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Streptomycin Resistance (*rpsL*) Produces an Absolute Requirement for Polyamines for Growth of an *Escherichia coli* Strain Unable to Synthesize Putrescine and Spermidine [Δ(*speA-speB*) Δ*specC*]

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The presence of certain *rpsL* (*strA*) mutations in a strain of *Escherichia coli* that cannot synthesize putrescine or spermidine because of deletions in ornithine decarboxylase, arginine decarboxylase, and agmatine ureohydrolase, converts a partial requirement for polyamines for growth into an absolute requirement.

Numerous studies have shown that the polyamines, putrescine, spermidine, and spermine, have a large variety of effects in vitro (reviewed in 1, 2, 8, 18, and 21). In an attempt to determine which of these effects are relevant to the physiological function of the polyamines in vivo, we have constructed strains of *Escherichia coli* that do not contain putrescine and spermidine when grown in amine-free media, because of deletions in the biosynthetic pathways, i.e., ornithine decarboxylase (*speC*), arginine decarboxylase (*speA*), agmatine ureohydrolase (*speB*), adenosylmethionine decarboxylase (*speD*), and lysine decarboxylase (*cadA*) (6, 11, 17). We were surprised to find that amine-deficient *E. coli* could grow indefinitely in such media, although at only one-third the rate observed in an amine-containing medium. Certain bacteriophages, such as T4 and T7, multiplied well in these amine-free strains, but bacteriophage λ exhibited little or no replication (6).

We have now found that the requirement for amines for growth can be made absolute in one Δ(*speA-speB*) Δ*speC* strain of *E. coli* by introducing a mutation in the *rpsL* (*strA*) gene; i.e., no growth is observed in the absence of added amines.

The methods used have been described previously (6, 14, 17). The strains used are listed in Table 1. Cells were grown at 37°C in a minimal medium (20) containing 0.4% glucose and the necessary auxotrophic requirements (100 μg/ml). Amines were added where indicated to a final concentration of 10^{-4} M putrescine and 10^{-5} M spermidine. Before transfer to an amine-deficient medium, cells were collected by centrifugation at room temperature and washed twice with an equal volume of 0.15 M NaCl.

When HT395, a Δ(*speA-speB*) Δ(*speC-glc*) strain, was removed from a medium containing polyamines, washed with saline, and diluted into fresh medium, it grew initially at about the same rate in the presence or absence of polyamines (Fig. 1A); after about 10 generations, the growth rate in the absence of polyamines had decreased to about one-third of that seen in the presence of polyamines, and this growth rate was maintained indefinitely. (The latter data, not shown in this paper, are similar to those in Fig. 4 in reference 6.) When HT414, an *rpsL9* transductant of HT395, was treated in a comparable manner, the growth without polyamines was very different; i.e., growth slowed very soon and ceased after 7 h (Fig. 1B). Surprisingly, the growth stopped while there was still a significant intracellular polyamine level. Addition of $MgSO_4$ (0.01 M) or of streptomycin (10 μg or 100 μg/ml) to the amine-deficient medium did not affect the growth rate; these negative experiments are of interest since previous studies showed that Mg^{2+} or streptomycin can often partially replace polyamines in in vitro studies on ribosome structure and protein biosynthesis (2, 3, 12).

It is clear that the introduction of the *rpsL9* mutation has resulted in an absolute requirement for polyamines. Similarly, incubation on agar plates containing minimal media confirmed this finding of an absolute requirement of HT414 for amine supplementation. However, suppressor mutants that no longer had an absolute requirement for polyamines for growth occurred at a frequency of about 1 in 10^6; these suppressor mutants were still streptomycin resistant.

The same mutation was responsible for the development of the absolute requirement for polyamines and for the resistance to streptomycin, since all of 96 streptomycin-resistant transductants (*rpsL9* transduced into HT395) showed both phenotypes. However, the introduction of different *rpsL* alleles resulted in different degrees of polyamine dependence. For example, an absolute amine requirement was found after transduction of HT395 to streptomycin resistance with bacteriophage P1 prepared on strains carrying *rpsL9, rpsL125,* or three undefined *rpsL* alleles. On the other hand, if the P1 was prepared on a strain containing the *rpsL25* mutation, the resultant strain showed much less dependence on amines. Four spontaneous streptomycin-resistant mutants of HT395 were also tested; only one showed an absolute amine requirement. We do not have sufficient data yet to state whether the phenotypic expression of the *rpsL* mutation presented in this paper is dependent on other factors in the genetic background of the strain containing the Δ(*speA-speB*) Δ*speC* deletions.

TABLE 1. *Strains*

Strain no.	Genotype	Source
HT395	*thr thi* Δ(*speA-speB*) Δ(*speC-glc*) *hsd sup*	*leu*$^+$ transductant of HT289
HT414	*thr thi* Δ(*speA-speB*) Δ(*speC-glc*) *hsd rpsL9 sup*	*rpsL* transductant of HT395[a]
HT289	*thr leu thi* Δ(*speA-speB*) Δ(*speC-glc*) *hsd sup*	Hfr cross of EWH331 and a *thyA* derivative of DK42
DK42	*thr leu thi hsd*	Obtained from D. Kopecko (13)
EWH331	Hfr *thr pro thi rpsL25 spc supE44* Δ(*speA-speB*) Δ(*speC-glc*) Δ*spcD*	Described in reference 6

[a] The *rpsL* defect was originally present in *E. coli* strain KL132 obtained from Barbara J. Bachmann of the *E. coli* Genetic Stock Center at Yale University.

Since it is well known that the *rpsL* mutation affects the S12 ribosomal protein (16), it seems likely that the effect of the *rpsL* mutation in increasing the polyamine requirement indicates polyamine involvement in ribosomal structure and protein biosynthesis, especially since there are previous reports (primarily in vitro) on the effects of polyamines in these systems (1–4, 7, 9, 10, 12, 15, 18, 19, 22). However, the exact biochemical mechanisms for our observation can-

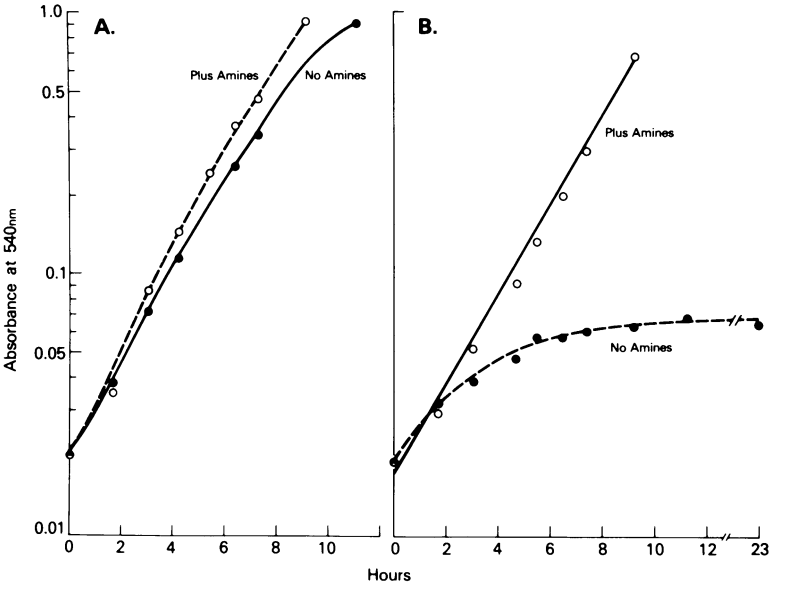

FIG. 1. *Growth rate of HT395 (A) and of its rpsL9 transductant (HT414) (B) in amine-free media and in media supplemented with 10^{-4} M putrescine and 10^{-5} M spermidine. Cells were grown in media containing 10^{-4} M putrescine and 10^{-5} M spermidine to approximately 10^9 cells per ml. The culture was centrifuged; the cells were washed twice with an equal volume of 0.15 M NaCl and then resuspended in 0.15 M NaCl. At zero time, this suspension was diluted approximately 50-fold into fresh medium (± amines) and incubated with shaking at 37°C.*

not be defined at present; *rpsL* mutation may also affect reactions not directly related to the ribosomal system because of its well-known effect on decreasing ambiguity in translation (5).

LITERATURE CITED

1. **Bachrach, U.** 1973. Function of naturally occurring polyamines. Academic Press, Inc., New York.
2. **Cohen, S. S.** 1971. Introduction to the polyamines. Prentice-Hall, Inc., Englewood Cliffs, N.J.
3. **Cohen, S. S., and J. Lichtenstein.** 1960. Polyamines and ribosome structure. J. Biol. Chem. **235:**2112-2116.
4. **Echandi, G., and I. D. Algranati.** 1975. Defective 30S ribosomal particles in a polyamine auxotroph of *Escherichia coli*. Biochem. Biophys. Res. Commun. **67:** 1185-1191.
5. **Gorini, L.** 1969. The contrasting role of *strA* and *ram* gene products in ribosomal functioning. Cold Spring Harbor Symp. Quant. Biol. **34:**101-109.
6. **Hafner, E. W., C. W. Tabor, and H. Tabor.** 1979. Mutants of *Escherichia coli* that do not contain 1,4-diaminobutane (putrescine) or spermidine. J. Biol. Chem. **254:**12,419-12,426.
7. **Igarashi, K., K. Sugawara, I. Izumi, C. Nagayama, and S. Hirose.** 1974. Effect of polyamines on polyphenylalanine synthesis by *Escherichia coli* and rat liver ribosomes. Eur. J. Biochem. **48:**495-502.
8. **Jänne, J., H. Pösö, and A. Raina.** 1978. Polyamines in rapid growth and cancer. Biochim. Biophys. Acta **473:** 241-293.
9. **Jelenc, P. C., and C. G. Kurland.** 1979. Nucleoside triphosphate regeneration decreases the frequency of translation error. Proc. Natl. Acad. Sci. U.S.A. **76:**3174-3178.
10. **Kyner, D., P. Zabos, and D. H. Levin.** 1973. Inhibition of protein chain initiation in eukaryotes by deacylated tRNA and its reversibility by spermine. Biochim. Biophys. Acta **324:**386-396.
11. **Maas, W. K.** 1972. Mapping of genes involved in the synthesis of spermidine in *Escherichia coli*. Mol. Gen. Genet. **119:**1-9.
12. **Mager, J., M. Benedict, and M. Artman.** 1962. A common site of action for polyamines and streptomycin. Biochim. Biophys. Acta **62:**202-204.
13. **Meselson, M., and R. Yuan.** 1968. DNA restriction enzyme from *Escherichia coli*. Nature (London) **217:** 1110-1114.
14. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
15. **Morch, M. D., and C. Benicourt.** 1980. Polyamines stimulate suppression of amber termination codons *in vitro* by normal tRNAs. Eur. J. Biochem. **105:**445-451.
16. **Ozaki, M., S. Mizushima, and M. Nomura.** 1969. Identification and functional characterization of the protein controlled by the streptomycin-resistant locus in *Escherichia coli*. Nature (London) **222:**333-339.
17. **Tabor, H., E. W. Hafner, and C. W. Tabor.** 1980. Construction of an *Escherichia coli* strain unable to synthesize putrescine, spermidine, or cadaverine: characterization of two genes controlling lysine decarboxylase. J. Bacteriol. **144:**952-956.
18. **Tabor, C. W., and H. Tabor.** 1976. 1,4-Diaminobutane (putrescine), spermidine, and spermine. Annu. Rev. Biochem. **45:**285-306.
19. **Tanner, M. J. A.** 1967. The effect of polyamines on the binding of aminoacyl transfer ribonucleic acid to ribosomes in a yeast system. Biochemistry **6:**2686-2693.
20. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithinase of *Escherichia coli:* partial purification and some properties. J. Biol. Chem. **218:**97-106.
21. **Williams-Ashman, H. G., and Z. N. Canellakis.** 1979. Polyamines in mammalian biology and medicine. Perspect. Biol. Med. **22:**421-453.
22. **Young, D. V., and P. R. Srinivasan.** 1972. Regulation of macromolecular synthesis by putrescine in a conditional *Escherichia coli* putrescine auxotroph. J. Bacteriol. **112:**30-39.