Vol. 86, No. 1, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 15, 1979 Pages 119-123

THERMOSENSITIVE CELL GROWTH AND PERMEABILITY CODED BY AN Rts1 PLASMID AND THEIR SUPPRESSION UPON INTEGRATION INTO THE CHROMOSOME IN Escherichia coli

Masanosuke Yoshikawa
Institute of Medical Science, University of Tokyo, Shiroganedai, Minato-ku, Tokyo, 108, Japan

Received November 10,1978

Summary : A multiphenotypically thermosensitive plasmid, Rts1, was found to confer upon the host cell of Escherichia coli K12 an increased permeability through the cell surface to Actinomycin D and rifampicin as well as a detrimental host cell growth at 42°C only when it existed autonomously but not in an integrated state.

A kanamycin resistance plasmid, Rts1, belonging to the T incompatibility group (1), is multiphenotypically thermosensitive with respect to its maintenance (2), conjugal transfer (2), host cell growth (3), and to "restriction" of T even phages (4). The temperature dependent detrimental effect upon the host cell growth was shown to be accompanied by a morphological and a permeability change of the cell surface (5).

On the other hand we had shown that Rts1 inhibited host cell growth at 42°C only when it existed autonomously but not in an integrated state (6). This observation together with some others (7) led us to a hypothesis that the control mechanism for chromosome replication inhibits that of integrated plasmid from functioning. In the present study an increased permeability to Actinomycin D and rifampicin at 42°C was found when Rts1 existed autonomously but not in an integrated state. The significance of this finding is discussed in relation to this hypothesis on the chromosome - plasmid interaction.

Materials and Methods

Three strains of Escherichia coli K12 , YC293, YC294 and YC295, which had been described previously (6) were mainly used. CRT46 (thi, thr, leu, ilv, thy, dnaA(Ts)) was the initial ancester for these three isogenic strains and carried a thermosensitive mutation in the dnaA locus respeonsible for the initiation of chromosome replication. YC294 was an ilv^+, $dnaA^+$ transductant of CRT46. YC295 was a derivative of YC294 carrying Rts1 autonomously. An Hfr strain with an integrated Rts1 plasmid was isolated by integrative suppression (8) with the use of CRT46 strain carrying Rts1 autonomously. The dnaA(Ts) mutation was removed by transducing the $dnaA^+$ genome to this Hfr. The strain, YC293, thus obtained , no longer carried any temperature sensitive mutation but the effect

0006-291X/79/010119-05$01.00/0

of Rts1. These three strains are all isogenic with respect to the chromosomal genetic constitution except for the plasmid Rts1. Two other strains, JC1569 (leu, his, arg, met, recA) and YC300 were recA- deficient strains, the latter carrying a prime - plasmid detached from YC293 (6). The recombination deficient strains were used to avoid re-integration of the detached Rts1 plasmid into the host chromosome.

Actinomycin D (Makor, Israel), rifampicin (Daiichi Seiyaku, Tokyo) and gentian violet (Wako Pure Chem., Osaka) were purchased commercially and used as inhibitors to examine their permeability through the cell surface of these cells with or without Rts1.

For sensitivity tests to these inhibitors logarithmic cultures in Bacto Penassay broth (Difco) supplemented with 10 mcg of thymine per ml (abbreviated as PAB-Thy) were inoculated at 2×10^6 per ml into 7 ml fresh PAB-Thy containing various concentrations of Actinomycin D or rifampicin as indicated in each figure. Each of the inoculated broth was divided into 2 tubes and one of them was grown at 30°C and another at 42°C with gentle shaking. After 18 hours incubation growth was read with a Shimadzu-Bausch-Lomb photometer at 600 nm. The growth was expressed in percentages of the optical density without any inhibitor.

Results and Discussion

Sensitivity was compared among three strains, YC293 (Rts1 integrated), YC294 (no plasmid), and YC295 (Rts1 autonomously), at 30 and 42°C to Actinomycin D, an agent which is generally recognized as a chemical to which Gram-negative rods are resistant due to penetration barrier. At 30°C all three strains showed relative insensitivity to the agent as expected (Fig. 1). However, as originally found by DiJoseph et al. (5), YC295, a strain carrying Rts1 autonomously, was markedly more sensitive to it at 42°C than was the plasmid-free parent, YC294 and than was any of these three strains at 30°C. This sensitivity at 42°C was not observed in YC293, an isogenic strain carrying the same plasmid in an integrated state. Essentially the same result was obtained with rifampicin, although this agent gave a little bit more complicated result presumably because it was active even to the plasmid-free strain, YC294, irrespective of the temperature used for growth (Fig. 2).

To exclude the possibility that the genetic locus on Rts1 responsible for the sensitivity to Actinomycin D and to rifampicin at 42°C had been lost during the process of integration into the chromosome, sensitivity of two recombination-deficient strains, JC1569 (no plasmid) and YC300 (with a prime-plasmid detachd from YC293) to Actinomycin D were examined. As shown in Fig. 3, YC300 was more sensitive to the agent at 42°C than the plasmid-free parent, JC1569. Thus, the detached plasmid behaved similarly to Rts1. It is clear that Rts1 makes the host cell permeability barrier damaged at high temperature when it exists autonomously but not when integrated into the normal host chromosome.

Gustaffson et al. (9) showed that Escherichia coli envelope mutants carrying either envA, galU or rfa mutation took up gentian violet more than the wild type at 37°C and concluded that the outer part of the bacterial envelope might be a penetration barrier

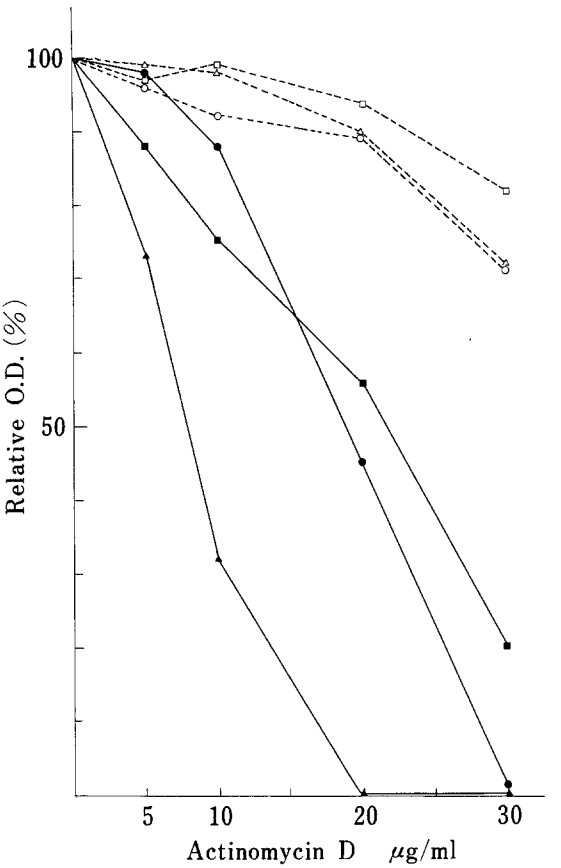
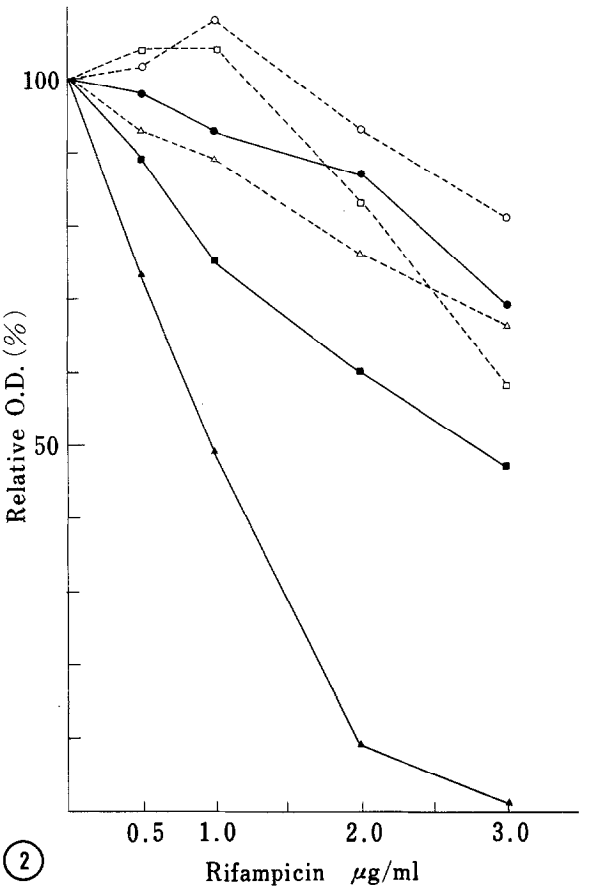


Fig. 1 Sensitivity of three isogenic strains of E. coli, YC294 (plasmid-free), YC295 (autonomous Rts1), and YC293 (integrated Rts1) to Actinomycin D. Logarithmic cultures of these strains were inoculated at 2 X 10^6 cells per ml of PAB-Thy containing various concentrations of Actinomycin as indicated. Identical inoculated cultures were divided into 2 tubes and one of them was grown at 30°C and another at 42°C with shaking. After 18 hours incubation the growth was measured turbidimetrically. The optical density of the culture without Actinomycin D was taken as 100 % and the growth of those with the inhibitor was expressed relatively. Closed symbols with solid lines; at 42°C , and open symbols with broken lines; at 30°C. Triangle; YC295, circle; YC294, and square; YC293.

to this dye. Therefore, the uptake of this dye was compared among YC293, YC294, and YC295 at both 30 and 42°C by the method described by Gustaffson et al. (9) but no difference was observed among them, suggesting that Rts1 does not alter the outer envelope at 42°C to the extent to destroy the outer penetration barrier to gentian violet.

In our previous communication (6) temperature sensitivity of conjugal plasmid transfer (or conjugal chromosome transfer in an Hfr strain) and that of T even phage "restriction" were found to be coded by some plasmid genes other than those coding thermosensitivity of plasmid replication and that of host cell growth. However, thermosensitivity of



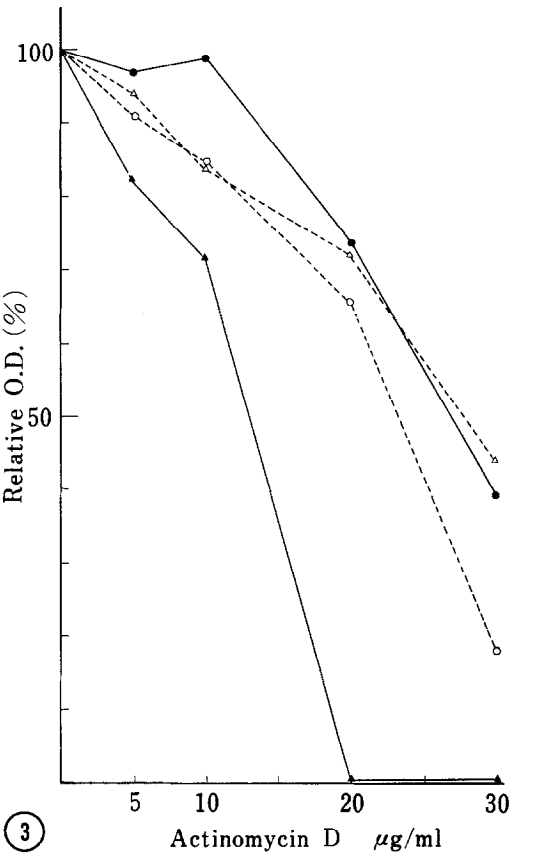


Fig. 2 Sensitivity of three isogenic strains, YC294 (plasmid-free), YC295 (autonomous Rts1), and YC293 (integrated Rts1) to rifampicin. The method was essentially the same as in Fig. 1. The symbols and the lines are also the same as in Fig. 1.

Fig. 3 Sensitivity of two isogenic RecA-deficient strains with or without an Rts1-prime plasmid derived from YC293. The method was the same as described in Fig. 1. Closed symbols with solid lines; at 42°C and open symbols with broken lines; at 30°C. Circle; JC1569 and triangle; YC300 (a recA strain with Rts1-prime plasmid autonomously).

plasmid replication seems to be somehow related to that of host cell growth. For example, an artificially isolated thermosensitive mutant of ColVBtrp plasmid also exhibited thermosensitivity in host cell growth and a revertant with respect to plasmid replication was found to have lost the thermosensitive nature of host growth (10). In Rts1, both of these two temperature sensitive natures were suppressed upon integration of the plasmid into the host chromosome (6). We supposed that Rts1 provoked a detrimental effect at 42°C when its replication mechanism was active presumably due to a defective replication apparatus. This defective apparatus may affect not only the replication of the plasmid itself in an autonomous state, resulting in its segregation, but replication of the chromosome or the

cell division. It was further supposed that the plasmid replication system was suppressed when it was integrated into a normally functioning chromosome and hence no detrimental effect was observed at 42°C when Rts1 was integrated into the host chromosome.

In the present study with Rts1 the abnormal permeability at 42°C, which seems also generally to be closely related to thermosensitive mutations of plasmid replication (10), was found to be also suppressed when this plasmid was integrated into the host chromosome. This may indicate that the abnormal permeability at 42°C is functionally related to the abnormal plasmid replication and/ or host growth or that these three functions are coded by a single plasmid gene or commonly regulated. It is interesting that abnormal replication of an independent replication unit is somehow related to abnormal permeability through the cell surface.

Acknowledgement

This study was supported in part by a grant provided by the Ministry of Education, Science, and Culture, the Japanese Government (Grant No. 357147)

References

1. Coetzee,J.N., Datta,N., and Hedges, R.W. (1972) J. Gen. Microbiol. 72, 543-552.
2. Terawaki, Y., Takayasu, H., and Akiba, T. (1967) J. Bacteriol. 94, 687-690.
3. Terawaki, Y., Kakizawa, Y., Takayasu, H., and Yoshikawa, M. (1968) Nature 219, 284-285.
4. Yokota, T., Kanamaru, Y., Mori, R., and Akiba, T. (1969) J. Bacteriol. 98, 863-873.
5. DiJoseph, C., G., Bayer, M. E., and Kaji, A. (1973) J. Bacteriol. 115, 399-410.
6. Yoshimoto, H., and Yoshikawa, M. (1975) J. Bacteriol. 124, 661-667.
7. Sotomura, M., and Yoshikawa, M. (1975) J. Bacteriol. 122, 623-628.
8. Nishimura, Y., Caro, L., Berg, C. M., and Hirota, Y. (1971) J. Molec. Biol. 55, 441-456.
9. Gustaffson, P., Nordström , K., and Normark, S. (1973) J. Bacteriol. 116, 893-900.
10. Koyama, A. H., and Yura, T. (1975) J. Bacteriol. 122, 80-88.