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Relative Map Location of the *rep* and *rho* Genes of *Escherichia coli*

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The *rep* gene of *Escherichia coli* was mapped between *ilvC* and *rho* by three-factor P1 transductional crosses and also by complementation with a set of lambda transducing phages that contain known amounts of bacterial DNA linked to *ilvC*. The physical distance between *ilvC* and *rep* and between *rep* and *rho* were calculated with an accuracy of ±0.4 kilobase to be $0 \leq ilvC\text{-}rep \leq 3.4$ kilobases and $2.0 \leq rep\text{-}rho \leq 6.0$ kilobases. It was shown that *rho-15* is Gro$^+$ for phage ST-1. An *ilv*::Tn*10* mutation was located in *ilvY*.

The *rep* and *rho* genes of *Escherichia coli* K-12 are both closely linked to the *ilv* region at 84 min (2, 3, 16), but the relative order of the three genes has not been determined. The product of the *rep* gene is a 65,000- to 70,000-dalton DNA-unwinding protein (19–21) that is required for the replication of certain phages, including φX174 (6) and the unrelated P2 (2). The product of the *rho* gene is an approximately 48,000-dalton protein needed for transcription termination (5, 9, 17, 18). There are functional similarities between the *rep* and *rho* proteins: mutations in each gene increase the UV sensitivity of the cell (2, 3, 6–8), and both proteins have associated ATPase activities (14, 15, 19). Furthermore, the *rho-15* mutation confers a Gro$^-$ phenotype for phages P2, Mu, and T2 (4).

We determined the map order of the *ilv*, *rep*, and *rho* genes by three-factor P1 transductional crosses. The sources of the donors and recipients are listed in Table 1. Specifically, the order was obtained from the relative number of *rep* and *rep*$^+$ alleles among selected *ilv-rho* recombinant types (Table 2). The only order consistent with the distribution of the *rep* alleles is *ilv-rep-rho*.

An independent determination of the gene order was made possible by the availability of a series of λ *ilv* transducing phages that contain different extensions of bacterial DNA (Fig. 1). The presence of the *rep* and *rho* genes on a lambda phage was determined by transducing an *ilv*::Tn*10* mutant to Ilv$^+$ and then observing whether the Ilv$^+$ transductant showed complementation of the defective alleles *rep-71*, *rep-85*, and *rho-15* (Table 3). The critical results were provided by λd37, which was shown to contain the *rep* gene but not *rho*, and by λd22, which was shown to contain both the *rep* and *rho* genes. The order of the genes, therefore, must be *ilv-rep-rho* (Fig. 1), in agreement with the three-factor transductional crosses.

The plaque-forming transducing phage λp29, which also carries a functional *rep* gene (Table 3), enabled us to infer that the *ilv*::Tn*10* mutation (Table 1) is in *ilvY*. Tn*10* had been located to the right of an *ilvA* mutation by three-factor P1 transductional crosses involving the outside markers *rbs* on the left and *cya* on the right (M. Levinthal, personal communication). This places Tn*10* in either *ilvY* or *ilvC*; *ilvA* had been eliminated as the site because the *ilv*::Tn*10* strains are Val$^-$, whereas *ilvA*, which codes for threonine deaminase, is not needed for valine biosynthesis. We ruled out *ilvC* as the location by the fact that we could not transduce IT1110 to *ilv*$^+$ with λp29, which contains the *ilvC* gene (11, 23). (By transduction of IT1378 to *ilv*$^+$, we verified that our lysate of λp29 could indeed transduce the *ilvC* gene.) Thus, by elimination, the Tn*10* must be located in *ilvY*. This mutation, *ilvY864*::Tn*10*, is the only known Ilv$^-$ mutation within that gene of *E. coli*.

We can further infer that λp29 does not contain an entire functional *ilvY* gene inasmuch as the phage failed to transduce *ilvY*::Tn*10* to Ilv$^+$.

One can estimate the physical distance between *ilv* and *rep* and between *rep* and *rho*. We assumed that the *rep* gene is 1.8 kilobases long, and the *rho* gene 1.3 kilobases. Gray et al. (11) have localized *rho* within the *Hin*dIII fragment bordered by the left and right *Hin*dIII sites, H_L and H_R (Fig. 1), and further suggest that *rho* may contain the *Eco*RI site (designated E in Fig. 1). Thus, the right end of *rho* should be between E and H_R, which defines the limits for both ends of the *rho* gene (dotted box in Fig. 1). The *rep* gene is limited to the region between the right end of *ilvC* (0 kilobases on the map) and the right end of λd37. We conclude, therefore, that the distance

TABLE 1. Bacterial strains

Strain[a]	Genotype	Source (reference)
E. coli K-12		
RH54	F⁻ *asnB3 relA1 spoT1 asnA31 ilv-864*::Tn*10*	R. D. Simoni (12)
PS1079	HfrPO53 *ara-42 rbs-115 xyl-7 lacY1 mglP1*	M. Levinthal
PS1901	HfrPO53 *ara-42 xyl-7 lacY1 mglP1 ilv-864*::Tn*10*	M. Levinthal, PS1079 × P1 · RH54
PS1088	HfrPO53 *ara-42 xyl-7 lacY1 mglP1 ilvC462*	M. Levinthal
IT1011	F⁻ *his-871 relA1 rpsL181 gal-3*	SA1030 from A. Das (3)
IT1012	As IT1011, but *rho-15*	AD1600 from A. Das (3)
IT1022	As IT1011, but *ilvY864*::Tn*10*	This paper, IT1011 × p1 · PS1901
IT1018	As IT1012, but *ilvY864*::Tn*10*	This paper, IT1012 × P1 · PS1901
IT1378	As IT1012, but *ilvC462*	This paper, Rho⁺ from IT1012 × P1 · PS1088
E. coli C		
EST571	F⁻ *rep-71*	Cla, E. S. Tessman (22)
EST303	F⁻ *metE4 his rha ilv-4 rep-85*	C1412, E. S. Tessman (22)
E. coli C × *E. coli* K-12 hybrid		
EST572	As PS1088, but *rep-71*	E. S. Tessman, Ilv⁺ from PS1088 × P1 · EST571
IT1101	As IT1022, but *rep-71*	This paper, Ilv⁺ from IT1022 × P1 · EST572
IT1110	As IT1101, but *ilvY864*::Tn*10*	This paper, IT1101 × P1 · IT1022
IT1139	As EST303 but *ilvY864*::Tn*10*	This paper, EST303 × P1 · IT1022

[a] All strains are λ⁻.

between *ilvC* and *rep* is within the limits of 0 to 3.4 kilobases, and the distance between *rep* and *rho* is 2.0 to 6.0 kilobases, with an accuracy of approximately ±0.4 kilobase. An additional small uncertainty arises in the location of *rho* because it is not known whether the assay for rifampin resistance that was used (11) to prove that *rho* is contained within the *Hin*dIII fragment (Fig. 1) requires the presence of the entire *rho* gene.

Because of the reported Gro⁻ phenotype of *rho-15* for certain phages, we measured the

TABLE 2. Cotransduction by phage P1 *vir* of *ilv*, *rho*, and *rep*[a]

Cross	Recipient	Donor	Selected genotype[b]	Rep[c] +	Rep[c] −
I	*ilv rep-71 rho*⁺	*ilv*⁺ *rep*⁺ *rho-15*	*ilv*⁺ *rho*⁺	25	35[d]
II	*ilv rep-71 rho*⁺	*ilv*⁺ *rep*⁺ *rho-15*	*ilv*⁺ *rho-15*	35	0[e]
III	*ilv rep*⁺ *rho-15*	*ilv*⁺ *rep-71 rho*⁺	*ilv*⁺ *rho*⁺	0	29

[a] Transduction with P1 *vir*, which had been irradiated with UV light at 250 J/m² to prevent infection with viable phage, was performed by standard techniques. The recipient strains were IT1110 (*ilv rep-71 rho*⁺) and IT1018 (*ilv rep*⁺ *rho-15*); the donors were IT1012 (*ilv*⁺ *rep*⁺ *rho-15*) and IT1101 (*ilv*⁺ *rep-71 rho*⁺). The *ilv*⁺ colonies were selected by their growth on minimal glucose plates at 30°C, and at the same time the *rho* alleles were selected on the same plates by colony size (*rho*⁺, large; *rho-15*, small). The *rho* genotype was confirmed in every case by the colony color on MacConkey-galactose plates (*rho*⁺, white; *rho-15*, red). The only phenotype scored was Rep, which gave the distribution of *rep* alleles for each selected genotype.

[b] The *rho* allele is about 65% cotransduced with *ilv*.

[c] Phage ST-1 was used to test the Rep phenotype because φX174 does not normally adsorb to K-12 strains. We have found that ST-1 has the same inability to grow on Rep⁻ strains as φX174, to which it is related.

[d] The observed number was 45, but it was corrected for the frequency of spontaneous *ilv*⁺ revertants observed in the recipient.

[e] We have not shown that the *rep-71 rho-15* combination is viable. That does not, however, alter the conclusion derived from all three crosses that *rep* is between *ilv* and *rho*.

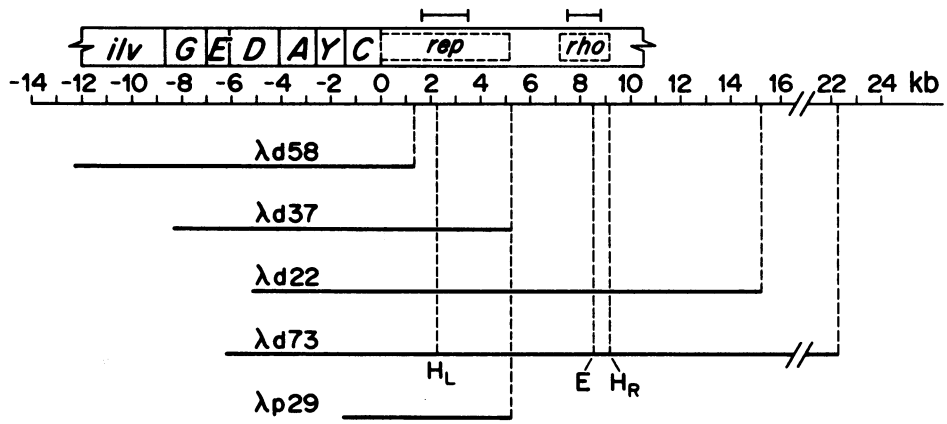

FIG. 1. Physical extent of the bacterial fragments contained within the λ *ilv* transducing phages λd58, λd37, λd22, λd73, and λp29 isolated by P. Jørgensen (1, 13). The defective phages were obtained from D. Calhoun via H. E. Umbarger, and the plaque-forming λp29 was from M. Uzan. All were in the form of dilysogens. We separated λp29 from the unneeded helper phage by transduction of IT1378 to Ilv$^+$ with a single-plaque lysate. The locations of the fragments (10) agree with independent observations of Uzan et al. (23), except that Uzan et al. found that their strain of λd73 has an unusual rearrangement of the bacterial genes on the phage chromosome. The 0-kilobase point is assigned to the end of the *ilvC* gene. H and E mark *Hin*dIII and *Eco*RI restriction sites, respectively. The dashed-line boxes show the limits to the possible locations of the *rep* and *rho* genes, whereas the bars centered above show the approximate sizes of the genes.

TABLE 3. Transductional capacity of the λ *ilv* phages[a]

Phage[b]	Complementation[c]		
	rep-71	*rep-85*	*rho-15*[d]
λd58	−	NT	−
λd37	+	+	−
λd22	+	NT	+
λd73	+	NT	+
λp29	+	NT	NT

[a] The *ilv* strains carrying *rep-71* (IT1110), *rep-85* (IT1139), and *rho-15* (IT1018) were transduced with the λ *ilv* phages, and *ilv*$^+$ lysogens were selected. These merodiploids were examined for the ability of the prophage genes to complement the bacterial *rep* or *rho* mutation. The Rep$^+$ phenotype was recognized by the ability of the transductant to support growth of the appropriate phage (ST-1 for IT1110, φX174 for IT1139). In the case of λp29 complementation of the *rep-71* mutation was also tested by coinfection with λp29 and ST-1. The Rho phenotypes were distinguished by the colony color on MacConkey-galactose plates and also by the ability of the cells to grow on plates containing mitomycin C (Sigma Chemical Co., lot 41F-0240). We observed that IT1018 (*rho-15*) grows marginally on plates containing 0.05 μg of mitomycin per ml and not at all on plates containing 0.1 μg of mitomycin per ml, the amount we used. IT1022 (*rho*$^+$) grows well on plates containing even 0.5 μg of mitomycin per ml.

[b] Lysates of λd58, λd37, and λd22 were prepared by heat induction of *E. coli* strains L58, L37, and L22, respectively (1), which were lysogenic for both the defective phage and a helper phage. The λd73 lysate was prepared from a monolysogen with the aid of a plasmid (gift of N. Sternberg) containing the lambda genes missing from the defective phage. No helper phage or plasmid was required for the preparation of λp29 lysates.

[c] The *rep-71* complementation results with λd58, λd37, λd22, λd73, and λp29 were based on an examination of 5, 4, 5, 2, and 2 lysogens, respectively, and in every case the merodiploids were either all wild type (+) or all mutant (−) in phenotype. Thus, a positive result indicates that the phage contains the entire functional gene. Complementation of *rep-71* by λp29 was also inferred from the ability of ST-1 to grow in IT1110 coinfected with λp29. The complementation proves *Rep*$^+$ is dominant for *rep-71* and *rep-85*. Each *rho-15* complementation result was based on an examination of at least 10 lysogens. NT, Not tested.

[d] The presence or absence of the *rho*$^+$ gene on each phage agreed with the observations of Gray et al. (11) and, excepting λd73 (see legend to Fig. 1), also with the observations of Uzan et al. (23).

ability of phage ST-1 to grow on strains carrying *rho-15*. The burst size of ST-1 in IT1012 at 39°C was 68, which was 80% of the burst size in the *rho*$^+$ parent (IT1011); at 39°C, IT1012 itself cannot grow. Thus, for this phage, *rho-15* does not have a Gro$^-$ or Rep$^-$ phenotype.

The mapping of *rep* with respect to *ilv* and *rho* and the localization of the gene within the bacterial DNA of the lambda transducing phages should be useful in subcloning the *rep* gene. This would be helpful for further exploration of the function of the *rep* protein. The mapping also

provides landmarks for the localization of other genes (11) that appear to be in this region.

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