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Characterization and Genetic Analysis of a Mutant of *Escherichia coli* K-12 with Rounded Morphology

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A morphological mutant of *Escherichia coli* K-12 that grows as round cells at 30, 37, or 42 C in a variety of complex and synthetic media has been isolated and characterized. The gene concerned, designated *rodA*, has been shown to be on the chromosome between the *purE* and *pyrC* loci and to be located at about minute 15. The *rodA* gene has been found to be co-transducible with the *lip* gene at a frequency of 95%. The *rodA* mutant showed an increased resistance to ultraviolet irradiation and a changed sensitivity to drugs. The resistance to ultraviolet irradiation and mitomycin C appears to be co-transducible with the *rodA* gene.

It is of great interest to study the genetic control and biochemical determinant(s) of bacterial morphology. Conditional or nonconditional *rod* mutants have been described in *Escherichia coli* (2, 10, 14, 17), *Bacillus subtilis* (3, 19, 20), *Bacillus licheniformis* (19, 20), and *Agrobacterium tumefaciens* (8). Adler et al. have found a morphological mutant of *E. coli* K-12 which is radiation resistant, and it is not known whether the unusual cell morphology and radiation resistance are a result of the same genetic alteration (2). Normark has reported a morphological *envB* mutant that has a mutated gene near the *strA* gene and shows a changed resistance to several antibiotics as well as an increased tolerance to ultraviolet (UV) irradiation (17).

We have found a morphological mutant of *E. coli* K-12 among a group of acridine orange-sensitive mutants. The cells grow as round cells at 30, 37, or 42 C in either liquid or solid media. In this report we describe a new gene, designated *rod A*, which controls the morphology of *E. coli* cells and is located at about minute 15 close to the *lip* gene (11). Resistance to UV irradiation and mitomycin C is co-transducible with the *rodA* gene.

Gene designation and time scale in this report are described according to Taylor (22).

MATERIALS AND METHODS

Bacterial strains. The properties of *E. coli* K-12 strains used are summarized in Table 1. The transducing phage P1*kc* was obtained from Y. Sugino.

Media. Bacteria were usually cultured at 37 C with shaking in nutrient broth or L-broth. Nutrient broth contained (per liter of deionized water): polypeptone (Daigo Eiyo Kagaku Co., Osaka, Japan), 10 g; Ehrlich meat extract (Kyokuto Seiyaku Co., Tokyo, Japan), 10 g; and NaCl, 2 g. L-broth contained (per liter of deionized water): polypeptone, 10 g; yeast extract (Kyokuto Seiyaku Co., Tokyo, Japan), 5 g; NaCl, 5 g; glucose, 1 g; and thymine, 10 g supplemented. Media were adjusted to pH 7.2 by addition of approximately 0.5 ml of 10 N NaOH. Davis minimal medium (21) was used for determination of auxotrophic markers. EM medium (21) and EMB medium (21) were used for determination and selection of defective sugar fermentation. K10 medium (6) was used for transduction experiments. For solidifying media 1.5% agar (Wako Pure Chemical Co., Osaka, Japan) was used.

Isolation of the morphological mutant. *E. coli* K-12 strain JE1011 was treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG), essentially under the conditions described by Adelberg et al. (1). The cells were then allowed to grow for about three generations in nutrient broth, and 0.1-ml samples were spread on plates of nutrient agar at a concentration giving about 300 colonies per plate (master plate). The plates were incubated at 37 C, and colonies were replicated on nutrient agar plates containing 50 μg of acridine orange (Chroma-Gesellschaft Schmid & Co., Germany) per ml. Colonies which did not grow on the replica plates were collected from the master plates, and the morphology of acridine orange-sensitive mutants was observed with a phase-contrast microscope.

UV sensitivity test. Bacteria in the logarithmic phase of growth (about 2×10^8 cells per ml) were diluted to 2×10^6 cells per ml in pH 6.8 buffer (0.13 M NaCl, 0.02 M sodium phosphate) and irradiated in petri dishes (84 mm in diameter) with a UV lamp

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TABLE 1. *Strains of Escherichia coli K-12 used*

Strain	Sex[a]	Genotype	Origin[b]
JE1011	F⁻	*thr, leu, trp, his, thy, thi, ara, lac, gal, xyl, mtl, strA*	Obtained from Y. Sugino
AOS15	F⁻	*thr, leu, trp, his, thy, thi, ara, lac, gal, xyl, mtl, strA, rodA*, acridine orange-sensitive	Isolated from JE1011 after treatment with NTG
AOS151	F⁻	*thr, leu, trp, his, thy, thi, ara, lac, gal, xyl, mtl, strA, rodA*	Acridine orange-resistant recombinant from AOS15 × W2252
W2252	Hfr C	*met*	Obtained from Y. Sugino
JE1031	Hfr H	*met, thi*	Obtained from Y. Sugino
W3807	Hfr 6	*metB1, mal-20, mtl-8, mut-2*	Lederberg Laboratory
KL99	Hfr	*thi-1, rel-1*	K. B. Low strain
AT1325 lip9	F⁻	*thi-1, his-4, purB15, proA2, mtl-1, xyl-5, galK2, lacY1, lip-9, str-35*	J. R. Guest strain
χ478	F⁻	*thi-1, metE70, lysA23, trpE38, purE42, proC32, leu-6, mtl-1, xyl-5, ara-14, lacZ36, tonA23, azi-6, tsx-67, sup-45, str-109*	R. Curtiss strain

[a] Each Hfr strain has the point of origin and direction of chromosome transfer as shown in Fig. 4.

[b] Strains W3807, KL99, AT1325 lip9, and χ478 were obtained from the collection of Coli Genetic Stock Center, Department of Microbiology, Yale University School of Medicine, New Haven, Conn. CGSC numbers of the strains are 4236, 4242, 4286, and 4212, respectively.

(Toshiba GL-15) at a distance of 50 cm. After dilution of the irradiated suspension (0.7 mm in depth), 0.1-ml samples were spread on plates of L-broth agar. The plates were incubated at 37 C overnight in the dark, and the colonies formed were counted.

Quantitative evaluation of sensitivity to drugs. After culturing overnight, the strains were diluted and 0.1-ml samples were spread on plates of L-broth agar containing different concentrations of each drug. The plates were incubated at 37 C for 1 or 2 days and the colonies were counted. The concentrations of drugs required to inhibit the colony formation to half that in the control plate were calculated.

Mating procedure. The method used was essentially that of Taylor and Thoman (23), which is described in full detail (21).

Transduction technique. The procedure used was based on that described by Lennox (15), and was as described previously (16) except that the temperature was kept at 37 C and the multiplicity of infection was 0.1. The cell shape of recombinants and transductants was examined with a phase-contrast microscope.

Electron microscopy. The preparative method of Kellenberger et al. (13) was used. Sections were cut on a Porter-Blum MT-2 ultramicrotome with a glass knife. The sections were collected on Formvar-coated copper grids and stained first with uranyl acetate and then with lead citrate (7). They were examined with a JEM-7A electron microscope.

RESULTS

Genetic characteristics of acridine orange-sensitive and morphological mutant strain AOS15. Forty-one acridine orange-sensitive mutants were isolated from strain JE1011 treated with NTG. Among them one mutant, strain AOS15, had rounded morphology and the same genetic markers as the parental strain. We determined whether the changed sensitivity to acridine orange and disturbed morphology were due to the same genetic alteration. Acridine orange-resistant revertants from AOS15 retained the disturbed morphology and could grow on plates containing 50 μg of acridine orange per ml. Acridine orange-resistant recombinants obtained by mating strain AOS15 with strain W2252 (Hfr C) also retained the rounded morphology. These results showed that the mutated gene(s) concerned with acridine orange sensitivity and cell morphology were not identical. Apparently this morphological mutant was obtained by chance out of a group of acridine orange-sensitive mutants.

The acridine orange-sensitive locus of strain AOS15 is presumably identical or close to the *mtc* gene (minute 12) (22), because in the interrupted mating between strain AOS15 and strain W2252 (Hfr C) the donor determinant of the acridine orange-resistant trait entered at about 5 min after the start of mating, and the *lac*⁺ allele was transfered at about 10 min (see Fig. 4). NTG is known to induce multiple mutations at the replicating region in the length between minute 1.5 and 2.0 (9). Therefore, the changed sensitivity to acridine orange and rounded morphology could be a result of comutation with NTG, taking into account that the mutated gene concerned with morphology of strain AOS15 is closely linked with the *lip* gene (minute 15) (11) as described below (see Fig. 4).

In the following experiments we used one of the acridine orange-resistant recombinants with disturbed morphology, strain AOS151, derived from a 10-min mating between strain AOS15 and strain W2252 (Hfr C). Strain AOS151 has the same genetic markers as the parental strain JE1011 except *rodA*.

General properties of strain AOS151. Strain AOS151 grows in the rounded morphology at 30, 37, or 42 C in a variety of complex and synthetic media (Fig. 1B), in contrast to the parental strain JE1011 which grows as a normal rod (Fig. 1A). At 25 C the cell shape of strain AOS151 becomes slightly lengthened and ab-

normal (Fig. 1C). These cells do not require isotonic osmotic conditions for their growth and under these conditions did not convert to rods. Strain AOS151 forms normal rough colonies on complex or synthetic agar plates. In complex and synthetic liquid cultures at 37 C, lysed cell debris can be observed, especially in the stationary phase of growth. However, the growth curve of strain AOS151 is normal in nutrient or L-broth, and the visible cells are almost all viable in any phase of growth. In L-broth cultures the generation time of strain AOS151 is 50 min, and that of the parental strain JE1011 is 35 min. In the case of *rodA*$^+$ and *rodA*$^-$ transductants selected with *lip*$^+$ from strain AT1325 lip9, the generation time is 29 and 33 min, respectively. In view of the long generation time of strain AOS151 in contrast to the parent strain, it is likely that strain AOS151 still contains additional NTG-induced mutation(s).

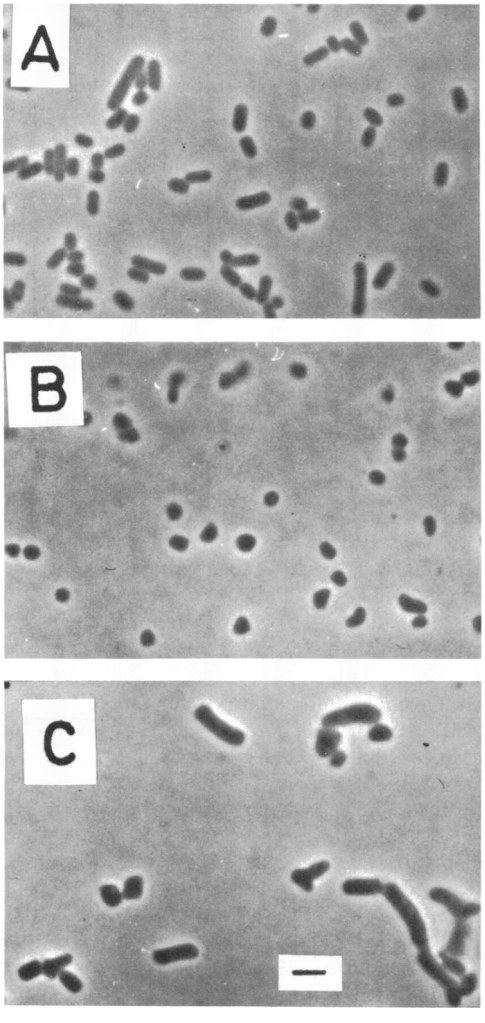

FIG. 1. *Phase-contrast photomicrographs of cells of (A) the parental strain JE1011 grown at 37 C and the rodA mutant AOS151 grown (B) at 37 C and (C) at 25 C. Medium used was L-broth. All sections of this figure are at the same magnification, and marker bar represents 3 μm.*

Electron microscopy examinations of ultrathin sections of strain AOS151 cells (Fig. 2) show no apparent defect in the cell membrane and wall. Normal symmetrical septum formation is observed in dividing cells of strain AOS151 (Fig. 2), which differs from that reported for the *envB* mutant with disturbed morphology (17).

Sensitivity of strain AOS151 to bacteriophages or colicins was examined by a cross-streak method. Strain AOS151 and the parental strain JE1011 were both sensitive to phages T2, T3, T4, T6, T7, BF23, λ, and P1*kc* and to colicins to E2 and K.

Sensitivity of strain AOS151 to UV irradiation and drugs. The morphological mutants reported by Adler et al. (2) and Normark (17) were both resistant to UV irradiation. Figure 3 shows that strain AOS151 is also more resistant to UV than the parental strain. The shape of the UV killing curve is similar to that obtained on plates of mineral-glucose agar and resembles that of the *envB* mutant described by Normark (17).

The sensitivity of strain AOS151 to antibiotics and drugs was examined (Table 2). The mean lethal doses (LD_{50}) of penicillin G, ristocetin, and phenethyl alcohol for the *rodA* mutant were one-half to one-third those of the

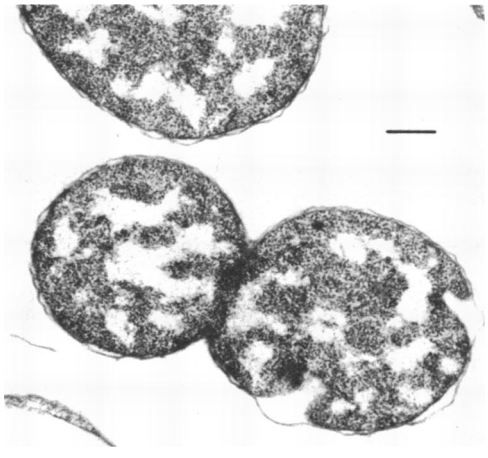

FIG. 2. *Electron micrograph of thin section of cells of the rodA mutant AOS151 grown in L-broth at 37 C. Marker bar represents 0.2 μm.*

parental strain. On the contrary, the LD_{50} values of D-cycloserine, novobiocin, nalidixic acid, and mitomycin C for the mutant were more than twice those of the parental strain. LD_{50} values of acridine orange and chloramphenicol were unchanged.

Mapping of the rodA gene. The point of origin and direction of chromosome transfer for Hfr strains used and several genetic markers are shown in Fig. 4. The gene carrying the mutation causing the morphological alteration in strain AOS151 is designated *rodA*. The *rodA*$^+$ allele was not transfered with an early marker in a mating experiment crossing strain AOS151 with strain W2252 (Hfr C). In crosses with strain JE1031 (Hfr H) for 1 h, the *rodA*$^+$ recombinants were 18 of 55 *lac*$^+$ (33%), 48 of 55 *gal*$^+$ (87%), and 17 of 54 *trp*$^+$ (31%). An interrupted mating was carried out by using strain W3807 (Hfr 6) at 3-min intervals, and times of entry of 12 and 18 min were obtained for *rodA*$^+$ and *gal*$^+$, respectively. The *rodA*$^+$ recombinants were selected on a fairly dense cell background as ristocetin-resistant recombinants which grew on nutrient agar plates containing 60 μg of ristocetin per ml and were almost all rod shaped. However, the donor determinant of the *rodA*$^+$ trait could not be transferred as an early marker by Hfr strain KL99. These results suggested that the *rodA* gene was on the chromosome between the *purE* and *pyrC* loci and located at about minute 15.

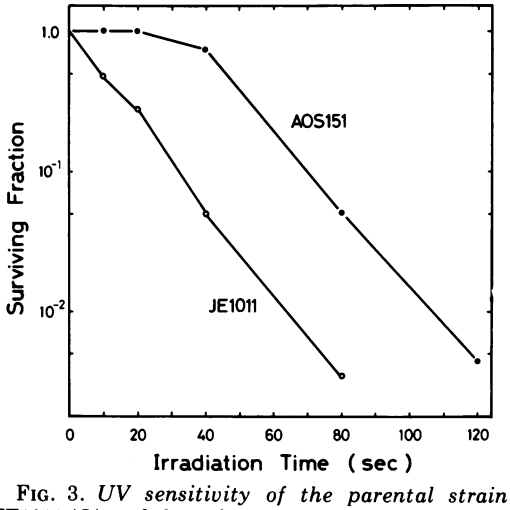

FIG. 3. *UV sensitivity of the parental strain JE1011* (○) *and the rodA mutant AOS151* (●).

TABLE 2. *LD_{50} values for E. coli strain JE1011 and its rodA mutant strain AOS151*

Drug tested	LD_{50} (μg/ml)	
	Strain JE1011	Strain AOS151
Penicillin G	27	13
Ristocetin	36	11
D-Cycloserine	13	23
Novobiocin	350	620
Nalidixic acid	2.7	7.5
Mitomycin C	0.11	0.98
Phenethyl alcohol[a]	0.28	0.13
Acridine orange	270	270
Chloramphenicol	3.3	4.8

[a] Results expressed as percent (vol/vol).

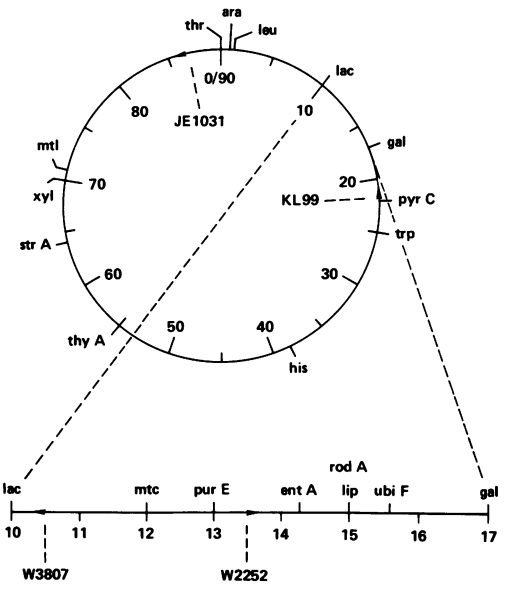

FIG. 4. *Genetic map and the origins and directions of chromosome transfer for Hfr strains, JE1031, W3807, W2252, and KL99.*

To test for co-transduction with the *lip* gene (11), P1*kc* lysate was prepared on *rodA*$^-$ strain AOS151 and used to transduce *lip*$^+$ into strain AT1325 lip9. The *rodA* gene was found to be co-transducible with *lip* at a frequency of 95% (159 *rodA*$^-$ of 168 *lip*$^+$ transductants). Figure 5 shows the cell shape of *rodA*$^+$ and *rodA*$^-$ transductants. However, no *rodA*$^-$ transductants were obtained when *purE*$^+$ was transduced into strain χ478 (0/263). The co-transduction frequency of the *ubiF* gene with *lip* is 30% (26), and that of *entA* and *entB* is 12% (25). At present the relationship of the *rodA* gene to these genes is being investigated.

These genetic analyses show that the *rodA* mutant is genetically different from the *envB* mutant mapped at a position close to *strA* (minute 64) (17) and may have some connection with the temperature-sensitive *rod* mutant re-

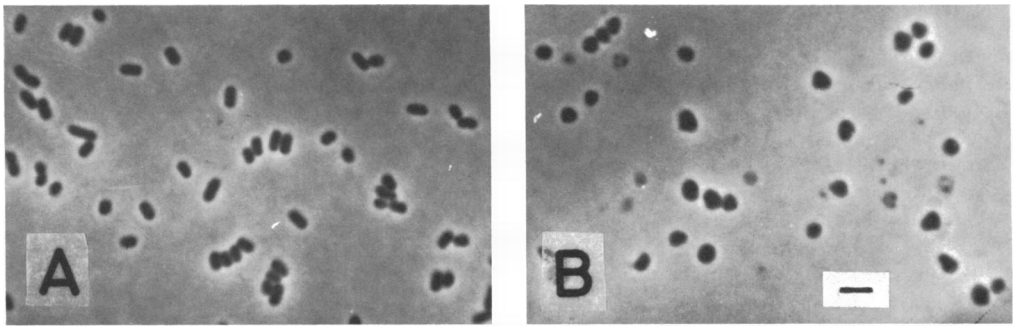

FIG. 5. *Phase-contrast photomicrographs of cells grown at 37 C in L-broth of (A) the rodA⁺ transductant AT1325-R1 and (B) the rodA⁻ transductant AT1325-S2. Sections of this figure are at the same magnification, and marker bar represents 3 μm.*

ported by Henning et al. which has the mutated gene near *gal* (10).

Co-transduction of resistance to UV irradiation and mitomycin C with the rodA gene. Strain AOS151 was more resistant to UV irradiation and some antibiotics than the parental strain, as described above. We determined whether these properties were transferred into the *rodA*⁻ transductants selected with *lip*⁺ from strain AT1325 lip9. Figure 6 shows that one of the *rodA*⁻ transductants, strain AT1325-S2, was more resistant to UV irradiation than one of the *rodA*⁺ transductants, strain AT1325-R1. Other *rodA*⁺ transductants were also sensitive, as were other *rodA*⁻ transductants resistant to UV irradiation (Table 3). The shape of the UV killing curve of strain AT1325-S2 is different from that of strain AOS151 (Fig. 3) and rather resembles that of the X-ray killing curve of the morphological mutant reported by Adler et al. (2). The *rodA*⁻ transductants were more tolerant to mitomycin C than the *rodA*⁺ transductants (Table 3), and the LD_{50} of mitomycin C was 0.50 and 1.8 μg per ml for strain AT1325-R1 and strain AT1325-S2, respectively. These results strongly suggest that resistance to UV irradiation and mitomycin C is co-transducible with the *rodA* gene and these phenotypes are controlled by the same gene.

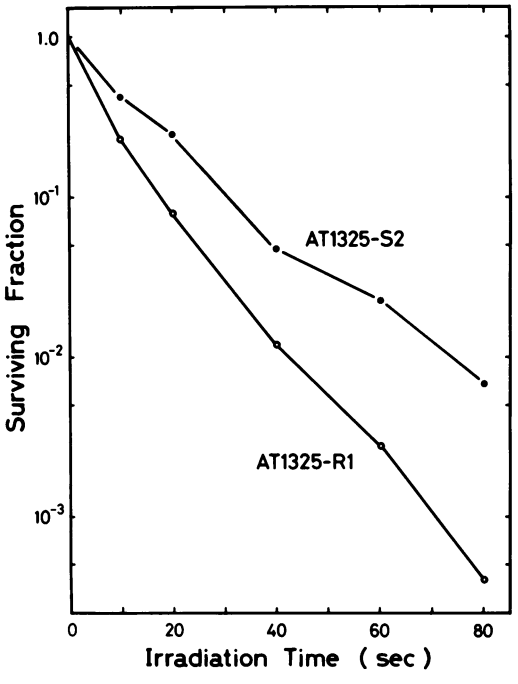

FIG. 6. *UV sensitivity of the rodA⁺ transductant AT1325-R1* (○) *and the rodA⁻ transductant AT1325-S2* (●).

DISCUSSION

Several mutants with disturbed morphology have been reported. Among them, *E. coli* mutants described by Adler et al. (2) and Normark (17) grow as round cells under normal conditions. The *rodA* mutant strain AOS151 also grows in the rounded morphology and is not corrected in supplemented isotonic osmotic medium, as described above. *B. subtilis rod* mutants have been reported by Rogers et al. which grow under nonrestrictive conditions as round cells and under restrictive conditions as normal rods (19, 20). As conditional and lethal *rod* mutants, there are temperature-sensitive mutants of *E. coli* described by Kohiyama et al. (14), Henning et al. (10), and H. Matsuzawa, M. Matsuhashi, and Y. Sugino (unpublished experiment), and a *B. subtilis* mutant described by Boylan and Mendelson (3). Several morphological mutants of *A. tumefaciens* have been isolated by Fujiwara and Fukui (8).

TABLE 3. *Sensitivity of rodA⁺ and rodA⁻ transductants to UV and mitomycin C*

Strain	Genotype of *rodA*	Surviving fraction after UV irradiation[a]	Growth on plate containing mitomycin C[b]
AT1325-R1	+	1.8×10^{-3}	–
AT1325-R2	+	3.3×10^{-3}	–
AT1325-S1	–	2.8×10^{-2}	+
AT1325-S2	–	1.7×10^{-2}	+
AT1325-S3	–	1.0×10^{-2}	+
AT1325-S4a	–	1.0×10^{-2}	+
AT1325-S4b	–	1.6×10^{-2}	+

[a] Cells were irradiated by UV for 60 s as described in Materials and Methods, and the colony number was compared with that of nonirradiated cells.

[b] A 1:100 dilution of an overnight culture of each strain was made and streaked on a plate of L-broth agar containing 4 μg of mitomycin C per ml. Presence (+) or absence (–) of visible colonies was recorded after overnight incubation at 37 C.

The genetic analyses of some morphological mutants have been described. As to the *rod* genes of *E. coli* K-12, one is the *envB* gene (17) mapped near *strA*, and the other, the temperature-sensitive *rod* mutant (10), is closely linked to *gal*. A third new gene, *rodA*, has been found to be 95% co-transducible with the *lip* gene as described in this report. The *rod* genes of *B. subtilis* have also been mapped. The *tag-1* mutation has been found to be approximately 40% co-transducible with *hisA1* (4). Salt-dependent *rod* mutants have also been mapped and are distributed into two small linkage groups designated *rodB* and *rodC* (12). These studies suggest that there are several genes and factors controlling bacterial morphology and morphogenesis. The viability or lethality resulting from each morphological mutation is of great interest, because the temperature lethality of the *tag-1* mutation appears to be dependent on the genetic background of the strain carrying the mutation, not on the *tag-1* mutation per se (18).

Our *rodA⁻* strains are more resistant to UV irradiation than the *rodA⁺* strains (Fig. 3 and 6; Table 3), as is the case of the morphological mutants described by Adler et al. (2) and Normark (17). It appears that the rounded morphology and UV resistance are a result of the same genetic alteration, because the resistance to UV irradiation of strain AOS151 is co-transducible with the *rodA⁻* gene (Fig. 6; Table 3). The kinetic difference in the UV killing curves of strain AOS151 (Fig. 3) and strain AT1325-S2 (Fig. 6) could be due to the large difference in the genetic background of these strains. However, at present it cannot be explained why these morphological mutants are resistant to UV irradiation.

The *envB* mutant is resistant to phage P1 (17), but the *rodA* mutant strain AOS151 is sensitive to P1*kc* and all other phages and colicins tested, and its sensitivity to drugs is not grossly altered (Table 2). These results suggest that the lipopolysaccharide, which has roles in phage adsorption and antibiotic resistance of *E. coli* K-12 (21), of the mutant envelope is not changed. Normal symmetrical septum formation is observed in dividing cells of strain AOS151 (Fig. 2), unlike the *envB* mutant (17).

There have been several reports concerned with the cause of shape alteration of morphological mutants. Henning et al. have reported that the temperature-sensitive *rod* mutant of *E. coli* is traced to an alteration in the cytoplasmic membrane, and round cells do not differ measurably in peptidoglycan from rod-shaped cells (10). In the case of the *rod* mutant of *B. subtilis*, *tag-1*, it has been found that the round cells contain much less teichoic acid than the rod forms (4, 5), but a teichoic acid-deficient mutant of *B. subtilis* with a rod shape does exist (24). Chemical analysis of cell envelope alteration in the *rodA* mutant is now in progress.

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