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Lethality of the Double Mutations *rho rep* and *rho ssb* in *Escherichia coli*

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The similarity of *rho* mutants to *rep* and *ssb* mutants in sensitivity to UV light and in recombination deficiency suggested that the function of the Rho protein might be related to that of Rep and Ssb. In support of that idea, we found that *rho rep* and *rho ssb* double mutants are either nonviable, or at best only marginally viable. Viability could be restored by suppressor mutations, one of which mapped either in the *rho* gene or close to its 5′-end. Rho may thus share a role with Rep and Ssb in replication and the structural maintenance of DNA; a multifunctional Rho protein could account for the diversity of the defects seen in *rho* mutants, some of which appear to have no relation to the defect in transcription termination.

Although isolated as suppressors of transcription termination, *rho* mutants often display an extensive array of other defects. The widely studied *rho* mutation, *rho-15* (6), is the most highly pleiotropic *rho* mutation known (7). In addition to its defect in transcription termination, the *rho-15* mutant is also temperature sensitive (6), rifampin supersensitive (14), radiation sensitive (6, 11), recombination deficient (6), defective in plasmid maintenance (1), defective in lysogenization by phages lambda and P1 (6), defective in the use of carbon sources such as succinate that are intermediates of the tricarboxylic acid cycle (7), Gro$^-$ for phages T2, P2, and Mu (7), able to hyperdegrade proteins (30a), and impaired in motility (S. Garges and S. Adhya, Abstr. Annu. Meet. Am. Soc. Microbiol., 1982, H16, p. 115). The defect in transcription termination may be the primary cause of the many phenotypic effects of *rho* mutations, but that has not been demonstrated. Alternatively, Rho may participate directly in several vital cellular processes.

The *rho-111* mutation of *Salmonella typhimurium* was isolated, like *rho-15*, as a suppressor of the IS2 termination signal in *gal-3*, and like *rho-15* it confers a temperature-sensitive phenotype upon the cell (15). Since *rho-15* and *rho-111* suppress the same strong termination signal to about the same extent, they presumably are about equally defective in transcription termination. However, they are not similarly defective in other respects. The *rho-111* mutant differs from *rho-15* in that it is not sensitive to UV light, is capable of growth on succinate as the sole carbon source, and is able to maintain plasmids normally (15). It therefore appears that the termination defectiveness of a *rho* mutation does not necessarily determine the nature of the other defects.

Another indication that the termination defect may not be related to some of the other Rho$^-$ defects is the phenotype of the *rho-15 rpoB101* double mutant (8). The *rpoB101* mutation was isolated as a suppressor of a *rho-15* defect, rifampin supersensitivity. The double mutant is termination proficient as well as Rif^r. Significantly, however, the strain retains other Rho-15 phenotypes (UV^s, Ts, Suc$^-$, and Gro$^-$ were noted [8]), again showing that there is no clear correlation between the magnitude of the termination defect and the scope of the pleiotropy. The differences in phenotypes among *rho* mutants might therefore have to be accounted for in a way that is not directly related to the transcription-termination defect. That Rho may have another function in the cell is also suggested by the fact that although the *rho* gene is essential for survival (6, 16), *rho* mutants severely defective in transcription termination are nevertheless viable.

In an attempt to identify another role of Rho in cellular metabolism that might account for the highly pleiotropic effects of *rho* mutations, the *rho-15* mutation was combined with mutations in the *rep* and *ssb* genes. Both the *rep* and *ssb* genes encode DNA replication proteins. The product of the *rep* gene is a DNA helicase which unwinds duplex DNA in an ATP-dependent reaction (17, 26). The *rep* protein is not essential for cell viability. The product of the *ssb* gene (12) is the major single-stranded DNA binding protein (30) of *Escherichia coli*. It is essential for cell viability and is important in repair of DNA (12, 34). The double-mutant combinations were expected to be revealing because *rep* and ssb mutations confer some phenotypic effects similar to those of *rho* mutations, notably UV sensitivity (5, 9, 12) and recombination deficiency (10, 12, 36). Both the *rho rep* and the *rho ssb* combinations turned out to be lethal or almost so. Rho may therefore be involved in some general way in the same functions as Rep and Ssb, for example, replication and the structural maintenance of DNA.

(A preliminary presentation of this work has been made [J. S. Fassler, I. Tessman, and E. S. Tessman, Abstr. Meet. Phage Bacterial Regulatory Mech., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1983, p. 137]).

MATERIALS AND METHODS

Bacterial strains. The strains used in this study are listed in Table 1. The recipients for all transductions described below were derived from SA1030 (*his-871 relA1 rpsL181 gal-3*).

P1 transductions. P1 transductions were performed by standard techniques; the phage were irradiated with a fluence of 40 J/m^2 to minimize infection with live phage. LB plates, LB plates containing 25 μg of tetracycline per ml (LB-Tet), or minimal A plates (20) supplemented with histidine, arginine, and methionine and containing either glucose or maltose were used to select, respectively, Ts$^+$ (Rho$^+$), Tet^r, Ilv$^+$, or Mal$^+$ (Cya$^+$). Selection for Ts$^+$ was performed at 42°C; otherwise, transduction plates were incubated at 30°C.

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TABLE 1. Bacterial strains

Strain	Relevant genotype	Source (reference)
E. coli K-12[a]		
IT1011	*gal-3*	SA1030 from A. Das (6)
IT1012	*gal-3 rho-15*	AD1600 from A. Das (6)
IT1018	*gal-3 rho-15 ilvY864*::Tn*10*	Our collection (35)
IT1021	*gal-3 rho-15 ilvY864*::Tn*10*	Independent isolate of IT1018
IT1022	*gal-3 ilvY864*::Tn*10*	Our collection (35)
IT1036	*gal-3 rho-15*	IT1022 × P1(IT1012)
IT1037	*gal-3 rho-15*	Independent isolate of IT1036
IT1069	Δ*cya*	CA8306 from P. Swenson (32)
IT1101	*gal-3 rep-71*	Our collection (35)
IT1110	*gal-3 ilvY864*::Tn*10 rep-71*	Our collection (35)
IT1520	*gal-3* Δ*cya*	IT1036 × P1(IT1069) (this paper)
IT1528	*gal-3* Δ*cya rho-15 ilvY864*::Tn*10*	IT1520 × P1(IT1018) (this paper)
IT1530	*gal-3 ssb-113*	Tet[s] derivative of IT1534[b] (this paper)
IT1534	*gal-3 ssb-113 zjb*::Tn*10*	IT1011 × P1(JGC248) (this paper)
IT1551	*gal-3 ilvY864*::Tn*10 rep-71 rho-15*(s1)[c]	IT1036 × P1(IT1110) (this paper)
IT1555	*gal-3 ilvY864*::Tn*10 rep-71* Δ*cya*	IT1528 × P1(IT1101) (this paper)
JGC248	*ssb-113 zjb*::Tn*10*	R. Meyer (12)

[a] The *rep-71* mutation was introduced from *E. coli* C.
[b] Procedure of Maloy and Nunn (19).
[c] s1, Partially suppressed *rho-15* mutant described further in the text.

Mutant scoring. Rep⁻ mutants of *E. coli* K-12 were recognized by their being Gro⁻ for phage St-1 (35). For our purposes, the defect in transcription termination defined the Rho⁻ character, which was therefore recognized in a *gal-3* background by the red color of the colonies on MacConkey-galactose plates. The *gal-3* mutation consists of an IS2 element encoding a transcription termination signal in the leader of the *gal* operon; the *rho-15* mutation produces a Gal⁺ phenotype by permitting transcriptional readthrough (6). In addition, the *rho-15* mutation makes cells Ts at 42°C on LB plates, mitomycin sensitive (0.25 μg/ml), and distinctively slow growing (a generation time roughly three times that of *rho*⁺). Δ*cya* mutants were recognized as white colonies on MacConkey-maltose plates, whereas *cya*⁺ colonies were red. *ssb-113* mutants were identified by their sensitivity to 0.5 μg of mitomycin C per ml.

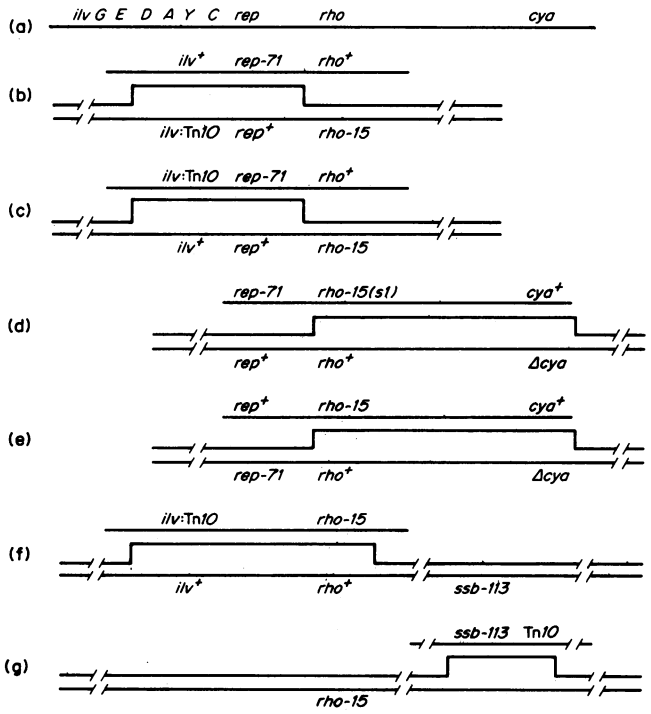

FIG. 1. Transductional crosses used in attempts to form *rho rep* and *rho ssb* double mutants. (a) Map order of *ilv-cya* region (35); (b) and (c) formation of *rho rep* recombinant; (d) separation of *rho-15*(s1) from *rep-71*; (e) formation of *rho rep* recombinant; (f) and (g) formation of *rho ssb* recombinant. ——/ /——, Extended region.

Media. LB plates (20) contained 5 g of NaCl per liter instead of 10 g per liter. TB contained 13 g of Difco tryptone and 7 g of NaCl per liter.

RESULTS

Inability to introduce the *rep-71* allele into a *rho-15* recipient. The *rep* and *rho* genes are linked (35) (Fig. 1a). The construction of *rep-71* and *rho-15* double mutants was attempted by P1 transduction of *rep-71* into a *rho-15* recipient (Table 2). In the first cross, the introduction of *rep-71* into a *rho-15* strain was attempted by linkage with *ilv*⁺ (Fig. 1b). Among 33 Ilv⁺ colonies selected on minimal plates, all were of the same class, Rep⁺ Rho⁻. In the control transduction with an isogenic *rho*⁺ recipient, 10 of 23 Ilv⁺ transductants were Rep⁻.

In a second type of P1 transduction (Table 2, cross 2) that was designed to construct a *rep-71 rho-15* double mutant, *rep-71* was closely linked in the donor strain to a Tn*10* element in *ilvY* so that *rep-71* could be selected by cotransduction with Tet[r] (Fig. 1c). Twenty-three Tet[r] transductant colonies were picked randomly and characterized. Of these, 15 were Rep⁺ Rho⁻ and 2 were Rep⁻ Rho⁺. Curiously, in another four, no live cells could be recovered from the colonies picked off the original LB-Tet transductant plates. The two remaining transductants were Rep⁻ Rho⁻, but in both cases the Rho⁻ phenotype was not fully characteristic of *rho-15*, which is Ts at 42°C and makes the *gal-3* strain form red colonies on MacConkey-galactose plates. One transductant made red colonies but was Ts⁺. The other was Ts, but isolated colonies that formed on MacConkey-galactose plates were pink rather than the red characteristic of *rho-15*.

TABLE 2. Transduction from *rep-71* donors into *rho-15* recipients[a]

Cross[b]	Recipient	Donor	Selected phenotype	No. of scored phenotypes[c]				
				Rep$^+$ Rho$^-$	Rep$^-$ Rho$^+$	Rep$^+$ Rho$^+$	Rep$^-$ Rho$^-$	Dead
1	*ilvY*::Tn*10 rep*$^+$ *rho-15*	*ilv*$^+$ *rep-71 rho*$^+$	Ilv$^+$	33	0	0	0	0
1C	*ilvY*::Tn*10 rep*$^+$ *rho*$^+$	*ilv*$^+$ *rep-71 rho*$^+$	Ilv$^+$	—[d]	10	13	—	—
2	*ilv*$^+$ *rep*$^+$ *rho-15*	*ilvY*::Tn*10 rep-71 rho*$^+$	Tet^r	15	2	0	2	4
2C	*ilv*$^+$ *rep*$^+$ *rho*$^+$	*ilvY*::Tn*10 rep-71 rho*$^+$	Tet^r	—	8	3	—	—

[a] The strains used were IT1021 (*ilvY*::Tn*10 rep*$^+$ *rho-15*), IT1101 (*ilv*$^+$ *rep-71 rho*$^+$), IT1022 (*ilvY*::Tn*10 rep*$^+$ *rho*$^+$), IT1036 (*ilv*$^+$ *rep*$^+$ *rho-15*), IT1110 (*ilvY*::Tn*10 rep-71 rho*$^+$), IT1011(*ilv*$^+$ *rep*$^+$ *rho*$^+$).
[b] C, Control cross.
[c] Rep$^-$ if Gro$^-$ for phage ST-1; Rho$^-$ colonies were red on MacConkey-galactose plates.
[d] —, Not relevant.

The transduction therefore yielded no live *rep-71 rho-15* combination that had the properties expected of *rho-15*. The transduction was also noteworthy in the reduced frequency of cotransduction of Rep$^-$ with Tet^r. Among live transductants, Rep$^-$ was cotransduced with Tet^r in 21% (4/19) of the cases whereas in the control experiment (cross 2C), in which the recipient as well as the donor was *rho*$^+$, *rep-71* was cotransduced with Tet^r in 73% (8/11) of the cases, a significant difference ($P = 0.05$). Dead colonies may well contain *rep-71*. In cross 2 it turned out to be possible to enrich for Rep$^-$ colonies by picking only exceptionally small transductant colonies from the LB-Tet transduction plates. Among 23 Tet^r colonies selected in this way, 3 yielded no live cells and 17 others were Rep$^-$. Of the 17 Rep$^-$, 14 appeared to be Rep$^-$ Rho$^+$; the remaining 3 might be loosely classed as Rep$^-$ Rho$^-$, but in each case the Rho$^-$ phenotype was not that of *rho-15*. One of the three, though forming characteristically red colonies (Gal$^+$) on MacConkey-galactose plates like *rho-15*, was nevertheless Ts$^+$; this isolate is referred to as IT1551 (Table 1) and its further characterization is described below. Another Rep$^-$ Rho$^-$ isolate was Ts but was pink rather than red on MacConkey-galactose plates, whereas the third was Ts but was white on MacConkey-galactose plates; these isolates were not further characterized. Cross 2 was repeated several more times, but among an additional 68 Tet^r transductants that were screened, no Rep$^-$ Rho$^-$ transductants with specifically *rho-15* characteristics were found (see Table 3).

These results can be summarized as follows. (i) The *rep-71 rho-15* double mutant did not appear in the crosses in which *rep-71* transductants were selected by linkage to *ilv*$^+$. (ii) A *rep rho* double mutant was rare when *rep-71* was selected by linkage to *ilv*::Tn*10*. (iii) The rare *rep rho* double mutants appeared to contain suppressors because they showed a variety of phenotypes, none of which corresponded completely to that of *rho-15*; it will be seen below that in one case studied further (IT1551) the suppressor mutation was found to be closely linked to the *rho* gene. (iv) On LB-Tet plates some transductional colonies consisted of dead cells; it is possible that these colonies originated as *rep-71 rho-15* double mutants.

TABLE 3. Distribution of transductant phenotypes in four independent transductions of a *rho-15* recipient by *rep-71* donors

Recipient[a]	Donor[b]	No. of selected Tet^r transductants	No. of scored phenotypes[c]				
			Rep$^+$ Rho$^-$	Rep$^-$ Rho$^+$	Rep$^+$ Rho$^+$	Rep$^-$ Rho$^-$	Dead
IT1036	IT1110-1	23[d]	15	2	0	2	4
IT1037	IT1110-1	21	20	1	0	0	0
IT1037	IT1110-2	21	7	10	1	3	0
IT1037	IT1110-3	26	9	15	2	0	0

[a] Strains IT1036 and IT1037 are independent *rho-15 rep*$^+$ isolates from the transduction described for IT1036 in Table 1.
[b] Strain IT1110 is *ilvY*::Tn*10 rep-71 rho*$^+$; -1, -2, -3 designate specific P1 lysates made on that strain.
[c] None of the five Rep$^-$ Rho$^-$ transductants shows the characteristic Rho-15 phenotype, namely red colonies on MacConkey-galactose and Ts at 42°C.
[d] Corresponds to cross 2 of Table 2.

Variability in frequency of *rep-71 rho*$^+$ transductants. In our experiments that showed that the *rep-71 rho-15* combination is nonviable, an unexpected variability was found in the frequency of cotransduction between *ilv*$^-$ and *rho*$^+$ that does not affect our conclusion but deserves some elaboration. For transductions in which the donor was *ilvY*::Tn*10 rep*$^+$ *rho*$^+$ and the recipient was *ilv*$^+$ *rep*$^+$ *rho-15*, the selected Tet^r transductants were Rho$^+$ in about 50% of the cases. The results, however, varied considerably when the donor contained the *rep-71* allele. In the first cross shown in Table 3, which is carried over from Table 2, only 2 of 23 Tet^r transductants were Rho$^+$. When this cross was repeated with the same P1 lysate (row 2), cotransduction of Rho$^+$ with Tet^r remained low (1 of 21). However, when the cross was repeated with two additional P1 lysates prepared on the same *rep-71* donor strain as the first lysate, Rho$^+$ was cotransduced with Tet^r in approximately 50% of the cases.

Similarly, P1 lysates grown on this donor varied in their frequency of Ilv$^+$ Rho$^+$ cotransduction. In cross 1 of Table 2, none of 33 Ilv$^+$ transductants were Rho$^+$. However, two other P1 lysates grown on strain IT1101 resulted in the 50% cotransduction frequency expected between *ilv* and *rho* (data not shown).

Despite this curious P1 lysate-dependent variation in the linkage between the *rho* and *ilvY* genes in transductions from *ilvY*::Tn*10 rep-71 rho*$^+$ and *ilv*$^+$ *rep-71 rho*$^+$ donors, it is clear from the control crosses that the lysates shown in Table 2 had the ability to cotransduce the *ilv* and *rep* genes that would have been necessary to introduce *rep-71* into a *rho-15* recipient.

Altered *rho-15* in rare *rep rho* double mutants. Crosses like cross 2 of Table 2 showed rare examples of Rep$^-$ Rho$^-$ transductants. As mentioned above, in these transductants the Rho$^-$ phenotype was always different in some respect from that of *rho-15*. Conceivably some of these were genuine *rep-71 rho-15* double mutants in which the presence of the *rep-71* allele altered the expression of the *rho-15* mutation. That was unlikely because of the variety of Rep$^-$ Rho$^-$ phenotypes found. More likely was the possibility that suppressor mutations arose that enabled the *rep-71 rho-15* combination to be viable. The following transduction experiment demonstrated that a suppressor closely linked to *rho-15* did indeed account for the altered Rho$^-$ phenotype in a Rep$^-$ Rho$^-$ double mutant.

TABLE 4. Cotransduction of *cya*, *rho*, and *rep*[a]

Cross	Recipient	Donor	Selected phenotype	Rho		Rep	
				+	−	+	−
1	*rep-71 rho*$^+$ Δ*cya*	*rep*$^+$ *rho*$^+$ *cya*$^+$	Cya$^+$	NT[b]	NT	0	18
2	*rep*$^+$ *rho-15* Δ*cya*	*rep-71 rho*$^+$ *cya*$^+$	Cya$^+$	30	90	NT	NT
3	*rep*$^+$ *rho*$^+$ Δ*cya*	*rep*$^+$ *rho-15 cya*$^+$	Cya$^+$	37	11	NT	NT
4	*rep-71 rho*$^+$ Δ*cya*	*rep*$^+$ *rho-15 cya*$^+$	Cya$^+$	72	0	1	47

[a] The strains used were IT1555 (*rep-71 rho*$^+$ Δ*cya*), IT1022 (*rep*$^+$ *rho*$^+$ *cya*$^+$), IT1528 (*rep*$^+$ *rho-15* Δ*cya*), IT1101 (*rep-71 rho*$^+$ *cya*$^+$), and IT1018 and IT1021 (*rep*$^+$ *rho-15 cya*$^+$). Strain IT1018 was the donor for 24 transductants in cross 4, and IT1021 was the donor for the remaining 48, which were also examined for their Rep phenotype. IT1021 was the donor in cross 3. All strains contained *gal-3*, which provided the test for the *rho* allele.
[b] NT, Not tested.

A P1 transduction was designed to retrieve the *rho* gene from the Rep$^-$ Rho$^-$ double mutant IT1551 (Gal$^+$ Ts$^+$). This strain served as the donor for introducing the *cya*$^+$ gene into IT1520 (*rep*$^+$ *rho*$^+$ Δ*cya*) (Fig. 1d). We selected 37 Mal$^+$ transductants and scored for their Rho phenotype on MacConkey-galactose plates at 35°C and LB plates at 42°C. Of the 37, 6 were Gal$^+$ (Rho$^-$); these were all Rep$^+$, and so the *rep-71* allele could not be responsible for their phenotypes. One of the six was Ts$^+$ like the IT1551 donor, implying that the suppressor mutation was linked to, and possibly in, the *rho* gene itself; five of the six were Ts like *rho-15*, which indicated that the suppressor mutation was distal to the *cya* gene. If the suppressor mutation in strain IT1551 is indeed in the *rho* gene, then it would be at the 5'-end relative to *rho-15*, inasmuch as the gene is transcribed from left to right in Fig. 1 (4). The example of strain IT1551 reinforces the conclusion that the combination of the original *rep-71* and *rho-15* mutations is not viable unless a suppressor mutation is present.

Inability to introduce the *rho-15* allele into a *rep-71* recipient. The apparent incompatibility of *rep-71* and *rho-15* was further confirmed by attempts to cotransduce *rho-15* and *cya*$^+$ into a *rep-71* Δ*cya* recipient (Fig. 1e). Although *rep* was rarely (≤6%) cotransduced with *cya* (Table 4, cross 1), *rho* was normally cotransduced about 25% of the time with *cya*, regardless of the *rho* allele (Table 4, crosses 2 and 3). However, when the donor contained *rho-15* and the recipient contained *rep-71* (Table 4, cross 4), none of the 72 Cya$^+$ (Mal$^+$) transductants examined was Rho$^-$, implying that the *rho-15* allele could not be combined with *rep-71*. However, when the selection in cross 4 was biased in favor of exceptionally small Cya$^+$ colonies, 7 of 62 such nonrandomly chosen transductants were found on MacConkey-galactose plates to be Gal$^+$, i.e., Rho$^-$. However, these exceptions proved the rule, as they were all Rep$^+$; they showed that cotransduction of the distantly linked *cya* and *rep* alleles, though rare, was still more frequent than the survival of *rep-71 rho-15* transductants. Furthermore, of seven rare Rep$^+$ Rho$^-$ transductants isolated, three did not exhibit the Ts phenotype of the *rho-15* mutant. This suggests that introduction of the *rho-15* allele into a cell initially containing the *rep-71* allele is aided by, and possibly even requires, a *rho-15* suppressor.

TABLE 5. Transductions to construct *rho-15 ssb-113* double mutants[a]

Cross	Recipient	Donor	Selected phenotype	No. of scored phenotypes	
				Rho$^+$	Rho$^-$
1	*gal-3 ilv*$^+$ *rho*$^+$ *ssb-113*	*ilvY*::Tn*10 rho-15*	Tet^r	48[b]	0
1C	*gal-3 ilv*$^+$ *rho*$^+$ *ssb*$^+$	*ilvY*::Tn*10 rho-15*	Tet^r	13	12
				Ssb$^+$	Ssb$^-$
2	*gal-3 rho-15 ssb*$^+$	*ssb-113 zjb*::Tn*10*	Tet^r	7	5
2C	*gal-3 rho*$^+$ *ssb*$^+$	*ssb-113 zjb*::Tn*10*	Tet^r	5	19

[a] Strains used were IT1530 (*gal-3 ssb-113*), IT1018 (*ilvY*::Tn*10 rho-15*), IT1011 (*gal-3*), IT1037 (*gal-3 rho-15*), and JGC248 (*ssb-113 zjb*::Tn*10*).
[b] Proof that these have a *rho*$^+$ genotype was established in 10 cases by transduction, as described in the text.

Incompatibility of *ssb-113* and *rho-15*. Attempts were also made to construct an *ssb-113 rho-15* double mutant by P1 transduction. In one set of experiments *rho-15* was in the donor and *ssb-113* was in the recipient (Fig. 1f). The recipient also contained *gal-3* to provide a phenotypic test of the presence of *rho-15*. It was seen (Table 5) that *rho-15* could be cotransduced with *ilvY*::Tn*10* in about 50% of the cases when the recipient was *ssb*$^+$ (cross 1C), but no cotransduction of *rho-15* was observed among 48 Tet^r transductants when the recipient was *ssb-113* (cross 1). However, we considered the possibility that *ssb-113* might suppress the Rho-15 phenotype and tested 10 of those 48 Rho$^+$ transductants for the hidden presence of the *rho-15* allele. Each served as donor in 10 independent P1 transductions into the recipient strain IT1011 (*gal-3 rho*$^+$). In each cross, 24 Tet^r transductants were selected and scored for the Rho phenotype on MacConkey-galactose plates. No red colonies were seen, proving that all 10 of the original Rho$^+$ transductants were genetically *rho*$^+$. We therefore conclude that *rho-15* could not be introduced into an *ssb-113* strain.

In a second set of experiments, *ssb-113* was linked to *zjb*::Tn*10* in the donor and *rho-15* was in the recipient (Fig. 1g). In these experiments it was not clear in advance whether the *rho-15 ssb-113* double mutant could be distinguished from the *rho-15* recipient, since *ssb* and *rho* mutants have similar phenotypes with respect to radiation sensitivity. Two classes of transductants were obtained (Table 5). The first class, Ssb$^+$ Rho$^-$, was in all respects indistinguishable from a *rho-15* mutant. The second class, Ssb$^-$ Rho$^-$, grew poorly in growth conditions known to be suitable for the growth of *rho-15* (Table 6). Therefore, although it appears that the Ssb$^-$ Rho$^-$ combination could be constructed, these transductants in fact showed severe growth defects relative to the single mutants.

It is possible that *ssb-113* and *rho-15* are actually completely incompatible and that the sickly double-mutant transductants survived only because of suppressor mutations. The Ssb$^-$ Rho$^-$ cells changed in just one passage in TB at 30°C to stationary phase, starting from a small inoculum. The original transductant colonies did not grow when directly streaked on MacConkey-galactose plates (Table 6),

TABLE 6. Plating properties of Ssb Rho transductants

Plate	Tet^r transductants[a]		
	Ssb$^+$ Rho$^-$	Ssb$^-$ Rho$^+$	Ssb$^-$ Rho$^-$
MacConkey-galactose	+[b]	+[c]	No growth
LB-Tet	+	+	Poor growth
Salt-free LB	+	+	No growth

[a] Ssb$^+$ Rho$^-$ and Ssb$^-$ Rho$^-$ were from Table 5, cross 2; Ssb$^-$ Rho$^+$ was from cross 1, except for the test on MacConkey plates for which IT1530 was substituted at a later time.
[b] Red colonies.
[c] White colonies.

but after the one passage in TB they did grow on the MacConkey plates; however, the colonies were pink rather than the red of the *rho-15* recipient strain, suggesting that the defective *rho* gene was changed in one passage when in the presence of *ssb-113*. Therefore, although the *ssb-113 rho-15* combination is at best marginally viable, its ultimate survival may require the acquisition of compensating mutations.

DISCUSSION

By using the alleles *rho-15*, *rep-71*, and *ssb-113* we attempted to isolate *rep rho* and *ssb rho* double mutants. Our inability to isolate the *rep-71 rho-15* double mutant implies that the combination is lethal. This was confirmed by the fact that the very few Rep$^-$ Rho$^-$ recombinants that were found had altered Rho-15 phenotypes and one that was examined further had a suppressor mutation close to, and probably in, the *rho* gene. Suppressor mutations may well preexist in the *rep*$^+$ *rho-15* parent, inasmuch as *rho-15* cultures, possibly because of the slow growth rate, accumulate suppressors rapidly.

The *ssb-113* and *rho-15* alleles also appeared to be incompatible. Efforts to introduce *rho-15* into an *ssb-113* recipient failed. The reverse cross, in which *rho-15* was the recipient, did produce Ssb$^-$ Rho$^-$ recombinants, but these had severe growth defects compared with the single mutants and might have survived only because of suppressors. We do not know why the results depended on the direction of the transductional cross, but it is possible that only when the recipient is *rho-15* does it sometimes contain extragenic suppressors that may be needed for the initial transductant to survive, however poorly.

The incompatibility of *rho-15* with *ssb-113* and *rep-71* indicates a functional relationship between Rho and both Ssb and Rep. Ssb is a moderately abundant replication protein (30) whose major known activity is that of binding to single-stranded DNA. Rep is also a replication protein that binds to single-stranded DNA (17), but it exists in only a few copies per cell (27). Rep works catalytically, energized by DNA-dependent hydrolysis of ATP, to separate the strands of duplex DNA (26, 27). Rho, an abundant protein (3, 28), binds like Rep and Ssb to single-stranded DNA and also binds to double-stranded DNA (2, 13, 21–23); the biological function of the DNA binding is not known. It too has an ATPase activity, but it depends on RNA rather than DNA (18). The fact that a *rep ssb* mutant combination is conditionally lethal (34) strengthens the argument that the three gene products are functionally related. (In contrast, the *rho-15 recA56* combination is viable [unpublished data].)

What function does Rho share with Rep and Ssb that is essential for the survival of the cell? Ever since the Rho protein was discovered by Roberts (25), it has been thought that its primary, if not sole, function is to effect transcription termination, and it is that function that has served here to define the Rho phenotype. It is conceivable that Rep and Ssb play a role in some aspect of that function, inasmuch as transcription termination is likely to be influenced by subtle changes in DNA conformation.

The obverse view, however, may be more promising: Rho could have a role in the cell that is similar to those known for Rep and Ssb, namely replication and determination of DNA conformation. A multifunctional Rho protein could account for the fact that several of the phenotypic effects of *rho* mutations described above, such as UV^s, Suc$^-$, Gro$^-$, and defects in plasmid maintenance are not always correlated with the defect in transcription termination. The binding of Rho to single-stranded DNA in addition to its binding to RNA provides a basis for another function that could account for the relation of Rho to Rep and Ssb.

How might Rho be related to Rep and Ssb so that although the single mutations are viable the double mutations are lethal? Overlapping functions could account for this. An instructive example of such a relation is suggested by the lethal combination of *rep* and *uvrD* mutations, both genes coding for a helicase, either one of which alone is apparently able to unwind the DNA helix, despite a defective partner (33). The possibility of a physical interaction of Rho with Rep or Ssb should also be considered. A physical interaction has already been proposed between Rho and the β subunit of RNA polymerase to explain the allele-specific suppression of a *rho* mutation by an *rpoB* mutation (8); that interaction was invoked in part because of its consistency with a theory, originally proposed by Richardson et al. (24), that Rho-dependent transcription termination involves the binding of Rho to RNA polymerase. It should now be apparent, however, that if Rho were indeed capable of altering the conformation of DNA, then a *rho* mutation could indirectly alter the function of the other proteins without the need for a direct physical interaction.

The search for lethal mutant combinations like those involving *rho-15* provides a straightforward genetic approach for revealing the existence of functional relationships between proteins without first requiring a detailed biochemical understanding of the functions. If we include the lethal pair *polA uvrD* (29, 31) with the mutant combinations *uvrD rep*, *rep ssb*, *rep rho*, *ssb rho*, and *rho rpoB*, then we have six proteins involved in the replication and transcription of DNA that are linked by a chain of genetic evidence suggesting intricate interrelationships among these proteins which remain to be explored biochemically.

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