Polar Mutations in the Left Arm of Bacteriophage λ i434

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By studying complementation between frameshift and nonsense mutants located in the structural genes for the head of bacteriophage λ i434, we found mutations in gene B which are polar on genes C and D and one mutation in gene E which is polar on gene F.

It is well known that nonsense and frameshift mutations can have a polar effect on the expression of distal genes in the same operon. In both cases, the polarity can be attributed to the generation of a nonsense triplet either as a direct result of the mutation or indirectly as a consequence of the frameshift (23).

The occurrence of polar effect in bacteriophage λ has been reported in genes coding for coat protein in amber and acridine-induced mutants (14, 22). On the other hand, a *cis-trans* effect has been demonstrated in some genes controlling tail protein (5, 6, 13).

Here we report a more detailed analysis of some of these acridine-induced mutants that behave in a polar fashion. We tested complementation of superinfecting *sus* mutants of λ by λ i434 prophages containing different acridine-induced or *sus* mutations. Both noninduced and induced lysogens were tested. To avoid complications due to the occurrence of recombinants, we also performed these experiments with *int*⁻ *red*⁻ phages in a *rec*⁻ bacterial host.

MATERIALS AND METHODS

Bacterial strains. Bacterial strains used were 594, *Su*⁻ (nonpermissive) *gal*-1 *gal*-2 *Sm*^r, derived from W3350 (6); CA244-1, *Lac*L125 *Try*⁻ *Su*⁻ *F*⁺, derived from CA244 (3); N100, *Su*⁻ *gal*-2 *UV*^s *recA* *Sm*^r, derived from W3102 (9); C600, *Su*II⁺ (2); CA244-1 (λ i434); CA244-1 (λ i434 *hed Aacr* 107); CA244-1 (λ i434 *hed Baer* 110); CA244-1 (λ i434 *hed Caer* 114); and CA244-1 (λ i434 *hed Eacr* 4) (reference 22).

The *recA* strains, lysogenic for acridine-induced mutants, were constructed by transferring the defective prophages in the strain N100 (see below).

Bacteriophages. Bacteriophages λ hed *Asus*11 *cIIc*, λ hed *Bsus*1 *cIc*, λ hed *Csus*39 *cIc*, λ hed *Dsus*129 *cIc*, λ hed *Esus*214 *cIIc*, λ hed *Fsus*204 *cIIc*57: (i) *sus* mutations were described by Campbell (6) or Thomas et al.

(19), (ii) *cIc* or *cIIc* mutants were induced by ultraviolet light, and (iii) *cIIc*857 was introduced by cross.

The red3 (15) *int*102 (Mousset) derivatives from the aforesaid phages were isolated during the course of this work. Bacteriophage λ hed *Bts*1 was kindly provided by W. Arber, and λ i434 was as described by Kaiser and Jacob (12).

Media. Tryptone media are as described by Adams (1). The tryptone broth was supplemented with thiamine (1 mg/liter) for bacterial growth and with MgSO_4 (10^{-2} M) when used for phage dilutions.

Superinfection experiments. Fresh, overnight cultures were diluted 1:100 in tryptone broth and grown to approximately 10^8 cells/ml at 37°C with aeration by shaking. The cultures were resuspended in 10^{-2} M MgSO_4 at about 10^9 cells/ml, assayed, and induced by ultraviolet light (520 ergs/mm²) when needed. Phages were added to give three infecting phages per bacterium. After adsorption for 15 min at 37°C, 5 ml of 10^{-2} M MgSO_4 was added and the solutions were centrifuged to eliminate unadsorbed phages. The pellets were resuspended in 5 ml of tryptone broth and aerated at 37°C for 30 min. Dilutions (10^{-2}) were then made in tryptone broth containing 10^{-2} M MgSO_4 . After 60 more min at 37°C, the lysates were treated with CHCl_3 , diluted, and plated on C600 and 594.

Transfer of acridine-induced (defective) mutants into the *rec*⁻ strain N100. Exponentially grown bacteria lysogenic for an acridine-induced mutant of λ i434 *cI*⁺ were irradiated with ultraviolet light (520 ergs/mm²), superinfected with λ i434 *cIc* at a multiplicity of infection (MOI) of three phage particles per bacterium, and incubated for 90 min at 37°C. After addition of CHCl_3 and centrifugation, the supernatants were used to infect (MOI of 1) a culture of N100 starved for 0.5 hr in 10^{-2} M MgSO_4 . After a 30-min incubation at 37°C the bacteria were spread on tryptone agar. After overnight growth, the colonies were tested for their immunity and their ability to produce phage. Colonies that are immune to λ i434 but produce no phage contain a defective prophage.

RESULTS

Complementation of λ sus mutants by hetero-immune prophages: complementation in *rec*⁺

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TABLE 1. *Heteroimmune superinfection of λi434 defective lysogens by amber mutants of λ^a*

Determination	Superinfecting phage	Superinfected bacterial strain ^b					
		594 I	594(λi434) II	594(λi434A _{sus} 11) III	594(λi434B _{sus} 1) IV	594(λi434C _{sus} 39) V	594(λi434E _{sus} 214) VI
Total phage production (phage/ bacterium)	Crosses A						
	1. λA _{sus} 11 cIIc	6.0×10^{-3}	1.04	3.0×10^{-3}	0.52	1.04	3.3
	2. λB _{sus} 1 cIc	1.3×10^{-3}	0.5	0.15	3.8×10^{-4}	0.16	0.88
	3. λC _{sus} 39 cIc	1.8×10^{-3}	0.4	0.14	3.2×10^{-2}	7.3×10^{-4}	1.6
	4. λD _{sus} 123 cIc	3.2×10^{-4}	0.44	0.11	6.2×10^{-2}	0.19	0.93
	5. λE _{sus} 214 cIIc	9.5×10^{-5}	0.38	0.15	0.13	0.44	1.3×10^{-4}
<i>sus</i> ⁺ Recombinant production (phage/bacterium)	6. λF _{sus} 204 cIrs857	1.8×10^{-5}	3	4.1	1.6	4.4	3.2
	1. λA _{sus} 11 cIIc	— ^c	0.32	1.1×10^{-5}	7.5×10^{-2}	8.0×10^{-2}	0.23
	2. λB _{sus} 1 cIc	—	0.6	5.1×10^{-3}	2.8×10^{-3}	1.2×10^{-2}	4.0×10^{-2}
	3. λC _{sus} 39 cIc	—	0.19	3.6×10^{-3}	4.9×10^{-3}	5.4×10^{-6}	2.4×10^{-2}
	4. λD _{sus} 123 cIc	—	0.19	1.5×10^{-2}	9.5×10^{-3}	7.5×10^{-3}	2.1×10^{-2}
	5. λE _{sus} 214 cIIc	—	0.2	9.5×10^{-3}	1.2×10^{-2}	8.7×10^{-3}	1.1×10^{-5}
Total phage production (phage/ bacterium)	6. λF _{sus} 204 cIrs857	—	0.22	2.5×10^{-2}	7.7×10^{-2}	1.8×10^{-2}	8.1×10^{-3}
	Crosses B						
	7. λA _{sus} 11 cIIc	4.0×10^{-3}	1.0	5.0×10^{-2}	0.6	1.0	0.25
	8. λB _{sus} 1 cIc	2.4×10^{-3}	0.45	0.17	4.3×10^{-3}	0.13	7.4×10^{-2}
	9. λC _{sus} 39 cIc	3.0×10^{-3}	0.5	0.17	3.6×10^{-2}	4.5×10^{-3}	0.12
	10. λD _{sus} 123 cIc	2.6×10^{-3}	0.55	9.4×10^{-2}	5.0×10^{-3}	7.1×10^{-2}	0.11
	11. λE _{sus} 214 cIIc	1.1×10^{-3}	0.35	0.14	8.7×10^{-2}	0.12	2.0×10^{-3}
	12. λF _{sus} 204 cIrs857	0.15	3.7	2.1	1.25	1.7	0.42
	7. λA _{sus} 11 cIIc	6.7×10^{-6}	0.35	1.2×10^{-2}	5.3×10^{-2}	0.12	3.8×10^{-2}
	8. λB _{sus} 1 cIc	1.6×10^{-5}	0.29	1.8×10^{-3}	1.1×10^{-3}	6.2×10^{-3}	10^{-2}
	9. λC _{sus} 39 cIc	3.7×10^{-5}	0.33	3.7×10^{-3}	5.0×10^{-4}	4.5×10^{-4}	2.9×10^{-3}
	10. λD _{sus} 123 cIc	—	0.36	9.0×10^{-3}	2.7×10^{-3}	4.4×10^{-3}	8.9×10^{-3}
<i>sus</i> ⁺ <i>acr</i> ⁺ Recombinant production (phage/bacterium)	11. λE _{sus} 214 cIIc	2.1×10^{-5}	0.22	4.8×10^{-3}	1.7×10^{-3}	2.3×10^{-3}	1.1×10^{-4}
	12. λF _{sus} 204 cIrs857	2.4×10^{-5}	0.30	2.1×10^{-2}	6.4×10^{-3}	7.7×10^{-3}	2.1×10^{-3}

Total phage production (phage/ bacterium)		594		
		I	II	III
Crosses C	13. λ Asus11 cIIc	9.5×10^{-4}	5.2×10^{-2}	4.4×10^{-2}
	14. λ Bsus1 cIc	6.4×10^{-4}	0.11	2.1×10^{-2}
	15. λ Csus39 cIc	1.1×10^{-3}	0.12	0.18
	16. λ Dsus123 cIc	1.4×10^{-3}	6.0×10^{-2}	6.0×10^{-2}
	17. λ Esus214 cIIc	6.2×10^{-4}	3.8×10^{-2}	2.6×10^{-2}
	18. λ Fsus204 cIrs857	4.2×10^{-4}	3.8	3.6
sus ⁺ ts ⁺ Recombinant production (phage/bacterium)	13. λ Asus11 cIIc	1.7×10^{-5}	10^{-2}	1.4×10^{-3}
	14. λ Bsus1 cIc	1.8×10^{-6}	9.0×10^{-3}	10^{-4}
	15. λ Csus39 cIc	3.5×10^{-6}	2.1×10^{-2}	1.2×10^{-3}
	16. λ Dsus123 cIc	7.8×10^{-6}	2.0×10^{-2}	1.2×10^{-3}
	17. λ Esus214 cIIc	1.8×10^{-6}	1.3×10^{-2}	1.4×10^{-3}
	18. λ Fsus204 cIrs857	2.9×10^{-6}	0.13	2.9×10^{-3}

^a For superinfected bacterial strain, crosses C, the experiment was performed at 42 C to maintain the λ i434Brs1 prophage in nonpermissive conditions.

^b Roman numerals indicate number of infection.

^c Phage production was too low to be calculated.

bacteria. λ i434 Prophages containing suppressible (*sus*) acridine-induced (*acr*) or thermosensitive (*ts*) mutations were superinfected with heteroimmune (λ) phages (Table 1). The resulting lysates were plated on C600 and 594 to measure total phages and recombinants (Table 1). Complementation is considered to be absent when the production of phages per bacterium is 100- to 1,000-fold less than the phage production obtained with λ i434 wild-type lysogen. Complementation is estimated to be weak when phage production is 7- to 10-fold less than in the cross-test.

As expected, there is no complementation when the prophage and the superinfecting phage are mutated in the same gene (Table 1; crosses A, B, and C; bold face).

There is significant complementation between the prophage and superinfecting phages when they contain *sus* and *ts* mutations in different cistrons (Table 1, crosses A and C). The only exception is found in crosses A (lines 3 and 4, infection IV); the mutation *Bsus1* in the prophage severely depresses complementation of superinfecting phages with *sus* mutations in genes C and D.

The presence of mutation *Bacr110* in the prophage also depresses complementation of the superinfecting phages λ C*sus*39 and λ D*sus*123 (crosses B, lines 9 and 10, infection IV); the mutation *Cacr114* depresses complementation by the adjacent gene D (crosses B, line 10, infection V) and mutation *Eacr4* depresses complementation by the adjacent gene F (crosses B, line 12, infection VI). The gene F is a catalytic gene and, therefore, fully complementable; it follows that a nine-fold decrease of phage production per bacterium is significant of a weak complementation.

Complementation in *rec*⁻ bacteria. The *recA*

lysogens containing mutant i434 prophages were superinfected with *red*⁻*int*⁻ phages by using the procedure described above. The number of recombinants was very low: approximately 10^{-5} per bacterium. It can be seen that complementation of the superinfecting *sus* mutants by nondefective λ i434 prophage is much lower in N100 (λ i434; Table 2) than in 594 (λ i434) (Table 1). Here, polar effects of *Bsus1*, *Bacr110*, *Cacr114*, and *Eacr4* are no longer detected.

Complementation between derepressed prophages and superinfecting phages in *rec*⁺ bacteria. The experimental conditions were the same as described in the preceding sections, except that the bacterial strains were induced by ultraviolet light before superinfection. Table 3 shows that there is no complementation when the prophage and the superinfecting phage are mutated in the same gene (bold face). There is significant complementation between the prophage and superinfecting phages when they contain *sus* and *ts* mutations in different cistrons (Table 3; crosses A and C). The mutation *Bacr110* reduces complementation by the adjacent genes C and D (crosses B, lines 27 and 28, infection IV). Mutation *Cacr114* depresses complementation by the adjacent genes D and E (crosses B, lines 28 and 29, infection V) and mutation *Eacr4* depresses complementation by the adjacent gene F (crosses B, line 30, infection VI).

Since the lambdoid phages are not inducible by ultraviolet light in a *recA* strain (4), experiments with ultraviolet induction cannot be performed on these strains.

DISCUSSION

The results presented in Tables 1 and 3 clearly indicate that some of the acridine-induced and

TABLE 2. Total phage production obtained after heteroimmune superinfection of *su*⁻ *recA* defective lysogens with *red*⁻ *int*⁻ amber mutants of λ

Superinfecting phage	Superinfected bacterial strain					
	N100	N100(λ i434)	N100(λ i434 <i>Bsus1</i>)	N100(λ i434 <i>Esus214</i>)	N100(λ i434 <i>Bacr110</i>)	N100(λ i434 <i>Eacr4</i>)
λ A <i>sus11 cIts857</i> <i>red</i> ⁻ <i>int</i> ⁻	1.7×10^{-3}	7.5×10^{-3}	1.7×10^{-2}	4.0×10^{-2}	1.4×10^{-2}	1.02×10^{-2}
λ B <i>sus1 cIts857</i> <i>red</i> ⁻ <i>int</i> ⁻	5.7×10^{-4}	2.3×10^{-2}	2.4×10^{-4}	6.3×10^{-2}	4.0×10^{-4}	2.6×10^{-2}
λ C <i>sus39 cIts857</i> <i>red</i> ⁻ <i>int</i> ⁻	1.4×10^{-3}	1.7×10^{-2}	1.4×10^{-2}	3.3×10^{-2}	9.2×10^{-3}	2.3×10^{-2}
λ D <i>sus123 cIts857</i> <i>red</i> ⁻ <i>int</i> ⁻	1.1×10^{-3}	5.5×10^{-3}	1.4×10^{-2}	8.4×10^{-3}	1.02×10^{-2}	4.5×10^{-3}
λ E <i>sus214 cIts857</i> <i>red</i> ⁻ <i>int</i> ⁻	8.5×10^{-4}	7.0×10^{-3}	2.1×10^{-2}	2.3×10^{-3}	1.8×10^{-2}	7.4×10^{-4}
λ F <i>sus204 cIts857</i> <i>red</i> ⁻ <i>int</i> ⁻	1.82×10^{-2}	1.4	0.33	1.0	3.3	0.34

TABLE 3. *Heteroimmune superinfection of λ i434 defective lysogens induced by ultraviolet light^a*

Determination	Superinfecting phage	Superinfected bacterial strain ^b					
		594 I	594(λ i434) ^c II	594(λ i434A ₈₅₇ 11) III	594(λ i434B ₈₅₇ 1) IV	594(λ i434C ₈₅₇ 39) V	594(λ i434E ₈₅₇ 214) VI
Total phage production (phage/ bacterium)	Crosses A						
	19. λ Asus11 cIIc	2.8×10^{-3}	1.7	1.4×10^{-4}	0.7	3.1	4.9
	20. λ Bsus1 cIc	5.6×10^{-4}	0.32	0.2	5.0×10^{-4}	0.42	1.5
	21. λ Csus39 cIc	9.3×10^{-4}	0.12	0.27	7.8×10^{-2}	1.6×10^{-3}	2.0
	22. λ DSus123 cIc	1.3×10^{-3}	0.37	0.45	0.14	0.33	0.92
	23. λ ESus214 cIIc	6.3×10^{-4}	0.27	0.66	0.35	0.73	4.3×10^{-4}
	24. λ FSus204 cIIs857	1.6×10^{-4}	4.6	2.5	2.6	5.5	4.8
	19. λ Asus11 cIIc	5.6×10^{-6}	0.45	1.3×10^{-6}	9.7×10^{-2}	0.21	0.32
	20. λ Bsus1 cIc	2.5×10^{-5}	0.15	6.3×10^{-3}	2.5×10^{-6}	2.1×10^{-2}	6.5×10^{-2}
	21. λ Csus39 cIc	1.1×10^{-5}	0.1	1.1×10^{-2}	8.3×10^{-3}	3.3×10^{-6}	6.5×10^{-2}
<i>sus</i> ⁺ Recombinant production (phage/bacterium)	22. λ DSus123 cIc	7.9×10^{-6}	0.23	2.0×10^{-2}	1.3×10^{-2}	9.5×10^{-3}	2.8×10^{-2}
	23. λ ESus214 cIIc	1.1×10^{-5}	7.0×10^{-2}	3.5×10^{-2}	1.8×10^{-2}	9.0×10^{-3}	1.8×10^{-5}
	24. λ FSus204 cIIs857	7.0×10^{-6}	0.13	7.8×10^{-2}	7.0×10^{-2}	6.0×10^{-2}	2.9×10^{-2}
Total phage production (phage/ bacterium)	Crosses B						
	25. λ Asus11 cIIc	5.0×10^{-3}	4.1	0.22	2.5	2.1	0.96
	26. λ Bsus1 cIc	3.7×10^{-3}	0.55	0.22	8.7×10^{-3}	0.2	0.3
	27. λ Csus39 cIc	2.0×10^{-3}	0.76	0.34	8.1×10^{-2}	8.4×10^{-3}	0.26
	28. λ DSus123 cIc	2.2×10^{-3}	0.86	0.23	8.4×10^{-2}	0.1	0.15
	29. λ ESus214 cIIc	7.3×10^{-4}	1.1	0.42	0.31	0.16	2.9×10^{-3}
	30. λ FSus204 cIIs857	0.39	4.7	7.7	2.15	2.7	0.63
<i>sus</i> ⁺ <i>ac</i> ⁺ Recombinant production (phage/bacterium)	25. λ Asus11 cIIc	1.1×10^{-5}	1.5	3.8×10^{-2}	0.28	0.27	0.13
	26. λ Bsus1 cIc	3.4×10^{-5}	0.32	1.3×10^{-2}	4.6×10^{-3}	1.3×10^{-2}	3.5×10^{-2}
	27. λ Csus39 cIc	3.6×10^{-5}	0.27	2.3×10^{-2}	7.5×10^{-3}	3.5×10^{-3}	9.5×10^{-3}
	28. λ DSus123 cIc	3.1×10^{-5}	0.6	2.5×10^{-2}	7.6×10^{-3}	8.2×10^{-3}	1.2×10^{-2}
	29. λ ESus214 cIIc	3.0×10^{-5}	0.26	3.4×10^{-2}	1.2×10^{-2}	1.5×10^{-2}	7.9×10^{-4}
	30. λ FSus204 cIIs857	2.8×10^{-5}	0.33	8.7×10^{-2}	3.3×10^{-2}	3.3×10^{-2}	6.0×10^{-3}

TABLE 3 (Continued)

Determination	Superinfecting phage	Superinfected bacterial strain ^b		
		594 I	594(λi434) ^c II	594(λi434Br/I) III
Total phage production (phage/ bacterium)	Crosses C			
	31. λAsus11 cIIc	9.0×10^{-4}	3.0×10^{-2}	0.16
	32. λBsus1 cIc	3.6×10^{-4}	2.8×10^{-2}	0.19
	33. λCsus39 cIc	7.3×10^{-4}	3.6×10^{-2}	0.4
	34. λDsus123 cIc	3.6×10^{-4}	4.8×10^{-2}	0.8
	35. λEsus214 cIIc	8.7×10^{-4}	6.4×10^{-2}	0.4
sus ⁺ ts ⁺ Recombinant production (phage/bacterium)	36. λFsus204 cI/s857	8.2×10^{-5}	1.75	1.9
	31. λAsus11 cIIc	1.6×10^{-6}	2.0×10^{-2}	2.6×10^{-3}
	32. λBsus1 cIc	—	1.4×10^{-2}	2.7×10^{-4}
	33. λCsus39 cIc	5.0×10^{-5}	3.2×10^{-2}	6.5×10^{-3}
	34. λDsus123 cIc	5.7×10^{-7}	3.2×10^{-2}	3.7×10^{-3}
	35. λEsus214 cIIc	5.7×10^{-7}	3.2×10^{-2}	7.5×10^{-3}
	36. λFsus204 cI/s857	1.4×10^{-6}	0.54	3.0×10^{-2}

^a For superinfected bacterial strain, crosses C, the experiment was performed at 42 C to maintain the λi434Br/I prophage in nonpermissive conditions.

^b Roman numerals indicate number of infection.

^c For 594(λi434) and CA244-1(λi434), phage production was calculated taking in account only superinfecting type; the induced prophage grows normally since it is not defective. So we can compare these values with others obtained in defective lysogens which produce few or no prophage type.

nonsense mutants we used have polar effects. The mutations *Bsu1* and *Bacr110* have a polar effect on genes C and D. Mutation *Cacr114* reduces the expression of gene D, and mutation *Eacr4* reduces the expression of gene F. These results confirm first data given by Toussaint and Lefebvre (22) with acridine-induced mutants and are to some extent in agreement with Parkinson's results (14).

However, where recombination cannot occur, complementation even by a nondefective prophage is strongly reduced, and the polar effects observed under conditions allowing recombination are no longer detectable. We shall discuss these two points successively.

It is known that all of the late genes of a prophage are *trans* activated after heteroimmune superinfection (17). This can be attributed to the action of the Q product synthesized by the superinfecting phage (7, 8, 11, 16) on a site of the prophage, located between gene Q and gene S (10, 21). However, where one deals with a so-called "stoichiometric function" (e.g., a function that needs a high number of proteins to be fully expressed), which is the case for the genes *hedA-E*, *trans* activation of a prophage gene can only exert a low effect on the level of production of mature phage, for obvious reasons of gene dosage. In such cases, the contribution of *trans* activation to the yield is small, compared with that of (non-defective) λ recombinants which have picked up wild-type gene. Nevertheless, after superinfection, the prophage is excised; it may be integrated by recombination into an λ genome and passively replicated (20). This will increase the gene dosage, resulting in more product since *trans* activation is the same whether the late genes which are activated are attached to a deoxyribonucleic acid which is or is not blocked by immunity.

Since the polar effect is no longer detected under conditions that prevent recombination, perhaps polarity is an artifact associated with recombination. For instance, one might argue that the presence of a B^- mutation in the prophage acts only by strongly depressing the number of nondefective λ recombinants formed (Fig. 1a). This would reduce the contribution by recombinants of B^+ function and simulate a polar effect of this B mutation. However, if there were no true polarity in this system, all of the λ recombinants carrying a C^+ allele (regardless of whether gene B is present as a B^+ or B^- allele) would express function C equally well. Since these recombinants are obviously equally frequent whether the prophage is B^+ or B^- (Fig. 1b), this hypothesis cannot be correct.

The lack of detectable polarity in the absence of recombinants may be interpreted in the following

FIG. 1. (a) $B^+C^+i\lambda$ recombinants formed between a B^-C^+ or a B^+C^+i434 prophage and a $B^+C^-i\lambda$ superinfecting phage. These recombinants are less frequent when the prophage is B^- than when it is B^+ since in the first case a double crossing-over is needed, whereas a single one is sufficient in the second case. (b) $B^-C^+i\lambda$ and $B^+C^+i\lambda$ recombinants formed between a $B^+C^-i\lambda$ superinfecting phage and a B^-C^+i434 or B^+C^+i434 prophage. These two kinds of recombinants are present in equal amount for the same C^- mutation.

manner: since the prophage can be integrated no more and, therefore, is replicated no more in the superinfectant genome, this prophage is the only one to be implicated in the complementation, which is thus very weak. Under these conditions, if a polar effect occurs, a supplementary decrease of complementation is not detectable.

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