JOURNAL OF BACTERIOLOGY, Jan. 1979, p. 661-663
0021-9193/79/01-0661/03$02.00/0

Vol. 137, No. 1

Regulation of Polyamine and Streptomycin Transport During Stringent and Relaxed Control in *Escherichia coli*

JOACHIM-VOLKER HÖLTJE

Institut für Biologie II, Lehrstuhl Mikrobiologie I, Universität Tübingen, D-7400 Tübingen, Federal Republic of Germany

Received for publication 31 July 1978

Inhibition of polyamine uptake was observed during amino acid depletion in a stringent strain of *Escherichia coli* CP78 but not in a relaxed strain (CP79). Chloramphenicol was shown partially to relieve the inhibition of uptake. Stringent cells which were induced for a transport system common to both polyamines and streptomycin were found to restrict the uptake of spermidine as well as streptomycin.

Bacterial cellular activities can be adjusted to varying demands by the deployment of finely tuned regulatory mechanisms. The mechanism termed stringent response couples several synthetic and transport processes, including stable RNA accumulation (3) and phospholipid synthesis (10) as well as purine and pyrimidine synthesis (9) and transport (5, 11), with the rate of protein synthesis. This control might be mediated by the guanosine nucleotides guanosine-5'-diphosphate-3'-diphosphate (ppGpp) and guanosine-5'-triphosphate-3'-diphosphate (pppGpp), which accumulate during amino acid starvation in stringent strains (*rel*$^+$) but not in relaxed strains (*rel*$^-$) (3, 4).

Since polyamines are evidently involved in various steps of transcription and translation (2, 16), the cellular requirement for putrescine and spermidine should be substantially reduced under conditions in which protein synthesis and RNA accumulation are restricted. Consistent with this notion is the report that the synthesis of polyamines is greatly impaired during stringent control of RNA synthesis (13).

In the case of nucleoside bases, not only the synthesis but also the transport of these compounds is stringently regulated. Several transport systems for putrescine and spermidine have been reported in *Escherichia coli* (12, 14, 15). One of these systems is inducible and has recently been shown to be utilized not only by putrescine and spermidine but also for the transport of the polycationic antibiotic streptomycin (12). It was, therefore, of interest to investigate whether, in analogy to purine and pyrimidine transport, the uptake of polyamines and streptomycin are also controlled via the pppGpp or the ppGpp level of the cell.

The effect of amino acid deprivation on the uptake of the polyamines putrescine and spermidine in the isogenic pair of stringent and relaxed strains of *E. coli* CP78 (*relA*$^+$) and CP79 (*relA*$^-$) (7) was determined. Addition of L-valine to cells growing in a minimal medium gives rise to isoleucine depletion by inhibiting an enzyme which is common to the biosynthetic pathways of valine and isoleucine (3). This technique of valine-induced isoleucine starvation was used because it requires less interference with the growing culture than washing the cells with a medium lacking a required amino acid. In the stringent strain (CP78), the uptake of spermidine was found to be severely restricted, whereas putrescine uptake was impaired to a minor extent during isoleucine starvation (Table 1). No decrease in polyamine uptake was seen in the relaxed strain (CP79) under the same conditions (Table 1). Arginine deprivation also inhibits the uptake of spermidine and putrescine in the *relA*$^+$ strain but not in the *relA*$^-$ strain of *E. coli* (data not shown). In both cases, the decrease in uptake was found to be no more than about 50% in the stringent cells. Thus, the effect is much less than inhibition of stable RNA accumulation in a *relA*$^+$ strain. This disparity may be explained by the fact that more than one uptake system is involved in the accumulation process of polyamines (12, 14, 15) and that they may be regulated differently.

Accumulation of ppGpp can be abolished by antibiotics that block protein synthesis by affecting the ribosomes, the site of ppGpp synthesis (3). Relief from repression caused by amino acid starvation in stringent cells was, therefore, expected to occur in the presence of chloramphenicol. Low concentrations of chloramphenicol (50 μg/ml) significantly enhance the uptake capacity for spermidine (Fig. 1).

In a previous report it was shown that the aminoglycoside antibiotic streptomycin is taken

TABLE 1. *Effect of isoleucine starvation on the uptake of putrescine and spermidine*

Strain[a]	Time (min)	Uptake (μmol/g of cells)			
		Putrescine[b]		Spermidine[b]	
		No addition	L-valine added[c]	No addition	L-valine added[c]
CP78	0.5	1.3	1.3	1.0	0.5
	5	4.0	3.4	3.3	2.4
	10	6.2	5.2	5.1	3.1
	15	8.6	6.3	7.0	4.0
	20	10.0	7.6	9.9	4.8
CP79	0.5	1.3	1.3	1.0	1.0
	5	4.3	4.2	3.4	3.0
	10	6.7	6.4	4.6	4.5
	15	9.0	8.8	7.3	6.9
	20	10.6	10.3	9.1	9.1

[a] *E. coli* CP78 (*relA*$^+$) and CP79 (*relA*$^-$) were grown in a low-phosphate tris(hydroxymethyl)aminomethane-buffered medium (6) to an absorbance of the culture at 578 nm of 0.25 (corresponding to 85 μg, dry weight, per ml). The medium was supplemented with 50 μg of each required amino acid per ml (7), 1 μg of thiamine per ml, and 0.4% glycerol.

[b] The uptake of polyamines was initiated by adding 50 μl of [^{14}C]spermidine or [^{14}C]putrescine (both at 5 μCi/ml; 2 mM), respectively, to 1-ml portions of the cultures. Incubation was continued, and 0.2-ml samples were withdrawn at the indicated intervals. The samples were treated as described previously (12).

[c] For isoleucine starvation, a portion of the culture received 250 μg of L-valine per ml, and incubation was continued for 10 min before [^{14}C]polyamine addition.

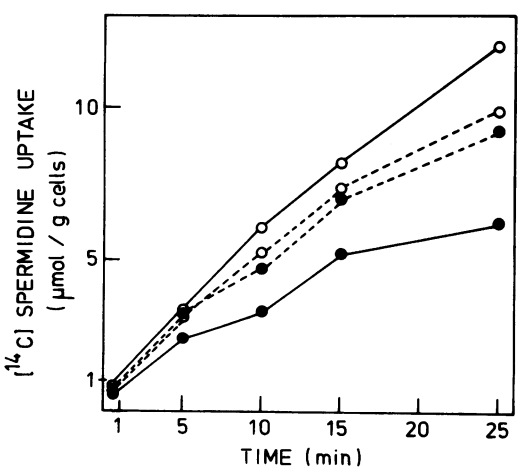

FIG. 1. *Effect of chloramphenicol on the uptake of spermidine during isoleucine starvation of Rel$^+$ E. coli CP78. Spermidine uptake was determined with isoleucine-deprived (●) and control (○) cells in the presence (- - - -) and absence (——) of 50 μg of chloramphenicol per ml. The experimental procedures were as described in Table 1.*

up by *E. coli* via an inducible transport system for polyamines (12). The induction is triggered by streptomycin itself during preincubation of the cells for 20 min in the presence of 5 μg of streptomycin per ml. The uptake of streptomycin and spermidine into induced cells was tested for response to amino acid deprivation. A distinct inhibition of the induced transport of both streptomycin and spermidine was observed when valine was added to the stringent strain grown in a minimal medium (Fig. 2). There was no diminished uptake detected in the relaxed control strain under identical conditions. Arginine deprivation could be shown to cause identical effects.

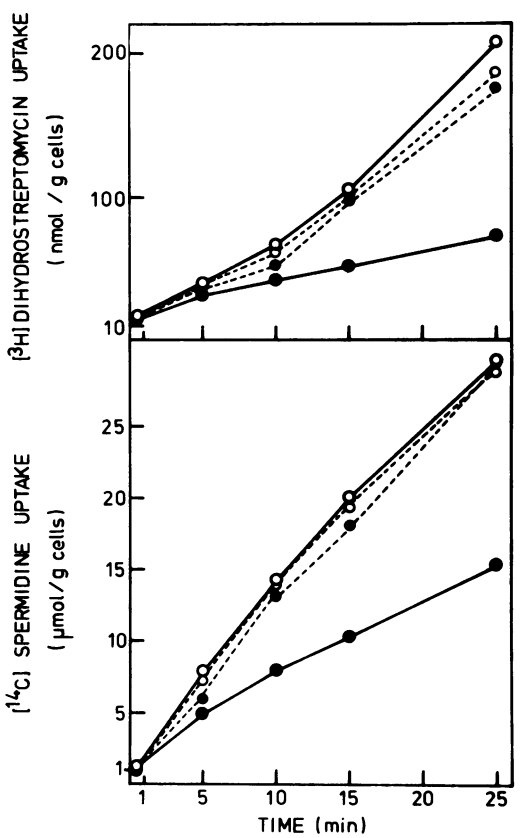

FIG. 2. *Effect of isoleucine starvation on the induced uptake of spermidine and streptomycin in Rel$^+$ (CP78) and Rel$^-$ (CP79) E. coli. Induction of transport was achieved by preincubation of the cells in the presence of 5 μg of streptomycin per ml for 20 min at 37°C. Uptake of streptomycin was initiated by adding 0.1 ml of [^{3}H]dihydrostreptomycin (100 μCi/ml; 342 μM) to 10-ml portions of the culture. Samples (1 ml each) were taken at the indicated intervals. Other experimental procedures were as described in Table 1. Symbols: (○) control; (●) isoleucine starved; (——) CP78; (-----) CP79.*

The results presented here demonstrate that culture conditions which are known to increase the level of ppGpp restrict the uptake of polyamines. Synthesis of polyamines has been reported to respond in a similar manner (13). Thus, accumulation of polyamines appears to be analogous to accumulation of stable RNAs. This observation may suggest that cells restricting their RNA accumulation have a greatly reduced requirement for polyamines compared to cells maintaining an unimpaired rate of RNA synthesis.

At present, it is not possible to determine whether there exists a direct interaction of ppGpp with the transport systems for polyamines. The possibility exists that restriction of polyamine uptake is not effected directly by changes in the ppGpp level but rather indirectly by the rate of synthesis of other stringently controlled compounds such as RNAs.

Stringent response could be shown not only for the constitutive uptake of spermidine but also for induced spermidine transport. Under the latter conditions, also, the uptake of streptomycin was stringently regulated. This is consistent with the previous results, recognizing the inducible polyamine uptake system as a common vehicle for the transport of both polyamines and streptomycin (12).

Accumulation of antibiotics inside the bacterial cell in sufficiently high concentrations is a necessary prerequisite for effective action of all antimetabolites having an intracellular target. An increasing number of antibiotics have been shown to be accepted and transported via the nutrient transport systems of the bacterium (8). Control of uptake of such antibiotics would likely depend upon the same regulatory mechanisms as those for the natural substrates (1).

The polyamine uptake system that is induced and utilized by streptomycin has been shown to be subject to catabolite repression (12). The uptake of various other antibiotics, similarly, seems to be under positive control by cyclic adenosine 5'-monophosphate (AMP) (1). This communication demonstrates that a second control mechanism, namely the stringent response, regulates the uptake of an antibiotic.

I thank Hans Zähner for his interest in these studies, and James W. Coulton for reading the manuscript. B. Bachmann kindly provided the strains of *E. coli* used in this study. The technical assistance of Judith Rinck is gratefully acknowledged.

This work was supported by the Deutsche Forschungsgemeinschaft.

LITERATURE CITED

1. **Alper, M. D., and B. N. Ames.** 1978. Transport of antibiotics and metabolite analogs by systems under cyclic AMP control: positive selection of *Salmonella typhimurium cya* and *crp* mutants. J. Bacteriol. **133**:149-157.
2. **Bachrach, U.** 1970. Metabolism and function of spermine and related polyamines. Annu. Rev. Microbiol. **24**:109-134.
3. **Cashel, M.** 1969. The control of ribonucleic acid synthesis in *E. coli*. J. Biol. Chem. **244**:3133-3141.
4. **Cashel, M., and J. Gallant.** 1969. Two compounds implicated in the function of the RC gene of *E. coli*. Nature (London) **221**:838-841.
5. **Edlin, G., and J. Neuhard.** 1967. Regulation of nucleoside triphosphate pools in *Escherichia coli*. J. Mol. Biol. **24**:225-230.
6. **Errera, M.** 1954. Induction of filamentous forms in ultraviolett-irradiated *E. coli* B. Br. J. Radiol. **27**:76-80.
7. **Fiil, N., and J. D. Friesen.** 1968. Isolation of "relaxed" mutants of *Escherichia coli*. J. Bacteriol. **95**:729-731.
8. **Franklin, T. J.** 1973. Antibiotic transport in bacteria. CRC Crit. Rev. Microbiol. **30**:253-272.
9. **Gallant, J., J. Irr, and M. Cashel.** 1971. The mechanism of amino acid control of guanylate and adenylate biosynthesis. J. Biol. Chem. **246**:5812-5816.
10. **Golden, N. G., and G. L. Powell.** 1972. Stringent and relaxed control of phospholipid metabolism in *Escherichia coli*. J. Biol. Chem. **247**:6651-6658.
11. **Hochstadt-Ozer, J., and M. Cashel.** 1972. The regulation of purine utilization in bacteria. V. Inhibition of purine phosphoribosyl-transferase activities and purine uptake in isolated membrane vesicles by guanosine tetraphosphate. J. Biol. Chem. **247**:7067-7072.
12. **Höltje, J.-V.** 1978. Streptomycin uptake via an inducible polyamine transport system in *Escherichia coli*. Eur. J. Biochem. **86**:345-351.
13. **Hölttä, E., J. Jänne, and J. Pispa.** 1974. The regulation of polyamine synthesis during the stringent control in *Escherichia coli*. Biochem. Biophys. Res. Commun. **59**:1104-1111.
14. **Munro, G. F., C. A. Bell, and M. Lederman.** 1974. Multiple transport components for putrescine in *Escherichia coli*. J. Bacteriol. **118**:952-963.
15. **Tabor, C. W., and H. Tabor.** 1966. Transport systems for 1,4-diaminobutane, spermidine, and spermine in *Escherichia coli*. J. Biol. Chem. **241**:3714-3723.
16. **Tabor, C. W., and H. Tabor.** 1976. 1,4-Diaminobutane (putrescine), spermidine and spermine. Annu. Rev. Biochem. **45**:285-306.