Genetic Regulation of Protein Biosynthesis at the Level of the Ribosome?

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Received January 8, 1963

A constitutive mutant of E. coli K-12 58-161 (methionineless) synthesizes β-galactosidase at a very high initial rate after methionine starvation in the presence and the absence of a carbon source (Yanagisawa, 1962a). This preferential synthesis of β-galactosidase was also observed in E. coli K-12 Ya 2 (Yanagisawa, 1962a) and in the original E. coli K-12 58-161 (both methionineless and inducible for β-galactosidase) only when methionine and a carbon source were both absent (Yanagisawa, 1962b).

Further study of this phenomenon led to the following conclusions:

(1) Preferential synthesis of β-galactosidase is caused by the accumulation of information for this enzyme during methionine and carbon source (glycerol) starvation.

(2) The information for β-galactosidase accumulates even in the absence of an inducer, IPTG, (isopropyl-β-D- thiogalacto pyranoside), during starvation.

(3) A molecule of β-galactosidase cannot be finished without the aid of the inducer even though the information for this enzyme is given abundantly.

Further, a dual action of the inducer was suggested. Although the action of an inducer at a later stage (or stages) than messenger RNA formation was indicated, inducer might still act at the gene level as well.

Figure 1 shows the amount of β-galactosidase 15 minutes after the restoration of methionine and glycerol (and IPTG, if necessary) following starvation for the times indicated on the abscissa. In the inducible strain, Ya 2, the amount of β-galactosidase increased until 45 minutes regardless of the presence or absence of the inducer during starvation. In the constitutive strain, 58-161, it reached, however, the highest amount only af-

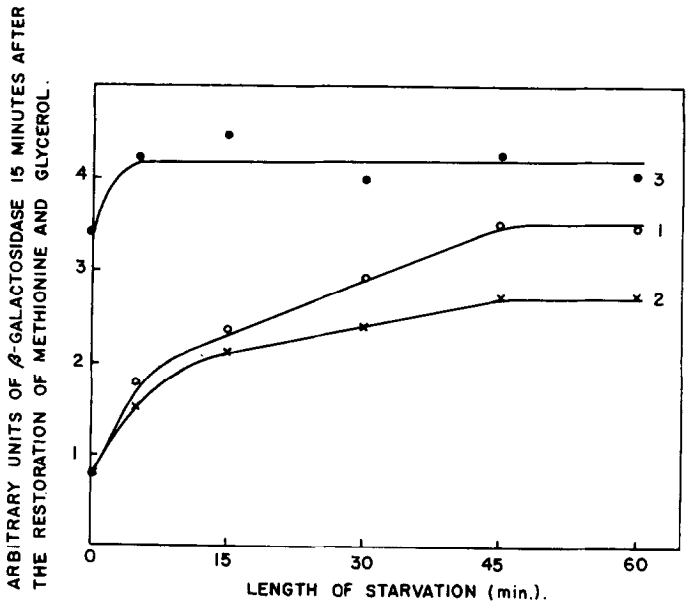

Fig. 1. Preferential synthesis of β-galactosidase after various lengths of starvation.

1. E. coli K-12 Ya 2, starved in the presence of inducer.
2. E. coli K-12 Ya 2 with inducer, methionine and glycerol added only after starvation.
3. E. coli K-12 58-161, constitutive mutant.

ter 5 minutes of starvation. With the inducible strain, 58-161, essentially the same curve was obtained as with Ya 2, although it leveled off at 30 minutes. The amount of β-galactosidase produced in the inducible strain, 58-161, is much less than that produced by Ya 2 and is somewhat variable. Therefore, Ya 2 was used exclusively for further experiments.

The preferential synthesis of β-galactosidase after methionine and glycerol starvation was completely eliminated by treatment with puromycin during the starvation period both in constitutive and inducible strains (Fig. 2). This chemical did not have any residual effect on the formation of β-galactosidase when it was removed. These two experiments led to conclusions (1) and (2).

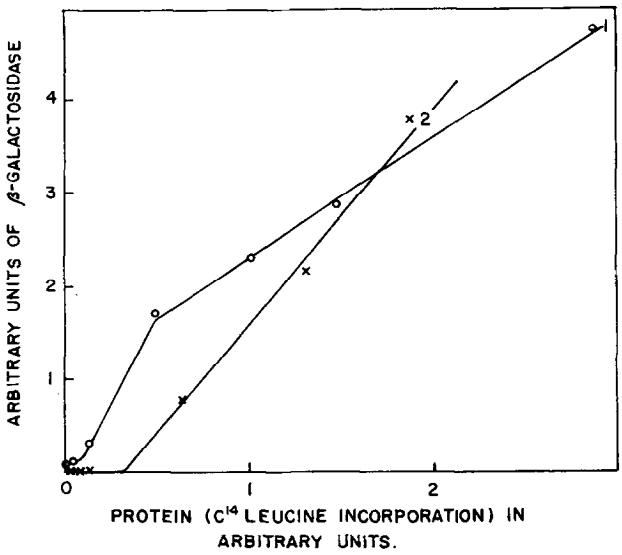

Fig. 2. Effect of puromycin on the preferential synthesis of β-galactosidase (E. coli K-12 Ya 2).

1. Control after 60 minutes starvation.
2. Treated during starvation with puromycin which was washed out prior to the restoration of methionine and glycerol.

To know the precise mode of β-galactosidase formation after starvation, samples were taken at 2 minute intervals after the restoration of methionine and glycerol. In the constitutive strain, 58-161, the amount of β-galactosidase reached the highest level as soon as methionine and glycerol were added (within 15 seconds), whereupon the synthesis of the enzyme ceased for a while. However, in the inducible strains, a lag of 3 to 4 minutes was observed before the enzyme started to form. Since this lag is not observed in the constitutive strain and its length coincides with that measured by Pardee et al (1960), it might be the same lag as is usually observed in β-galactosidase induction. This lag is observed regardless of the presence or absence of the inducer during starvation. Furthermore, when inducer is present during starvation but absent after it, no enzyme is formed. The first two experiments have already shown that the information for β-galactosidase accumulated in the starved cells, and the last two experiments indicate that the intervention of the inducer is still essential to finish the synthesis of active β-galactosidase molecules. Thus we are brought to conclusion (3).

Figure 3 shows the differential rates of β-galactosidase formation with and without 5 minute starvation. Without starvation the differential rate is the same in the constitutive strain, 58-161, and in the inducible strain, Ya 2. Comparing the amount of the enzyme after 5 minutes starvation between 58-161 (constitutive) and Ya 2 (inducible but without inducer during starvation) the activity of the β-galactosidase locus is calculated to be at least 6 times higher in the constitutive strain. This, of course, is

a minimal value, since the enzyme level could have reached the maximum well before 5 minutes (cf. Fig. 1). Also there is no definite evidence that this difference is caused solely by differences in the i or o loci between the two strains.

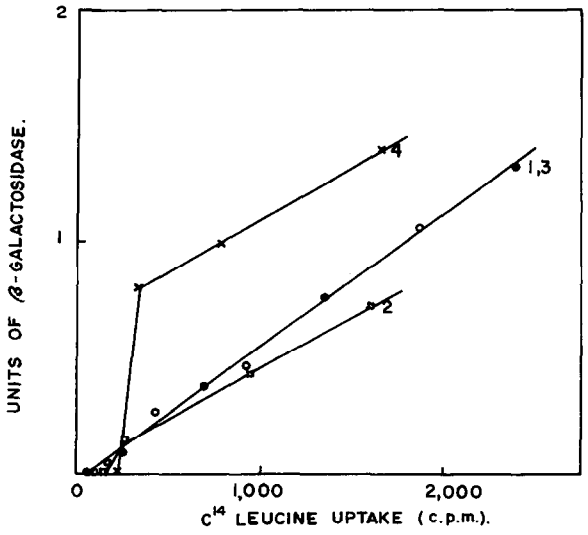

Fig. 3. Differential rate of β-galactosidase synthesis after 5 minutes starvation for methionine and glycerol.

1. E. coli K-12 Ya 2; control, no starvation.
2. E. coli K-12 Ya 2; 5 minutes starvation in the absence of inducer.
3. E. coli K-12 58-161 (constitutive); control, no starvation.
4. E. coli K-12 58-161 (constitutive); 5 minutes starvation.

These data are consistent with the hypothesis that the repressor combines, at least in part, with the ribosome or with messenger RNA.

Acknowledgment

The author is grateful to Professor F. J. Ryan for his guidance throughout the course of this work which was supported in part by grants from the National Science Foundation and the U. S. Public Health Service.

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