CELL SEPTATION AND THE SYNTHESIS OF CATABOLITE REPRESSIBLE ENZYMES IN *Escherichia coli*

Michel PIOVANT, Jeanine BUSUTTIL, Claude LAZDUNSKI

CENTRE DE BIOCHIMIE ET DE BIOLOGIE MOLECULAIRE, C.N.R.S.
31, chemin Joseph Aiguier - 13274 MARSEILLE Cedex 2

Received December 2, 1974

Summary:- In casamino acids containing minimal medium-glycerol there is a slow increase of intracellular cyclic AMP level during growth. Concomitantly, β-galactosidase and tryptophanase synthesis are initiated. If, in the same medium, cell septation is blocked, either by using non permissive conditions with conditional thermosensitive mutants or by addition of Penicillin G, one observes an early escape of tryptophanase and β-galactosidase from catabolite repression. This effect is not related to an alteration of intracellular cyclic AMP level upon cessation of cell division. However, it seems to be specific to catabolite repressible enzymes.

As bacterial cells progress through their division cycle in cultures undergoing steady-state growth, the quantity of all macromolecular components must double. Some macro-molecules, such as ribosomal and transfer RNA (1)(2) and certain enzymes (3) (4) are synthesized continuously throughout the division cycle. On the other hand, some enzymes have been reported to be synthesized during only a brief period of the bacterial division cycle (5) (6)(7). The synthesis of lactose operon products is more complex. It can be transcribed at all times during the bacterial cell cycle. However, according to Goldberg and Chargaff (8), Hfr strains of *E. coli*, grown synchronously in the presence of an inducer, produce β-galactosidase in a rhythmically discontinuous fashion. β-galactosidase synthesis is interrupted when the cells are dividing actively. From this, it appears that some events related to cell division might exert a control on the synthesis of catabolite-repressible enzymes. Alternatively, the two phenomena might be regulated by a single mechanism. In fact, a metabolic control of cell septation was recently suggested by D. Zusman et al. (9) from the study of a filament-forming mutant the division of which is caused by inhibition of peptide-bond formation. This regulation might also be effective on the synthesis of catabolite-repressible enzymes. The present studies were done to examine this possibility.

MATERIALS AND METHODS

Bacterial strains and Media : The following strains were employed in this study : PA 3092 T 8416 F^-, thr^- leu^- arg^- his^- try^- str^s thi^- DAP^- lys^- FtsA; MFT 1083 F^-, thr^- leu^- arg his^- str^s thi^- DAP^- lys^- FtsC; PA 3097 F^-, thr^- leu^- arg^- his^- thi^- str^s DAP^- lys^- parental strain. These are the generous gift of Dr. Y. Hirota. The strain C 90, PhoT has been originally isolated by A. Garen.

Enzyme assays

β-galactosidase and tryptophanase were assayed as previously described (10)(11). β-galactosidase was induced with 1mM isopropyl β-D thiogalactoside (IPTG); one enzyme unit catalyzes the hydrolysis of 1 nmole of O-nitrophenyl β-D galactoside per minute at 28°C pH 7. Tryptophanase was induced with 1.5 mM L-tryptophan; one enzyme unit is the amount that will produce 1 nmole of indole per minute under these conditions. Measurements of cyclic AMP levels (12), as well as counting of cell numbers with a Coulter counter were carried out as previously described (13).

RESULTS AND DISCUSSION

We have investigated a possible relationship between cell septation and the synthesis of catabolite-repressible enzymes in conditional thermosensitive mutants of *E. coli* defective in cell septation. For this study, logarithmic rather than synchronous cultures were used since recent evidence has shown that synchronization techniques can disturb cellular metabolism (14). Cells were grown on a medium that produces a slow increase of the intracellular cyclic AMP level, because of the sequential utilization of the catabolites added (12). Under these conditions the derepression of β-galactosidase and tryptophanase was followed (Figure 1). The endogenous molarity of cyclic AMP can be evaluated in cells at any time during the growth from the number of cells per milliliter of culture. Using 7.5×10^{-13} ml as the accessible volume per bacterium, one can calculate that at the beginning of growth this molarity (0.8×10^{-5}M) is not sufficient for the induction of β-galactosidase and tryptophanase (15). This expectation is coroborrated when the mutant FtsA is grown at the permissive temperature 30°C

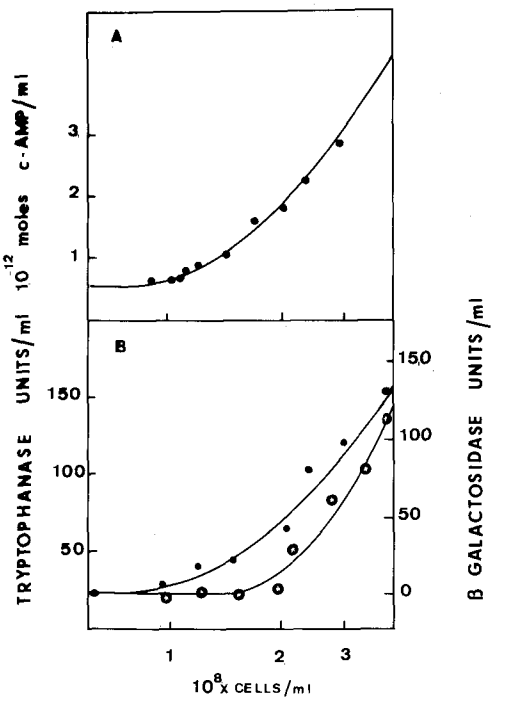

Figure 1 - Increase of intracellular cyclic AMP concentration per ml of culture during growth - Effect on β-glactosidase and tryptophanase synthesis. A culture of the strain PA 3097 was grown on minimal salts medium-glycerol (0,4%) supplemented with 0.5% casamino acids. Aliquots were removed at intervals and, A) cyclic AMP was assayed (●), B) the number of units of β-galactosidase (●) and tryptophanase (●) per ml of culture was determined at each cell density (measured by Coulter counts).

(Figure 1B). During growth the catabolites responsible for repression are progressively used up and the endogenous cyclic AMP level increases (Figure 1A). Concomitantly β-galactosidase and tryptophanase synthesis are initiated. However, they are not equally sensitive to catabolite repression. A detailed study of this phenomenon has been reported elsewhere (16). Since we deal with cells that form filaments at the restrictive temperature enzyme units have been plotted versus the radioactivity incorporated into proteins rather than versus the cell density of the culture. In the parental strain used as a control, the initiation of tryptophanase synthesis occurs at the same cell protein content whether the cells are grown at 30°C or at 41°C (Figure 2A). With conditional thermosensitive mutants defective in cell septation we obtained a different result regardless of whether the mutation

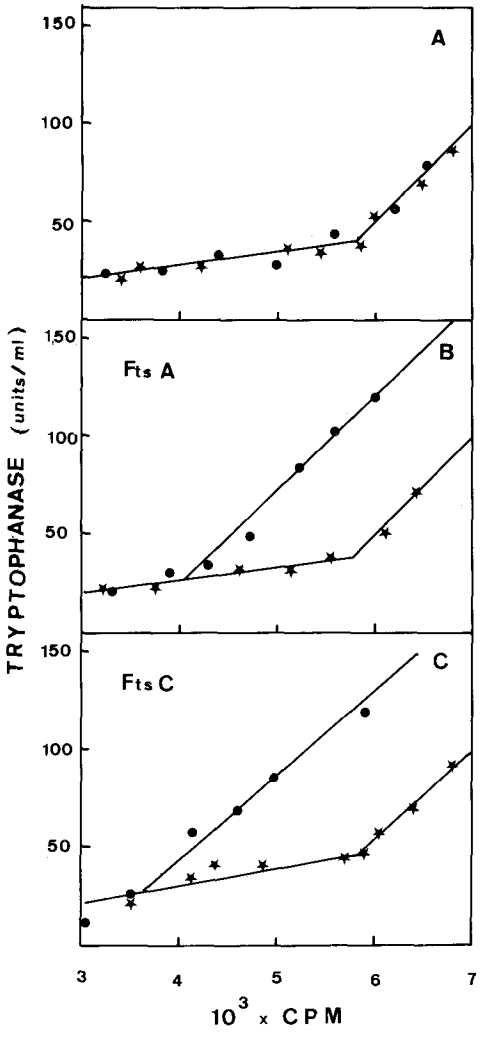

Figure 2 - Effect of filamentation on the induction of tryptophanase synthesis. Cells were grown under the conditions described in Figure 1. Aliquots were removed at intervals. The radioactivity incorporated into proteins was determined as well as the number of units of tryptophanase in the aliquots. A) Control with the parental strain grown at 30°C (✶) and 41°C (●)- B) Mutant Fts A grown at 30°C (✶) and 41°C(●) Mutant FtsC grown at 30°C (✶) and 41°C (●).

---

was in the FtsA or FtsC gene. Tryptophanase synthesis escaped catabolite repression much earlier in growth at 41°C in the filaments than in normally dividing cells grown at 30°C. This does not reflect a faster use of catabolites at 41°C than at 30°C since the specific radioactivity of [^{3}H] - leucine is the same at both

temperatures. The same result, although less marked, was found for β-galactosidase synthesis. Consequently, we conclude that inhibition of cell septation causes or is accompanied by an early escape from catabolite repression. This is not specific to mutation of one gene but rather related to the block in cell division since the same result was found in Fts-C as well as in Fts-A

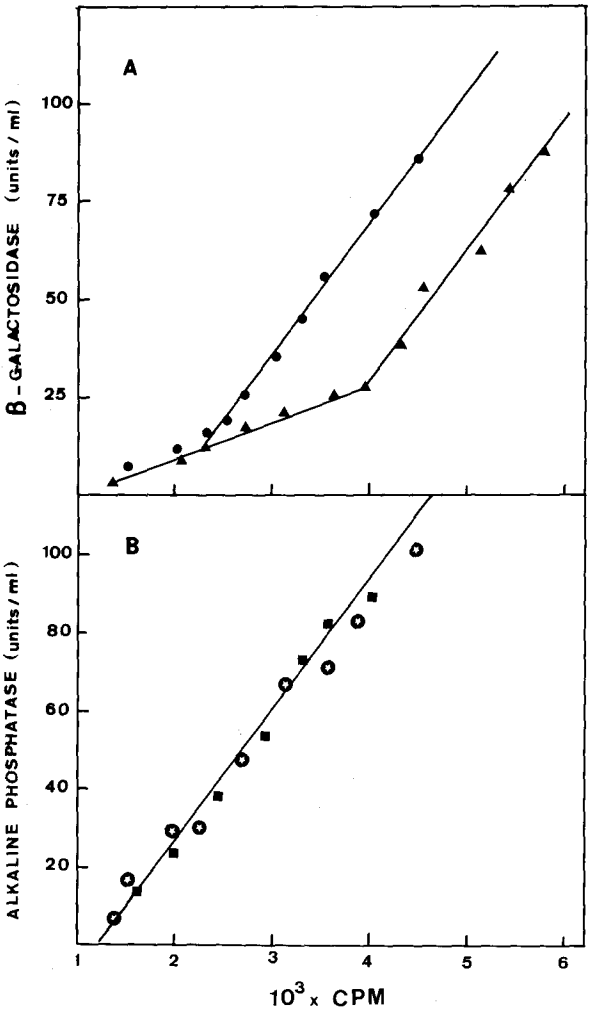

Figure 3 - Selective effect of the inhibition of septation on protein synthesis - A culture of the strain C90 was grown at 37°C on the same medium as described in figure 1, except that 12% sucrose was added. At OD 600 nm = 0.1 the culture was divided. One part was kept without addition as a control - 50 units/ml of Penicillin G was added to the other part. A) β-galactosidase synthesis and B) alkaline phosphatase synthesis were compared in the presence (●■) or absence (▲✪) of Penicillin G.

mutants (Figure 2B and C). Moreover, we found that inhibition of cell division by low concentrations of Penicillin G has exactly the same effect as the mutations Fts A and Fts C. This is shown in figure 3A where β-galactosidase synthesis was followed in cultures of the strain C90 grown in the presence or absence of Penicillin G (50 units/ml). Again filamentation is accompanied by an early escape from catabolite repression. The strain C90 was chosen because it is constitutive for alkaline phosphatase synthesis. When this enzyme is assayed under the same growth conditions as β-galactosidase its differential rate of synthesis is not altered at all by blocking cell division (Figure 3B). Consequently, the effect of the block in septation on protein synthesis seems to be specific to catabolite-repressible enzymes. One possible hypothesis was that inhibition of cell division was accompanied by an increase in the endogenous cyclic AMP level. This might have explained the effects observed. And we did observe that under the conditions used the addition of exogenous cyclic AMP causes exactly the same effect as the inhibition of cell division. Therefore, we measured the effect of specific inhibition of cell division on intracellular levels of cyclic AMP in the mutant Fts-A. When a well-balanced culture of this mutant is shifted from 30°C to 41°C, cell division stops immediately (Figure 4). The biosynthesis of RNA, DNA, and protein are not affected at the restrictive temperature. The intracellular cyclic AMP concentration per ml of culture was followed before and after the temperature shift. Figure 4 shows that the ratio of cyclic AMP concentration to the radioactivity incorporated into protein remains constant after cessation of cell division at 41°C. Thus cessation of cell division has no influence on the intracellular level of cyclic AMP. Although we did not measure the amounts of the mRNAs of tryptophanase and β-galactosidase produced, this suggest that the control mechanism of cell division on the synthesis of the enzymes submitted to catabolite repression is exerted at the level of the translation of mRNA rather than at the transcriptional level. This would be in agreement with the results of Goldberg and Chargaff (8) who have shown that the lac mRNA is produced at any time during the cell cycle, but not translated during cell division in synchronous cultures, upon continuous induction by 1 mM isopropyl β-D galactoside. Such an effect should allow us to observe not only an early initiation of the synthesis of catabolite repressible enzymes in

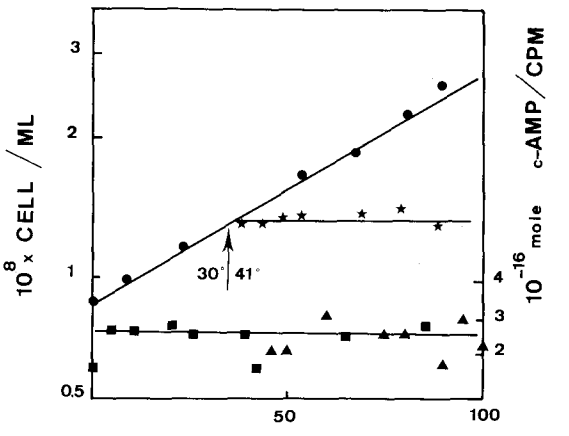

Figure 4 - Effect of the block in septation on the intracellular cyclic AMP level : An exponential culture of the Fts A mutant was divided into two subcultures at the arrow. One was kept at 30°C as a control (●) the other one was shifted to 41°C. The ratio of intracellular cyclic AMP concentration to the radioactivity incorporated into proteins was determined for the cells kept at 30°C (■) and 41°C (▲).

filamenting cells but also a higher differential rate of synthesis after blocking cell division. This last difference was not detected. Thus, it is likely that the control of cell division on the synthesis of catabolite-repressible enzymes is only transitory and cannot be established permanently. It is tempting to suggest that cell septation and synthesis of catabolite repressible enzyme share an element of control which might be the metabolic control described by D. Zusmann et al. (9). This control would operate at the translational level. The most likely site of action would then seem to be the ribosomes. Additional experiments are presently being carried out to elucidate these points.

ACKNOWLEDGEMENTS

We are grateful to Dr. M. Delaage for providing facilities for cyclic AMP assays and to R. Gaillard for use of the Coulter counter. We are undebted to Dr. D. Grossman for careful reading of this manuscript. This investigation was supported in part by the "Délégation Générale à la Recherche Scientifique et Technique".

REFERENCES

(1) - Dennis, P. (1971)
Nature New Biol. 232, 43-47
(2) - Dennis P.(1972)
J. Biol. Chem. 247, 204-208
(3) - Abbo, F.E. and A.B. Pardee (1960)
Biochem. Biophys. Acta 39, 478-485
(4) - Masters, M. and W.D. Donachie (1966)
Nature 209, 476-479
(5) - Kuempel, P.L., M. Masters and A.B. Pardee (1965)
Biochem. Biophys. Res. Commun. 18, 858-867
(6) - Kogoma, R. and A. Nishi (1965)
J. Gen. Appl. Micribiol. 11, 321-328
(7) - Goodwin, B.C. (1969)
Europ. J. Biochem. 10, 511-514
(8) - Goldberg, R.B. and E. Chargaff (1971)
Proc. Nat. Acad. Sci., U.S.A. 68, 1702-1706
(9) - Zusman, D.R.; M.Inouye and A.B. Pardee (1972)
J. Mol. Biol. 68, 119-136
(10) - M. Piovant J. Busuttil, C. Ladzunski (1974)
Ann. Microbiol. Inst. Pasteur, in press
(11) - Bilezikian J.P., R.O. Kaempfer and B. Magasanik (1967)
J. Mol. Biol. 27, 495-506
(12) - M. Piovant, H. Cailla, C. Ladzunski (1974)
F.E.B.S. Letters 46, 281 284
(13) - M. Piovant, H. Cailla, C. Ladzunski (1974)
F.E.B S. Letters 46, 42-44
(14) - Bellino F.L. (1973)
J. Mol. Biol. 74, 223-238
(15) - Buettner M.J., Spitz E., and Rickenberg H.V. (1973)
J. Bacteriol. 114 1068-1073
(16) - M. Piovant, C. Ladzunski (1974)
Submitted for publication.