INVOLVEMENT OF GENE *49* IN RECOMBINATION OF BACTERIOPHAGE T4

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ABSTRACT

The role of T4 gene *49* in recombination was investigated using its conditional-lethal amber (*am*) and temperature-sensitive (*ts*) mutants. When measured in genetic tests, defects in gene *49* produced a recombination-deficient phenotype. However, DNA synthesized in cells infected with a *ts* mutant (*tsC9*) at a nonpermissive temperature appeared to be in a recombinogenic state: after restitution of gene function by shifting to a permissive temperature, the recombinant frequency among progeny increased rapidly even when DNA replication was blocked by an inhibitor. Growth of a gene *49*-defective mutant was suppressed by an additional mutation in gene *uvsX*, but recombination between *rII* markers was not.

DURING the course of abortive development caused by conditional-lethal mutants in gene *49* of bacteriophage T4, characteristic phenotypes are recognized. First, DNA replication proceeds normally, but replicating DNA does not mature (MINAGAWA and FUJISAWA 1968; FUJISAWA and MINAGAWA 1971a), and forms a very-fast-sedimenting complex (VFS DNA) whose sedimentation coefficient is greater than 1000 S (FRANKEL, BATCHELER and CLARK 1971; KEMPER and JANZ 1976). Electron microscopic observations revealed that VFS DNA is a huge mass of condensed DNA surrounded by a loose arrangement of strands in which branched structures are sporadically observed (KEMPER and BROWN 1976). Second, the mutation affects neither the synthesis of detectable phage proteins nor the formation of capsids, but maturation of phage heads is blocked (KING 1968; LUFTIG, WOOD and OKINAKA 1971), and partially DNA-filled heads accumulate (LUFTIG, WOOD and OKINAKA 1971; GRANBOULAN, SÉCHAUD and KELLENBERGER 1971; LAEMMLI, TEAFF and D'AMBROSIA 1974; BLACK and SILVERMAN 1978). Upon restoration of gene-*49* function, preformed capsids and DNA are converted to complete heads. Thus, the capsid-DNA complex was postulated to be an intermediate in the maturation of phage beads. Third, the abortive infection does not induce the synthesis of a characteristic endonuclease which specifically cleaves VFS DNA (FRANKEL, BATCHELER and CLARK 1971). The nuclease has been isolated and has the following activities: cleavage of gapped DNA as well as VFS DNA, weak cleavage of single-stranded DNA at high enzyme concentration (MINAGAWA and RYO 1978; NISHIMOTO, TAKAYAMA and MINAGAWA 1979; KEMPER and GURABETT 1981; KEMPER, GURABETT and COURAGE 1981) and cleavage and resolution of Holliday structures in the recombination

intermediate (MIZUUCHI *et al.* 1982). The finding that a *ts* mutant of the gene-induced synthesis of a temperature-sensitive nuclease (NISHIMOTO, TAKAYAMA and MINAGAWA 1979) suggested that gene *49* is a structural gene, although it is as yet unknown whether the gene encodes the entire enzyme or only a subunit.

It is a characteristic feature of the T4 reproductive cycle that DNA recombination is essential (e.g., BROKER and DOERMANN 1975). The structures susceptible to the gene-*49* endonuclease may arise from DNA recombination as well as replication processes. The formation of VFS DNA and the number of branches in it were influenced by various gene products involved in recombination (MINAGAWA and RYO 1979; MINAGAWA *et al.* 1983). It is possible that the gene-*49* product pertains to T4 DNA metabolism, although gene-*49* function depends on the expression of genes controlling late protein (FRANKEL, BATCHELER and CLARK 1971). In this paper we have investigated the effect of gene-*49* mutations on genetic recombination. Parts of these results were presented at meetings of the Japanese Society of Genetics (MIYAZAKI, RYO and MINAGAWA 1974, 1976).

MATERIALS AND METHODS

Bacterial and phage strains: Escherichia coli BB and B^E are the nonpermissive hosts, and B40*su1* is permissive for amber mutants. *E. coli* K12 and its derivatives K12S F^+, CR63, C600 and Y-mel are Su^-, Su^-, $Su1^+$, $Su2^+$ and $Su3^+$, respectively. CR63(λh), a λ lysogen of CR63, was used as the selective host for rII^+ phage.

Bacteriophage T4 strains were: wild type; *amE727x1* and *tsC9*, amber and temperature-sensitive mutants, respectively, of gene *49*; *tsL109* (gene *46*: exonuclease); *tsY213* (gene *5*; baseplate component); *tsL90* (gene *24*: capsid component); and *amxb* (gene *uvsX*: DNA recombination and repair). The *rII* mutants *rM16*, *rM36* and *r596* are derivatives of T4B. They were backcrossed four times against T4D before use. Multiple mutants were constructed by recombination. Occasionally, mutants are cited by gene number, for example, *amE727x1* as 49^- or as 49^{am}.

Media and buffers: Peptone-glucose (PG) medium contained 1% Polypeptone (Daigo Chemical Company), 0.3% NaCl and 0.1% glucose; the pH was adjusted to 7.4 with NaOH. PG top and bottom agars were PG solidified by adding 0.5 and 1.0% agar, respectively. M9A medium contained 7 g Na_2HPO_4, 3 g KH_2PO_4, 1 g NH_4Cl, 2 g glucose, 2 g Casamino acids (Difco), 1 μM $FeCl_3$, 0.1 mM $CaCl_2$ and 1 mM $MgSO_4$ per liter of distilled water.

Phage crosses: In experiments with *am* mutants, host cells were grown in M9A at 37° to 5×10^8 cells/ml. NaCN was then added to 4 mM. After 2 min, the cells were coinfected with *rII* mutant phages at a multiplicity of five each. After 10 min, 0.1 volume of anti-T4 rabbit serum (K = 20) was added to the infected suspension, which was further incubated for 5 min. The infected cells were then diluted 10^4-fold into prewarmed M9A and incubated at 37° for 70 min. In crosses with *ts* mutants, the infected cells were centrifuged for 5 min at 5000 rpm. They were resuspended in the original volume of ice-chilled M9A, diluted 10^3-fold into M9A prewarmed at various temperatures and incubated at the respective temperature for appropriate times. After the cultures were lysed by adding chloroform, the total progeny and rII^+ recombinant phage were scored by plating on CR63 (or B40*su1*) and on CR63 (λh), respectively. Plates were incubated at 37° overnight, or at 28° for *ts* mutants. The frequency of rII^+ recombinants was expressed as the percentage of rII^+ among total progeny. The burst size was calculated by dividing the total number of progeny by the number of infected host cells.

RESULTS

Effect of gene 49 mutation on frequencies of recombinants: BERNSTEIN (1967, 1968) and BERGER, WARREN and FRY (1969) explored the roles of a number of T4 early genes in genetic recombination by limiting the activity of the genes.

Similar experiments were carried out using conditional-lethal mutants of gene 49. Two *rII* mutants, *rM16* and *r596*, each carrying an *am* mutation in gene 49 (*amE727x1*), were crossed in various host cells, and the rII^+ recombinant frequencies were determined (Table 1). The am^+ control crosses were done simultaneously in each of the host cells. Judging from burst sizes, the *am* mutation was fully suppressed in two hosts, CR63 and Y-mel, and frequencies of recombinants in these cells were about ⅔ of the control values. In C600, the 49^- burst size was weakly suppressed, and the recombinant frequency was reduced to ⅓ of the control. The recombinant frequency in a Su^- host (K12S F^+) was decreased to ¼ of the am^+ control value. The decreased recombinant frequencies when gene-49 function was limited suggest that this gene may be classified as a recombination gene according to the criterion used by BERNSTEIN (1967, 1968) and BERGER, WARREN and FRY (1969).

The same result was obtained from experiments with a *ts* mutant. The double mutants *rM16* 49^{ts} and *rM36* 49^{ts} were crossed in CR63 cells at various temperatures. Control crosses in which gene 49 was wild type were performed under the same conditions. The incubation period at each temperature was chosen to yield a burst size greater than 100 for the wild type, as shown in the legend for Figure 1. Within the range of temperatures used, recombinant frequencies were constant in the ts^+ control, but in 49^{ts}, burst sizes and recombinant frequencies were decreased along with decreased gene function. For comparison, effects of mutations in gene 5 (*tsY213*), gene 24 (*tsL90*) and gene 46 (*tsL109*) were examined by the same procedures. The results, together with that for gene 49, are summarized in Figure 2, in which recombinant frequency is plotted against burst size on a logarithmic scale. When the functions of the genes were weakly restricted and more than 100 progenies were produced per infected cell, effects on recombinant frequency produced by mutations in these genes were weak and indistinguishable from each other. When the activities of these genes were further limited, different effects of the gene mutations were observed. The effect of mutation in gene 46 was the greatest, in good agreement with BERNSTEIN (1968), and that in gene 49 was slightly lower than that in gene 46. The slope of the curve for gene 46 is −1, showing direct proportionality between the decrease in burst size and in recombinant frequency. The slope for gene 49 is half of that for gene 46; compared with the decrease in burst size, the decrease in the recombinant frequency was less sensitive to limiting gene function. Effects on the recombinant frequency by mutations in gene 5 and 24 were low compared with effects on burst size.

Effect of resumption of gene-49 function on recombinant frequencies: DNA formed by 49^{ts} at high temperature is partially packaged in phage capsids and matures into complete phage DNA after shifting to low temperature (LAEMMLI, TEAFF and D'AMBROSIA 1974; BLACK and SILVERMAN 1978). Therefore, a clue to the stage at which recombination is blocked may be obtained by examining the frequency of recombinants among the progeny formed after resumption of gene function. CR63 cells coinfected with *rM16* 49^{ts} and *rM36* 49^{ts} were incubated at 40.5°. After 27 min fluorodeoxyuridine (FUdR) and uridine were each added to final concentrations of 20 μg/ml to abolish the synthesis of DNA, and the same

TABLE 1

Effect of gene-49 amber mutation on burst size and frequency of rII$^+$ recombinants in nonsuppressing and suppressing hosts

Host	*rM16* × *r596*		*M16 49^{am}* × *r596 49^{am}*		Relative frequency of recombinants
	Burst size	% *rII$^+$*	Burst size	% *rII$^+$*	
K12S(*Su$^-$*)	71	2.7	2.0	0.74	0.27
CR63(*Su1$^+$*)	118	3.2	97	2.2	0.68
C600(*Su2$^+$*)	119	3.1	26	1.1	0.34
Y-mel(*Su3$^+$*)	96	3.2	114	2.2	0.69

All values are averages of two experiments. Relative frequencies of recombinants were calculated as the ratios of the frequency of *rII$^+$* recombinants in crosses of *rM16 49^{am}* × *r596 49^{am}* and *rM16* × *r596*.

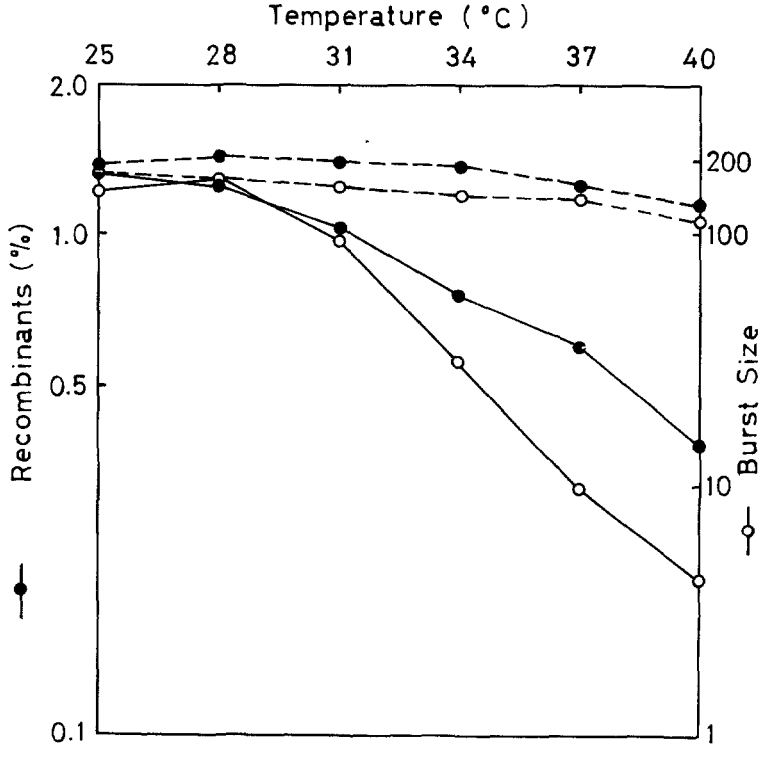

FIGURE 1.—Effect of temperature on recombinant frequencies and burst sizes in the crosses rM16 × *rM36* and *rM16 49^{ts}* × *rM36 49^{ts}*. Experimental details are described in MATERIALS AND METHODS. Incubation periods were 160 min at 25°, 120 min at 28°, 90 min at 31°, 80 min at 34°, 70 min at 37° and 90 min at 40°. ● = recombinant frequency; ○, burst size; - - = *ts$^+$*; —— = *ts*.

volume of water was added to the control culture. The concentration of FUdR was high enough to block DNA replication almost completely (FUJISAWA and MINAGAWA 1971b). At 30 min, cultures were transfered to 29°, and samples were withdrawn at intervals, lysed with chloroform and plated on CR63 and CR63(λh) to score total plaque formers and recombinants, respectively (Figure

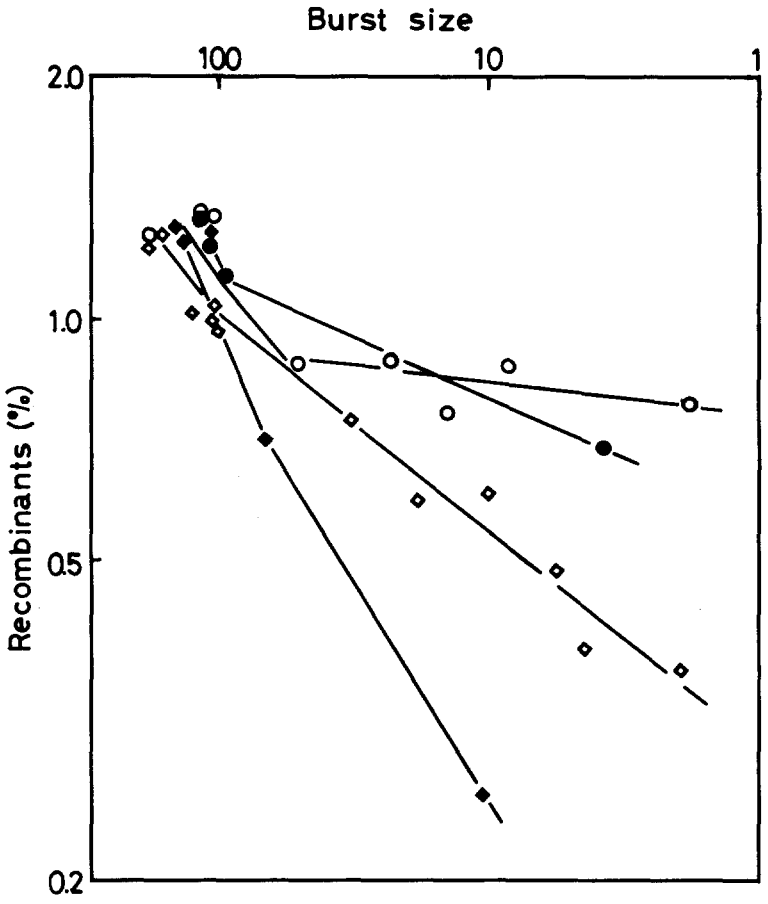

FIGURE 2.—Comparison of effect of *ts* mutation in various genes on recombination frequency. Crosses with *rM16* × *rM36* were performed in the background of *tsY212* (●, gene 5), *tsL90* (○, gene 24), *tsL109* (◆, gene 46), or *tsC9* (◇, gene 49) at various temperatures between 25° and 40°, except for *tsL90*, which was incubated between 25° and 35° because burst sizes were extremely low at higher temperatures. Recombinant frequencies were plotted against burst sizes on logarithmic scales.

3). At the time of the temperature shift, few phage particles had formed and the frequency of rII^+ recombinants was low (at the same level as in Figure 1). This indicates that burst size and recombinant frequency do not increase appreciably after 30 min at the restrictive temperature. Phage in the FUdR-inhibited culture formed slowly after the temperature shift and their production ceased at 25 min, whereas in the control it increased at the same slow rate as in FUdR for 5 min but thereafter increased at a higher rate to 25 min. Contrary to phage production, the increase of recombinant frequency started at a similar maximum rate, without a lag in both cultures after the shift, and continued for 15 min, after which the frequency in the control became slightly higher than the frequency in FUdR. These results imply that DNA accumulated in the absence of the gene-49 function is in a state ready to produce recombinant progeny when the function is restored. This may differ, for example, from the situation with

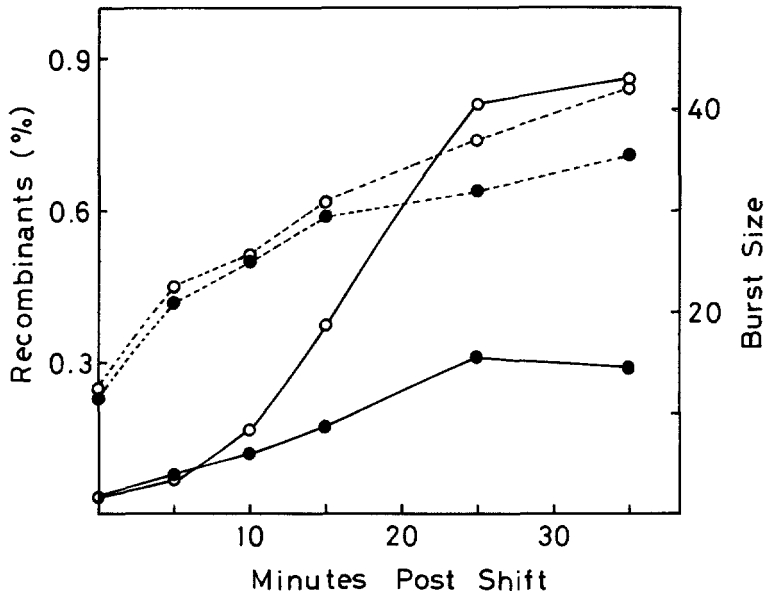

FIGURE 3.—Increase of recombinant frequencies and burst sizes after temperature shift-down. Two cultures of *E. coli* CR63 coinfected with both *rM16 49*^{ts} and *rM36 49*^{ts} were incubated at the nonpermissive temperature (40.5°). At 27 min after infection, FUdR and uridine were each added to 20 μg/ml to one culture; the same volume of distilled water was added to the other. At 30 min, both cultures were shifted to the permissive temperature (29°). At various times thereafter, total plaque formers and *rII*$^+$ recombinants were assayed by plating on appropriate hosts. ● = FUdR + uridine; ○ = distilled water only; - - - = recombinant frequency; ——, burst size.

mutations in genes *42* (BERNSTEIN 1968) and *58* (BERGER, WARREN and FRY 1969), as well as gene *46* (Figure 2), in which a clear correlation was observed between decreases in burst sizes and recombination frequencies.

Effect of a mutation in gene uvsX *on the recombination of* 49$^-$ *phage:* The lethality of a gene-*49* defect is suppressed by mutation in gene *uvsX*, the suppression being specific to gene-*49* mutations (DEWEY and FRANKEL 1975; CUNNINGHAM and BERGER 1977; SHAH and DELORENZO 1977). Mutations in gene *uvsX* decrease recombination frequencies and resistance to DNA damage but are not lethal (CUNNINGHAM and BERGER 1977; BERNSTEIN 1981). The gene product has been purified to electrophoretic homogeneity. It has a single-stranded DNA-dependent ATPase activity and promotes pairing of complementary single-stranded DNA, as well as single-stranded DNA and homologous duplex DNA (T. YONESAKI and T. MINAGAWA, unpublished results). We inquired whether a *uvsX*$^-$ mutation also suppresses recombination in a *49*$^-$ background. Crosses between *rM36* and *r596* were performed in *49*$^-$, *uvsX*$^-$ and *49*$^-$ *uvsX*$^-$ backgrounds as shown in Table 2. The *uvsX*$^-$ mutation appreciably raised the burst size of the *49*$^-$ mutant but failed to increase the recombination frequency.

DISCUSSION

In the absence of gene-*49* function, DNA accumulated as VFS DNA was shown to be a compact complex whose strands are inter- or intramolecularly

TABLE 2

Epistatic interaction of gene uvsX(amxb) *with gene* 49(amE727x1)

Host	r36 × r596 in the background of	Burst size	rII^+ progeny (%)
$B^E(Su^-)$	$uvsX^+$ 49^{am}	1.8	0.70
B40Su1	$uvsX^+$ 49^{am}	90	2.1
$B^E(Su^-)$	$uvsX^{am}$ 49^+	42	0.46
B40Su1	$uvsX^{am}$ 49^+	105	2.0
$B^E(Su^-)$	$uvsX^{am}$ 49^{am}	45	0.50
B40Su1	$uvsX^{am}$ 49^{am}	83	2.0

All values are averages of four experiments.

linked and to be a specific substrate for the gene-49 nuclease (FRANKEL, BATCHELER and CLARK 1971; KEMPER and BROWN 1976; KEMPER and JANZ 1976; MINAGAWA and RYO 1978; NISHIMOTO, TAKAYAMA and MINAGAWA 1979; KEMPER, GURABETT and COURAGE 1981; MIZUUCHI *et al.* 1982). VFS DNA was also shown to contain randomly distributed Y-shaped branches, the average number of branches per unit T4 DNA length approximating that of sites susceptible to the nuclease (MINAGAWA *et al.* 1983). Formation of VFS DNA, and numbers of enzyme-susceptible sites of branches, can be affected by mutations in other genes involved in recombination: they were reduced by mutations in gene *46* or *uvsX* (which cause recombination deficiency) and increased by a mutation in gene *30* (which causes recombination proficiency) (MINAGAWA and RYO 1979; MINAGAWA *et al.* 1983). The formation of VFS DNA is supposed to depend on recombination. However, the results in Table 1 and Figure 1 show that, when gene-*49* function is limited, the *rII* recombinant frequency in progeny phage was reduced to ⅓ to ¼ of the 49^+ control value. It seems from the results in Figure 2 that the recombination-deficient phenotype of mutations in gene *49* may differ from recombination deficiency caused by mutation in gene *46*. When the gene-*46* function is defective, recombinational intermediate molecules are not formed (BROKER 1973); the formation of concatemeric DNA (HOSODA, MATHEWS and JANSEN 1971; SHAH and BERGER 1971) as well as VFS DNA (MINAGAWA and RYO 1979) are prohibited. In contrasts in 49^--infected cells VFS DNA forms, seemingly dependent on a recombination process.

A model of the lethality of gene-*49* mutations has been proposed in which DNA packaging is interrupted by an unresolved recombinational branch, resulting in the accumulation of partially filled heads; the interruption is relieved upon restoration of the gene-*49* function (LUFTIG, WOOD and OKINAKA 1971; LAEMMLI, TEAFF and D'AMBROSIA 1974; BLACK and SILVERMAN 1978). Since the average number of branches in VFS DNA can be estimated, from results reported thus far, to be two or three per T4 unit length (MINAGAWA and RYO 1978; KEMPER, GURABETT and COURAGE 1981), the complexed DNA would carry enough branches to regularly interrupt DNA packaging. The results in Table 1 and Figures 1–3 show that mutations in gene *49* are slightly leaky and have a recombination-deficient phenotype. Phage may be infrequently produced when VFS DNA is packaged at regions where branch structures more than one unit length are not present. Such regions may be generated by dimerization of two

daughter genomes originating from a common parental genome (WATSON 1972; BROKER 1973) or, infrequently, by chain migration of branched recombinational intermediates. Such genomes would have been less frequently subjected to genetic recombination. This pathway is supported by the fact that the yield of 49^- phage was increased by a secondary mutation in gene *uvs*X, but recombination was not (Table 2). The formation of VFS DNA in this infection was retarded, but when the DNA was formed it contained fewer branches (MINAGAWA and RYO 1978; MINAGAWA *et al.* 1983). Such VFS DNA may be more readily packaged into capsids.

The temperature-shift experiment in Figure 3 shows that recombinant progeny increased immediately after the shift even under the condition in which DNA replication was severely blocked. This implies that DNA accumulated as VFS DNA is in a state ready to produce recombinant progeny upon resumption of the gene-*49* function. As already mentioned, the production of progeny is inhibited by branches in VFS DNA, debranching is brought about by the gene-*49* nuclease, and branch formation depends on recombination. It seems, therefore, that, in the absence of the gene-*49* function, the recombination process is initiated but the recombinational intermediate remains unresolved.

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