JOURNAL OF BACTERIOLOGY, Mar. 1978, p. 1543-1545
0021-9193/78/0133-1543$02.00/0

Vol. 133, No. 3
Printed in U.S.A.

Ribonuclease III Is Involved in Motility of *Escherichia coli*

DAVID APIRION* AND NED WATSON

Department of Microbiology and Immunology, Division of Biology and Biomedical Sciences, Washington University Medical School, St. Louis, Missouri 63110

Received for publication 4 November 1977

Mutants of *Escherichia coli* deficient in ribonuclease III are nonmotile. All transductants and revertants that regained ribonuclease III also regained motility, and all transductants that remained or became *rnc* are nonmotile, although only some of the revertants that regained motility also became ribonuclease III$^+$.

Motility of *Escherichia coli* is a very complex process that depends on the cooperation of many gene products; some are involved in producing components of the sensory system, while others are involved in producing components of the flagella itself (for review, see 1, 9, 13, 15). Here, we shall report findings that indicate that the enzyme ribonuclease (RNase) III is also involved in motility.

For purposes of mapping a new temperature-sensitive mutation, we used motility markers. In one such cross, an *rnc* (RNase III$^-$) mutation (5) was involved. When the cross was analyzed in semisolid agar plates (7), it became apparent that the *rnc-105* mutation might be segregating as a nonmotile mutation. Since in this cross the strain carrying the *rnc* mutation also carried another mutation in a new RNase, as well as some other unidentified mutations, it became necessary to test this proposition critically. Therefore, we tested many *rnc*- and *rnc*$^+$-related strains, as well as revertants from *rnc* strains (revertants for better growth at elevated temperatures, some that remained *rnc*, and some that became RNase III$^+$, see 3). It should be emphasized that these studies were carried out with the single *rnc-105* allele. At present, this is the only mutation available in the *rnc* gene. We tested 15 strains, and invariably found that all the *rnc*$^+$ strains swarmed on semisolid agar plates and were motile (by watching for swimming under a light microscope), whereas all *rnc* strains were nonmotile in both tests. Occasionally in *rnc* cultures, we could see a swimming bacterium at a frequency of perhaps 1 in 3,000 by using a microscope. One of the *rnc* strains, N2077 (5), was also tested by M. Simon (University of California, San Diego) and found to be nonmotile under all growth conditions tested (25 to 37°C) and to exhibit a very low degree of flagellation. No more than 1 in 1,000 to 10,000 cells had sufficient flagella to be motile. When tested by electron microscopy, no flagella were detected in cells from strain N2077 (M. Simon, personal communication).

We carried out transduction experiments in which an *rnc* strain was either the recipient or the donor, using, as partners, strains with the cotransducible markers *nadB* (69%) and *purI* (64%; see 5, 18). (The *rnc* gene maps in between these two markers at min 55 on *E. coli* map 8.) In these crosses, about 300 transductants selected for either Nad$^+$ or Pur$^+$ were analyzed. The results invariably were similar; all the *rnc* recombinants were nonmotile, whereas all the *rnc*$^+$ recombinants were motile. Motility was tested under a light microscope and in semisolid agar plates (7), whereas RNase III was tested by enzymatic assays (4) and by gel patterns of rRNA (3, 12). RNase III$^-$ strains show an unusual pattern of rRNA that contains, after a short time of labeling, 30S, 25S, 18S, 17S, and 16S rRNA's (3, 12).

There is a certain variability in the swarming capacity of various *E. coli* laboratory strains. The *rnc-105* mutation was introduced into strain PA3306 (5), which swarms somewhat less than other strains. All *rnc*$^+$ revertants and transductants swarmed to the same extent as strain PA3306, whereas the *rnc* transductants did not swarm (see also Fig. 1).

One explanation of these results could be that the original strain AB301-105, into which the *rnc-105* mutation was introduced, contained a mutation that is very closely linked to the *rnc-105* mutation, renders the carrier strain nonmotile, and was always cotransduced with the *rnc-105* mutation. Since the original strain AB301-105 contains a large number of mutations (4), this possibility could not be dismissed, even though no motility genes have thus far been located in the neighborhood of the *rnc* gene (see 8). To test this possibility, we isolated *rnc*$^+$ strains from two *rnc* strains (N2077 and N2097) (for details, see 3). Sixteen *rnc*$^+$ strains were isolated, and all were motile. Moreover, by plat-

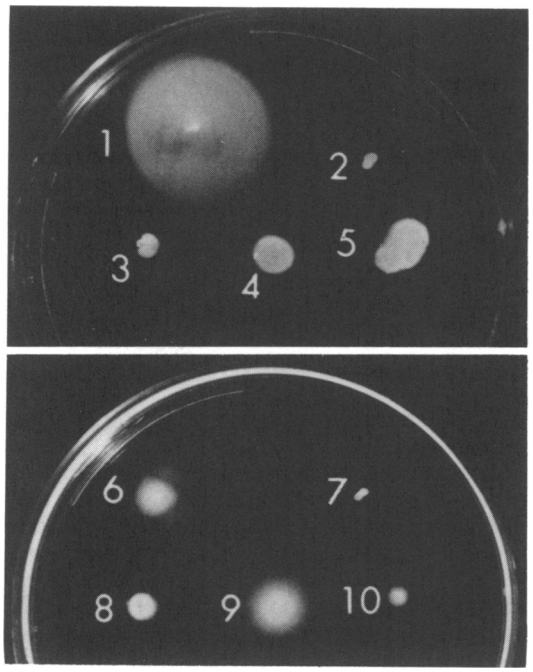

FIG. 1. *Growth of various strains in semisolid agar medium. Cultures were grown in broth liquid cultures, inoculated into semisolid agar plates (7), and incubated at 37°C. (1) PA3306 rnc⁺; (2) AW405 mot-488; (3) AW405 hag-585; (4) AW405 cheB690; (5) AW405 cheA593. This plate was photographed after 30 h of incubation. (6) PA3306 rnc⁺; (7) AW405 mot-488; (8) AW405 cheA593; (9) N2076 rnc⁺; (10) N2077 rnc. This plate was photographed after 12 h of incubation. Notice that strain PA3306 in (6) sectored. Some such sectors are due to mutations, which cause faster spread of strains in semisolid agar medium. The strains carrying mot, che, and hag mutations were obtained from J. Adler. All of these mutants cannot swarm in semisolid agar medium; the mot and hag mutants are nonmotile, whereas the che mutants are.*

ing a large number of *rnc* cells in semisolid agar plates, it was possible to isolate motile revertants. Three out of eight such isolated motile revertants were RNase III⁺ and behaved in every test like *rnc*⁺ strains, whereas the other five remained RNase III⁻. Thus, although it is clearly possible for a strain to be both motile and RNase III⁻, it is apparently impossible to be both RNase III⁺ and nonmotile, provided that all the other motility genes are intact.

These experiments support the notion that RNase III is involved in motility. This involvement, however, could be indirect, for under a large number of adverse conditions, bacterial cells apparently fail to produce flagella, and it is possible that *rnc* cells might fall into this category. However, the difference in growth rate between *rnc* and *rnc*⁺ cells is less than a factor of two, and the nonmotile *rnc* cells do grow exponentially (5). Thus, the interesting possibility remains that RNase III may be involved in the obligatory processing of an mRNA transcribed from one of the many genes required for motility. Thus far, although RNase III was found to be involved in the maturation of T7 RNA (11), λ RNA (14), T4 RNA (Ko and Apirion, unpublished data), and host (*E. coli*) rRNA (2, 10, 12), it was not found to affect any other host RNA beside rRNA. It would be useful to identify in *E. coli* a message that requires obligatory processing, thus making *E. coli* a further useful model for the analysis of mRNA processing, a phenomenon which thus far has not been recognized in *E. coli* mRNA.

If RNase III does participate in the obligatory processing of some mRNA that is involved in motility, the revertants that regained motility, but remained RNase III⁻, suggest that perhaps this processing can be circumvented or can be achieved in another way, and the mutations that caused reversion could facilitate this event. In the processing of tRNA, for instance, it is quite evident that in the absence of RNase P, another enzyme could process, albeit slowly, precursors that are normally cut by RNase P (16).

It has been reported that flagella formation in *E. coli* is regulated by cyclic AMP and its receptor protein (17, 20). However, since the *rnc-105* mutation does not seem to have a general effect on the synthesis of catabolite sensitive operons, it is not possible to argue that RNase III is involved in the processing of a messenger common to all such operons. For instance, β-galactosidase can be induced in *rnc* strains (6), and bacteriophage λ can be lysogenized in *rnc* strains (14).

Recently it was shown that some mutants in RNA polymerase are also defective in flagella formation (19). In this case, again, the effect was rather specific and did not extend to other catabolite sensitive operons. Although in the case of RNase III, as mentioned above, the possibility that a particular messenger might require processing by this enzyme could explain the dependency of flagellation on RNase III, other explanations are necessary to elucidate the failure of RNA polymerase mutants to form flagella.

We are most grateful to J. Adler and M. Simon for sending us strains and for patiently advising us about motility in *E. coli*, and to M. Simon for testing strain N2077 for motility and flagella.

This research was supported by grant PCM-76-81665 from the National Science Foundation.

LITERATURE CITED

1. **Adler, J.** 1975. Chemotaxis in bacteria. Ann. Rev. Biochem. **44**:341–356.
2. **Apirion, D.** 1974. The fate of mRNA and rRNA in *Escherichia coli*. Brookhaven Symp. Biol. **26**:286–306.
3. **Apirion, D., J. Neil, and N. Watson.** 1976. Revertants from RNase III negative strains of *Escherichia coli*. Mol. Gen. Genet. **149**:201–210.
4. **Apirion, D., and N. Watson.** 1974. Analysis of an *Escherichia coli* strain carrying physiologically compensating mutations one of which causes an altered ribonuclease III. Mol. Gen. Genet. **132**:89–104.
5. **Apirion, D., and N. Watson.** 1975. Mapping and characterization of a mutation in *Escherichia coli* that reduces the level of ribonuclease III specific for double-stranded ribonucleic acid. J. Bacteriol. **124**:317–324.
6. **Apirion, D., and N. Watson.** 1975. Unaltered stability of newly synthesized RNA in strains of *Escherichia coli* missing a ribonuclease specific for double stranded RNA. Mol. Gen. Genet. **136**:317–326.5.
7. **Armstrong, J. B., J. Adler, and M. M. Dahl.** 1967. Nonchemotactic mutants of *Escherichia coli*. J. Bacteriol. **93**:390–398.
8. **Bachmann, B. J., K. B. Low, and A. L. Taylor.** 1976. Recalibrated linkage map of *Escherichia coli* K-12. Bacteriol. Rev. **40**:116–167.
9. **Berg, H. C.** 1975. Chemotaxis in bacteria. Ann. Rev. Biophys. Bioeng. **4**:119–136.
10. **Dunn, J. J., and F. W. Studier.** 1973. T7 early RNAs and *Escherichia coli* ribosomal RNAs are cut from large precursor RNAs *in vivo* by ribonuclease III. Proc. Natl. Acad. Sci. U.S.A. **70**:3226–3300.
11. **Dunn, J. J., and F. W. Studier.** 1974. Processing, transcription, and translation of bacteriophage T7 messenger RNAs. Brookhaven Symp. Biol. **26**:267–276.
12. **Gegenheimer, P., N. Watson, and D. Apirion.** 1977. Multiple pathways for primary processing of ribosomal RNA in *Escherichia coli*. J. Biol. Chem. **252**:3064–3073.
13. **Komeda, Y., M. Silverman, and M. Simon.** 1977. Genetic analysis of *Escherichia coli* K-12 region I flagellar mutants. J. Bacteriol. **131**:801–808.
14. **Lozeron, H. A., P. J. Anevski, and D. Apirion.** 1977. Antitermination and absence of processing of the leftward transcript of coliphage lambda in the RNase III-deficient host. J. Mol. Biol. **109**:359–365.
15. **Parkinson, J. S.** 1975. Genetics of chemotactic behavior in bacteria. Cell **4**:183–188.
16. **Schedl, P., P. Primakoff, and J. Roberts.** 1974. Processing of *E. coli* tRNA precursors. Brookhaven Symp. Biol. **26**:53–76.
17. **Silverman, M., and M. Simon.** 1974. Characterization of *Escherichia coli* flagellar mutants that are insensitive to catabolite repression. J. Bacteriol. **120**:1196–1203.
18. **Studier, F. W.** 1975. Genetic mapping of a mutation that causes ribonuclease III deficiency in *Escherichia coli*. J. Bacteriol. **124**:307–316.
19. **Yamamori, T., K. Ito, T. Yura, T. Suzuki, and T. Iino.** 1977. Ribonucleic acid polymerase mutant of *Escherichia coli* defective in flagella formation. J. Bacteriol. **132**:254–261.
20. **Yokota, T., and J. S. Gots.** 1970. Requirement of adenosine 3',5'-cyclic phosphate for flagella formation in *Escherichia coli* and *Salmonella typhimurium*. J. Bacteriol. **103**:513–516.