was 25 μc per 16 μg per ml of culture and that of H^3-leucine was 3.1 μc per 3.8 μg per ml of culture. (B) H^3-leucine was added to a culture to give a final specific activity of 0.63 μc per 20 μg per ml of culture at the beginning of synchronized growth, and the incorporation of radioactivity into cellular protein was followed.*

mained almost constant during the cell cycle (curve a), whereas the radioactivity of the protein fraction decreased temporarily during the fission period (curve b), so that the difference between curve a and b showed characteristic peaks during the period of cell division (curve c). These results show definitely that a characteristic modification of protein metabolism occurs during the fission period.

Similar results were also obtained when the cells were pulse-labeled at the beginning of the synchronized culture. The results obtained in eight experiments, in which the labelings were performed prior to or just after the filtration of the cells, are presented in Table 1. Although the degree of protein degradation occurring during the fission period fluctuated from one experiment to another, it appeared not to be affected by the time of labeling. The maximal rate of protein degradation in question is roughly estimated to be around 10% under the present experimental conditions.

Some characteristics of the degradation products. The cold trichloroacetic acid-soluble fractions were obtained from the synchronized cells which had been labeled with C^{14}-phenylalanine, and the chemical nature of radioactive substances in the fraction was analyzed by paper chromatography. The distribution of radioactivity on the paper chromatograms is shown in Fig. 6. Cells harvested at the fission period obviously contained much more of the radioactive substances in the trichloroacetic acid-soluble fraction than did those collected during the interdivision phase. The radioactive materials showing lower R_F values than that of phenylalanine were assumed to be phenylalanyl peptides, because they showed the same R_F value as phenylalanine after hydrolysis with 6 N HCl.

As described by Britten and McClure (1962), the pool amino acids in *E. coli* cells are easily exchangeable with environmental amino acids. To examine the exchangeability of the protein degradation products formed during the fission period, the following experiments were performed. Tritiated leucine-labeled cells were synchronized in a culture medium containing a sufficient amount (200 μg/ml) of unlabeled leucine, and the radioactivity of the trichloroacetic acid-insoluble fraction of cells was followed. The course of change observed was quite similar to those shown in Fig. 5, with almost 10% protein degradation occurring during the fission period (Table 2). The degradation products appeared to be reconstructed into the acid-insoluble form without any appreciable leakage of radioactivity from the bacterial cells.

DISCUSSION

The experimental data presented show that in synchronized *E. coli* cells both inducible enzyme synthesis and incorporation of labeled substances into protein proceed without interruption during the fission period. Our separate experiments, in

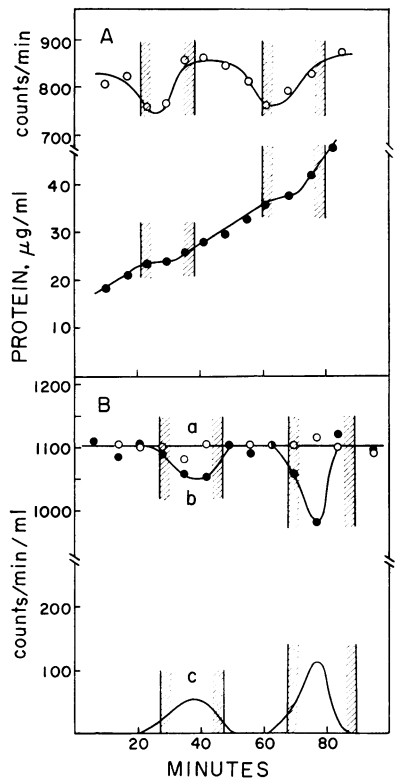

FIG. 5. *Change in radioactivity of the protein fraction during the course of synchronized culture of Escherichia coli ML 30 which had previously been labeled with C^{14}-amino acids. (A) Hot trichloroacetic acid-insoluble fractions were obtained at intervals and their radioactivities were followed. (B) Changes in radioactivities of intact cells (curve a, ○) and cold trichloroacetic acid-treated cells (curve b, ●) are shown. Curve c was obtained by subtracting b from a.*

which the sucrose density-gradient technique of Gros et al. (1961) was used, demonstrate that before and during the fission period there appears a rapidly labeled ribonucleic acid (RNA) in the cells of *E. coli*. Recently, Rudner, Prokop-Schneider, and Chargaff (1964) showed with synchronized *E. coli* that the synthesis of pulse-labeled RNA occurred steadily during the whole course of the cell cycle. Kuempel, Masters, and Pardee (1965) also showed that certain enzymes can be induced or derepressed at all times during the cell cycles of *E. coli* and *Bacillus subtilis*. These observations indicate, as already remarked by Abbo and Pardee (1960), that the rate of protein synthesis increases steadily during the course of the bacterial cell cycle. In contrast to these observations, our experimental results indicate that the net increase of bulk protein as well as of the

TABLE 1. *Rates of protein degradation occurring during the fission periods in synchronized cultures of Escherichia coli**

Time of addition of C^{14}-amino acids	Expt no.	Minimal levels of radioactivity observed during fission period (%)†	
		1st division	2nd division
30 min before cell filtration	1	87	93
	2	95	96
	3	93	90
	4	91	90
	5	92	92
	Mean	91.5	92
At the beginning of synchronized growth	6	86	92
	7	94	97
	8	92	80
	Mean	90.5	90

* Experimental procedures were the same as those employed in experiments shown in Fig. 5A.

† Radioactivity of trichloroacetic acid-insoluble fraction of cells at the interdivision phase was taken as 100.

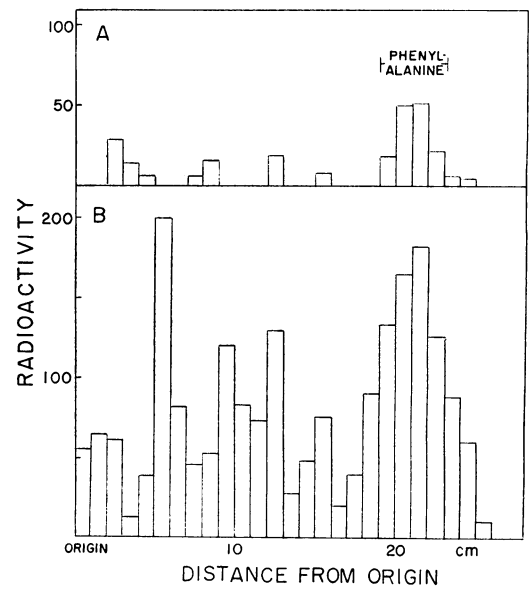

FIG. 6. *Distribution of radioactivity on paper chromatograms of protein degradation products. C^{14}-phenylalanine-labeled cells were synchronized and samples were taken at 8 min before (A) and during (B) the fission period. The trichloroacetic acid-soluble materials were developed on filter paper with phenol-water (4:1). The position of the phenylalanine spot is shown in A.*

TABLE 2. *Rates of protein degradation during the fission periods in the presence of excess amount of environmental amino acids**

Expt no.	Minimal levels of radioactivity (%)†	
	1st division	2nd division
1	92	93
2	88	—

* H^3-leucine (10 mμc/ml) was added to a logarithmically growing culture of *Escherichia coli*. After the consumption of H^3-leucine, 200 μg/ml of unlabeled leucine were added, and cultivation was continued for 1 hr. Cells were then subjected to a filtration procedure as synchronized in the presence of the same amount of unlabeled leucine, and the radioactivity of the protein fraction was followed as in Fig. 5A.

† Expressed as in Table 1.

constitutive enzyme took a stepwise course with a distinct cessation occurring during the fission period. If de novo protein synthesis is a process occurring steadily throughout the course of cell cycle, one has to assume that a partial degradation of protein occurs during the fission period. In the present study, evidence has been marshalled showing that a partial degradation (up to 10%) of protein takes place during the fission period.

It is known that the protein turnover in bacterial cells occurs markedly in resting cells but only slightly, if at all, in growing cells (Mandelstam, 1960). Some evidence for this conclusion has been obtained from experiments in which the rate of replacement of pool amino acids by exogenous amino acids was examined. As demonstrated in the present experiments, the products of the protein degradation occurring during the fission period were not replaced by environmental amino acids. The degradation products, unlike usual pool amino acids in bacterial cells, did not leak out from cells, thus indicating that they are different in nature from simple amino acids taken up by cells from the culture medium. The fact that, when cells were labeled prior to synchronization, the radioactivity of intact cells remained practically constant during the cell cycle (Fig. 5B) indicates that the degradation products accumulated during the fission period are kept in the cells until they are reutilized for the protein synthesis occurring in the ensuing growth phase.

Sylvén et al. (1959) reported that protease and peptidase activities of synchronously growing yeast cells were accelerated prior to cell division. As will be reported elsewhere, we also observed with our system that activity of proteases increases during the fission period; thus, their role in the enhanced protein degradation may possibly be related in some way to the phenomenon of cell division in this organism.

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