JOURNAL OF BACTERIOLOGY, June 1975, p. 1387-1390
Vol. 122, No. 3

NOTES

Rifampin Resistance Mutation of *Bacillus subtilis* Altering the Electrophoretic Mobility of the Beta Subunit of Ribonucleic Acid Polymerase

THOMAS LINN, RICHARD LOSICK,* AND ABRAHAM L. SONENSHEIN

The Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138, and Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Massachusetts 02111*

Received for publication 26 March 1975

The rifampin-resistance mutation of LS3, an asporogenous mutant of *Bacillus subtilis* 3610, leads to altered mobility of the β subunit of ribonucleic acid polymerase in sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis. This finding argues that the rifampin-resistance mutation is located in the structural gene coding for the β polypeptide.

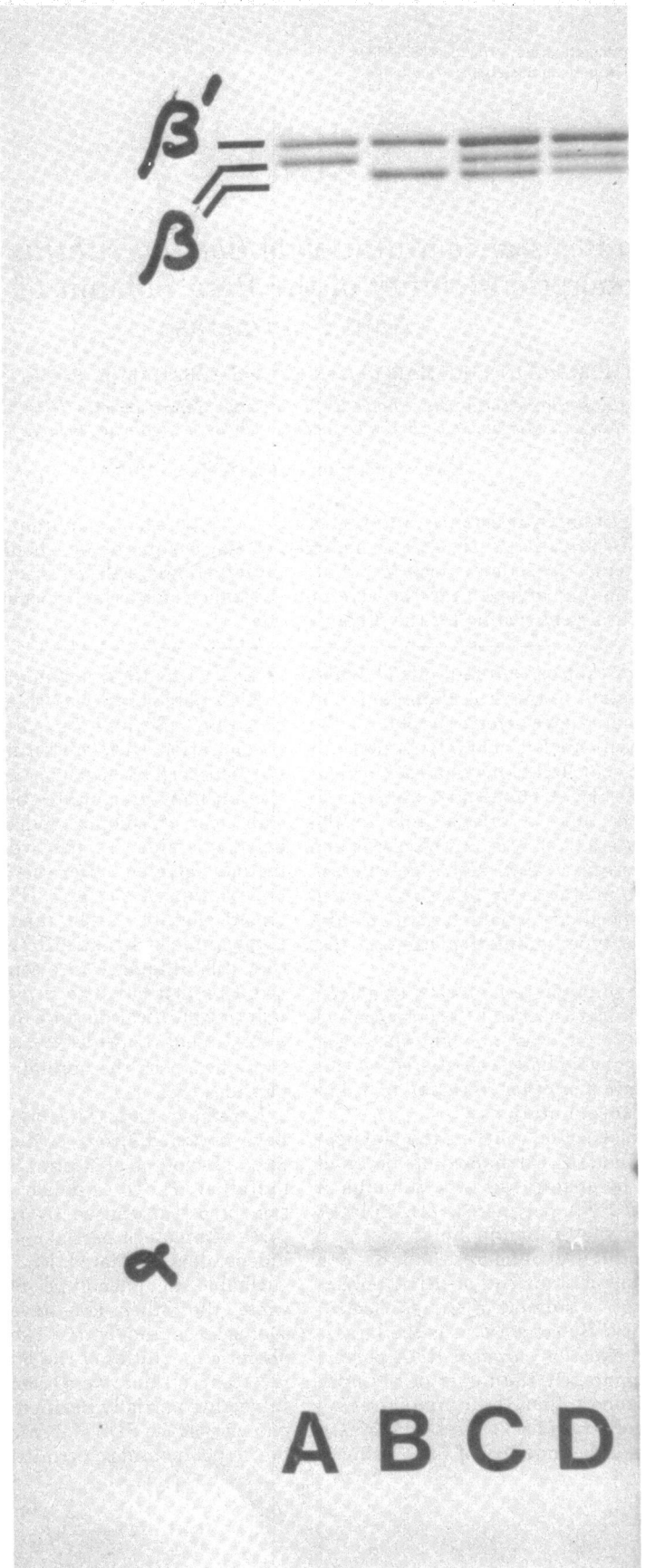
The discovery that certain mutants of *Bacillus subtilis* resistant to the drug rifampin fail to sporulate normally gave strong support to the idea that deoxyribonucleic acid (DNA)-dependent ribonucleic acid (RNA) polymerase (nucleoside triphosphate:RNA nucleotidyl-transferase; EC 2.7.7.6) may play a critical role in the sporulation process (2, 3, 7, 8). In the case of one such mutant strain (LS3), RNA polymerase fails to undergo in stationary phase the change in template specificity characteristic of the enzyme in wild-type sporulating bacteria (6a, 7, 8).

Although the rifampin resistance of LS3 RNA polymerase could be ascribed to a component of core enzyme (7), it was not known which subunit of the enzyme had been altered by this particular mutation or other rifampin- or streptolydigin-resistance mutations.

B. subtilis holoenzyme contains the polypeptides α, σ, β, and β' (4) named in order of increasing size by analogy with the subunits of *Escherichia coli* RNA polymerase (1). Zillig et al. (14) showed that rifampin binds to the β subunit of *E. coli* RNA polymerase and that one rifampin-resistant mutant had an RNA polymerase containing a β subunit of altered electrophoretic mobility. By contrast, it is the largest subunit of *Lactobacillus curvatus* RNA polymerase that is apparently the target of rifampin (10). Here we report that the rifampin-resistance mutation of strain LS3 causes an increase in the electrophoretic mobility of the β subunit of *B. subtilis* RNA polymerase.

RNA polymerase was isolated from an extract of strain LS3 and its parent strain 3610 by precipitation with antiserum directed against purified core RNA polymerase (4). Analysis of the antibody precipitates by electrophoresis on slab gels of sodium dodecyl sulfate (SDS)-polyacrylamide in tris(hydroxymethyl)aminomethane-glycine buffer (11) revealed that the β polypeptide of the LS3 RNA polymerase migrated slightly farther than the corresponding polypeptide of strain 3610 (Fig. 1). It is unlikely that this difference in electrophoretic mobility could be attributed to in vitro modification of β during purification since enzyme isolated from a mixture of the wild-type and mutant bacteria contained both the normal and altered β polypeptides (Fig. 1).

Reversion and transformation experiments have provided strong evidence that the Rfm^R Spo^- phenotype of strain LS3 is due to a single mutation (7). To establish whether the alteration of β was caused by the same mutation, three complete or partial revertants of LS3 were independently isolated. LS3 Rev 7 was indistinguishable in phenotype from the wild-type while the other two revertants were oligo sporogenous and Rfm^R (Table 1). The electrophoretic mobilities of the β polypeptides of the revertant strains were compared by directly subjecting partially purified extracts to electrophoresis on the SDS slab gels. (The large size of the β polypeptides permitted their resolution


β'
β
α
A B C D

from other bacterial proteins without purification by antibody precipitation.) The experiment of Fig. 2 shows that the β polypeptides of all three revertants were indistinguishable in mobility from the wild-type subunit.

The return of the β subunit of the spontaneous Spo$^+$Rfm^S revertant LS3 Rev7 to wild-type mobility confirmed that the same mutation caused both the altered β mobility and the Spo$^-$Rfm^R phenotypes of LS3. Since the partial revertants LS3 Rev13 and LS3 Rev17 also showed wild-type β mobility, we conclude that in these cases the reverting mutation returned the β polypeptide to wild-type mobility without restoring the wild-type amino acid sequence. This could be caused, for example, by a reverting mutation at a different site in the β cistron than the original rifampin-resistance mutation.

A possible explanation for the increased mobility of the mutant polypeptide is that the LS3 β subunit might be a truncated version of the

TABLE 1. *Strain phenotypes*

Strain	Sporulation[c]	Rifampin sensitivity[d]
3610	1.0	S
LS3[a]	10^{-6}	R
LS3 Rev7[b]	1.1	S
LS3 Rev13[b]	10^{-1}	R
LS3 Rev17[b]	10^{-1}	R

[a] LS3 is a spontaneous rifampin-resistant mutant of *B. subtilis* strain 3610 (7).

[b] Spontaneous revertants were found as survivors of heat treatment at 80 C for 10 min of a culture of strain LS3 grown overnight in 121B sporulation medium (9).

[c] Spore production given is the ratio of spores per milliliter produced by mutant or revertant to spores per milliliter produced by parent after 24 h of incubation in medium 121B at 37 C.

[d] Rifampin sensitivity was defined by ability of the strains to grow on Difco sporulation medium plates or in Difco sporulation medium broth (7) containing 5 μg of rifampin per milliliter.

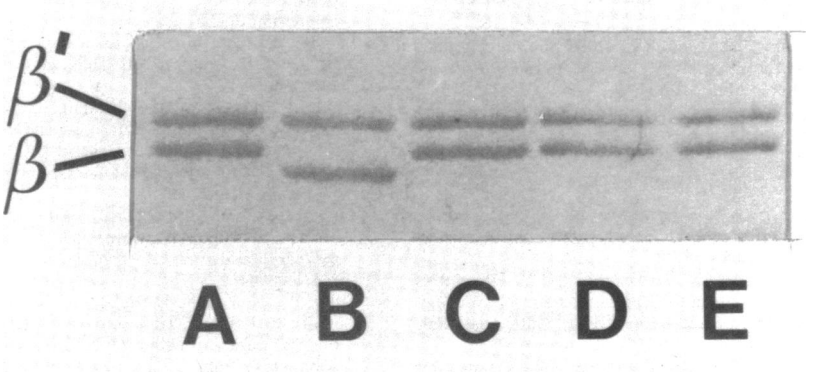


FIG. 2. *SDS-polyacrylamide gel electrophoresis of the β' and β subunits. Bacteria were grown in 60 ml of 121A medium (9) without radioactive label and harvested in late logarithmic growth. Extracts were prepared as described in the legend to Fig. 1 and approximately 40 μg of total protein was applied directly to an SDS slab gel. The gel was stained for 1 h with 0.1% Coomassie Brilliant Blue (14) and destained overnight in 7.5% acetic acid and 5% methanol. The figure shows an enlargement of the β region of the stained gel: (A) B. subtilis 3610; (B) LS3; (C) LS3 Rev7; (D) LS3 Rev13; and (E) LS3 Rev17.*

FIG. 1. *SDS-polyacrylamide slab gel electrophoresis patterns of RNA polymerase. Bacteria were grown in 60 ml of 121A medium (9) radioactively labeled with 10 mCi of [^{35}S]-sulfate (4) and harvested in late logarithmic growth. Extracts were prepared by sonication, high-speed centrifugation, and ammonium sulfate fractionation as previously described (5). RNA polymerase was isolated from the extracts by precipitation with antiserum against core RNA polymerase (4). The precipitates were solubilized and subjected to electrophoresis on a 25-cm-long slab gel containing SDS in a tris(hydroxymethyl)aminomethane-glycine buffer and a 5 to 10% gradient of acrylamide. An autoradiogram of the gel is shown: (A) RNA polymerase from the parent strain 3610; (B) RNA polymerase from the mutant LS3; (C) a mixture of RNA polymerase separately purified from the parent and mutant; and (D) RNA polymerase purified from a mixture of parent and mutant cells.*

wild-type polypeptide as would be generated by a deletion or nonsense mutation in the promoter-distal region of the β structural gene. However, SDS electrophoresis in tris(hydroxymethyl)aminomethane-glycine buffer does not necessarily separate polypeptides solely on the basis of molecular weight (12). It is possible, therefore, that the altered mobility of the mutant β could result from a missense mutation conferring a change in the net charge of the β polypeptide. Unfortunately, SDS electrophoresis in phosphate buffer, a reliable procedure for separating proteins according to molecular weight (13), is not of high enough resolution to be useful here. It should be noted that our attempts to suppress the LS3 phenotype by the selection of spontaneous suppressor mutations or the introduction of a known suppressor have been unsuccessful.

In summary, our findings strongly suggest that the rifampin-resistance mutation of strain LS3 as well as other Rfm^R mutations mapping at the same locus are located in the gene coding for the β subunit of *B. subtilis* RNA polymerase.

This work was supported by grants from the National Institutes of Health to R. L. (GM-18568-02 from the National Institute of General Medical Sciences) and to A. L. S. (5 RO1-GM19168), and to R. L. from the Camille and Henry Dreyfus Foundation, Inc.

LITERATURE CITED

1. Burgess, R. R. 1969. Separation and characterization of the subunits of ribonucleic acid polymerase. J. Biol. Chem. **244**:6168–6176.
2. Doi, R. H., L. R. Brown, G. Rogers, and Y. Hsu. 1970. *Bacillus subtilis* mutants altered in spore morphology and in RNA polymerase activity. Proc. Natl. Acad. Sci. U.S.A. **66**:404–410.
3. Leighton, T. J. 1973. An RNA polymerase mutation causing temperature-sensitive sporulation in *Bacillus subtilis*. Proc. Natl. Acad. Sci. U.S.A. **70**:1179–1183.
4. Linn, T. G., A. L. Greenleaf, R. G. Shorenstein, and R. Losick. 1973. Loss of the sigma activity of RNA polymerase of *Bacillus subtilis* during sporulation. Proc. Natl. Acad. Sci. U.S.A. **70**:1865–1869.
5. Losick, R., and A. L. Sonenshein. 1969. Change in the template specificity of RNA polymerase during sporulation of *Bacillus subtilis*. Nature (London) **224**:35–37.
6. Losick, R., R. G. Shorenstein, and A. L. Sonenshein. 1970. Structural alteration of RNA polymerase during sporulation. Nature (London) **227**:910–913.
6a. Segall, J., and R. Losick. 1975. Effect of asporogenous mutation on rate of bacteriophage ϕe transcription in stationary-phase *Bacillus subtilis* cells, p. 221–225. *In* P. Gerhardt, R. N. Costilow, and H. L. Sadoff (ed.), Spores VI. American Society for Microbiology, Washington, D.C.
7. Sonenshein, A. L., B. Cami, J. Brevet, and R. Cote. 1974. Isolation and characterization of rifampin-resistant and streptolydigin-resistant mutants of *Bacillus subtilis* with altered sporulation properties. J. Bacteriol. **120**:253–265.
8. Sonenshein, A. L., and R. Losick, 1970. RNA polymerase mutants blocked in sporulation. Nature (London) **227**:906–909.
9. Sonenshein, A. L., and D. H. Roscoe. 1969. The course of phage ϕe infection in sporulating cells of *Bacillus subtilis* strain 3610. Virology **39**:237–248.
10. Stetter, K. O., and W. Zillig. 1974. DNA-dependent RNA polymerase from *Lactobacillus curvatus*. Eur. J. Biochem. **48**:527–540.
11. Studier, F. W. 1973. Analysis of bacteriophage T7 early RNAs and proteins on slab gels. J. Mol. Biol. **79**:237–248.
12. Swaney, J. B., G. F. Vande Woulde, and H. L. Bachrach. 1974. Sodium dodecylsulfate-dependent anomalies in gel electrophoresis: alterations in the banding pattern of foot-and-mouth disease virus polypeptides. Anal. Biochem. **58**:337–346.
13. Weber, K., and M. Osborn. 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. **244**:4406–4412.
14. Zillig, W., K. Zechel, D. Rabussay, M. Schachner, V. S. Sethi, R. Palm, A. Heil, and W. Seifert. 1970. On the role of different subunits of DNA-dependent RNA polymerase from *E. coli* in the transcription process. Cold Spring Harbor Symp. Quant. Biol. **35**:47–58.