VIROLOGY 42, 777–779 (1970)

Temperature-Sensitive Mutants of Serratia Phage Kappa

Temperature-sensitive (*ts*) mutants of phages are well known to be very useful for recombinational and physiological studies (*1*). *Ts* mutants of phage Kappa were first isolated in 1966 (U. Winkler, unpublished data). The wild type of Kappa forms plaques at 30 and 38° whereas the mutants grow only at the low temperature. The present paper deals with the isolation and preliminary characterization of further *ts* mutants of Kappa. Bacterial strains, methods, and media were as described earlier (*2*) if not stated otherwise.

Isolation of *ts* mutants followed the method of Edgar and Lielausis (*1*) with slight modifications. Mutants were induced by treating extracellular phage with either ultraviolet light (about 4800 erg/mm^2) or nitrous acid (0.01 *M*, pH 6.0, 20 minutes). In both cases the survival of phage was approx. 6×10^{-4}. The frequencies of *ts* mutants among the survivors were 3.0% (30/1000; ultraviolet light) and 1.4% (28/2000; nitrous acid) considering only mutants with a reversion frequency less than 2×10^{-4}. All mutants were numbered. The prefixes (capital letters) mark the cistrons and the suffixes *u* and *n* stand for ultraviolet light or nitrous acid, respectively, as inducing agent.

In Fig. 1 the mean burst size of cells infected with either wild type phage or one of two *ts* mutants is plotted as a function of incubation temperature. The optimum is 34° for the wild type and 30° for the *ts* mutants. Referring to the burst size at 30° that at 38° is reduced to nearly 10% (wild type), 1% (*ts* Z57u), and 0.1% (*ts* S49n). The low mean burst size at 38° could be due to a few temperature-resistant complexes among the mass of infected cells giving no infectious progeny. However, in single burst experiments with wild type phage the fraction of complexes producing infectious progeny as well as the randomness of their burst sizes were the same at 30 and 38°. Growth of uninfected cells was nearly the same at 30 and 38°.

Temperature pulse experiments were carried out because intracellular growth of wild type phage might not be sensitive to the depressing effect of 38° for the total latent period. It turned out to be the last 10 minutes of the latent period only that were sufficient to reduce the mean burst size to 30%. Therefore it seems to be the maturation process that is inhibited by the elevated temperature. Surprisingly the mean burst size was nearly doubled if a 38° pulse

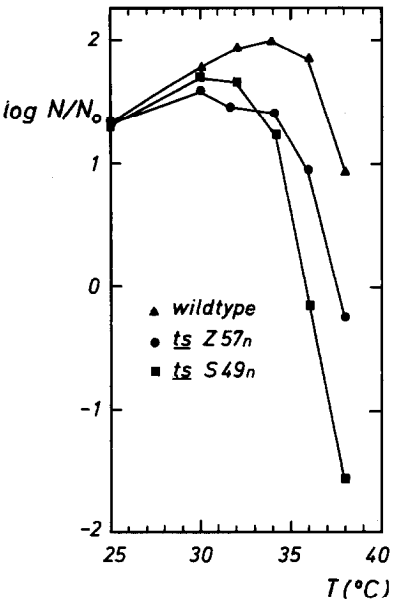

FIG. 1. Mean burst size (N/N_0) of cells infected with phage Kappa as a function of the incubation temperature (T). Growing cells of *S. marcescens* HY were infected with phages at a multiplicity of 0.1 and incubated at the temperatures indicated (deviation ± 0.1°). After 5 minutes the cultures were diluted 1:100 into prewarmed broth and incubated for additional 95 minutes at the different temperatures. Then chloroform was added and phage titers (N) were determined at 30°. N_0 gives the number of infectious centers at the end of the adsorption time (prelysis plating at 30°).

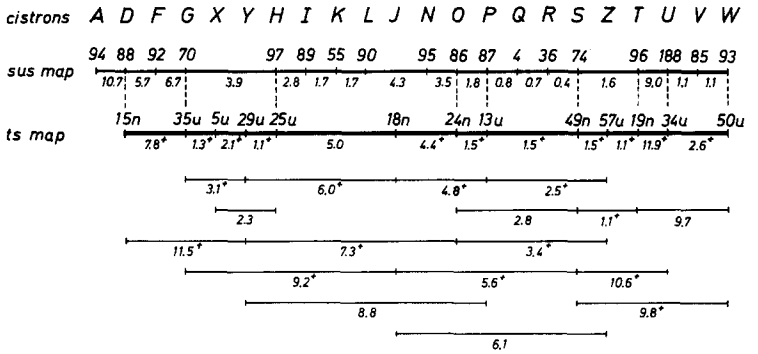

FIG. 2. Genetic map of phage Kappa obtained by two factor crosses between *ts* mutants and its relation to the *sus* map (*2*). Numbers above the main lines give the relative position of the mutant loci. Numbers below the lines represent distances measured in recombination units (RU), i.e., twice the fraction of wild type recombinants multiplied by 100. RU values with an asterisk are averages of two to four crosses. *ts* and *sus* markers connected by a dotted line belong to the same cistron as shown by complementation and crosses (see Table 1, I).

For crossing procedure see (*2*), except that (i) crosses were performed also in su^- cells, (ii) in some experiments the complexes were incubated for 70 instead of 60 minutes and (iii) the recombinants were assayed at 38°.

was given between the 5th and the 10th minute of the latent period.

The diverse response of intracellular wild type phage to a temperature pulse at different phases of the latent period made it difficult to examine our *ts* mutants with temperature shift experiments to see whether "early" or "late" genes are blocked. Preliminary experiments indicated that most of our *ts* mutants except *ts* U34u are probably blocked in "late" genes. This might be due to our isolation procedure.

A spot test for complementation was performed with all *ts* mutants at 38°. The method used was as described earlier (*2*) except that the *ts* mutants had titers of 2×10^8/ml and plates were seeded with 5×10^8 cells. According to positive complementation 13 different groups were distinguished. These groups probably represent different cistrons as concluded from the relatively long distances between the corresponding mutant loci as found in crosses (Fig. 2). So it seems that intragenic complementation which is possible on principle among *ts* mutants (*3*) did not interfere with the test for intergenic complementation.

In further complementation tests between suppressor-sensitive (*sus*) mutants (*2*) of phage Kappa and our 13 "standard" mutants anltogethe 22 complementatior

TABLE 1

PHAGE CROSSES[a]

Parents	RU	Parents	RU
I. Intragenic crosses (*ts* × *sus*)			
D15n × D88	0.54	P13u × P87	0.23
G35u × G70	0.02	S49n × S74	0.04
H25n × H97	0.07	T19n × T96	0.19
O24n × O86	0.19	U34u × U188	.0.02
		W50u × W93	.0.50
II. Intragenic crosses (*ts* × *ts*)			
J18n × J36u	0.08	Z57u × Z5n	0.08
O24n × O6n	0.37	Z57u × Z6u	0.28
T19n × T20n	0.40	Z57u × Z12n	0.23
III. Intergenic crosses (*ts* × *sus*)			
T19n × U188	7.7	U34u × T96	11.5
O24u × N95	4.1	J18n × O86	4.7

[a] For procedure see legend of Fig. 2.

groups were found. Nine of these groups (cistrons) are represented by *sus* as well as *ts* mutants. The four new cistrons marked by *ts* mutants only were named J, X, Y, and Z.

Two factor crosses were carried out with the 13 *ts* mutants in order to construct a genetic map (Fig. 2). The shortest distance between two mutations located in different cistrons was 1.1 RU (recombination units). Summing up the distances between all 13 *ts* markers the total length of the map is

41.8 RU. The corresponding value of the *sus* map is 46.8 RU (*2*).

The nine complementation groups represented by *ts* as well as *sus* mutants were examined by crosses between the corresponding mutants (dotted lines in Fig. 2). The recombination units obtained were less than 0.6 (Table 1, I) which are in the range of intragenic crosses of the type *ts* × *ts* (Table 1, II). Some intergenic crosses between *ts* and *sus* mutants gave recombination units as expected from either the *ts* or the *sus* map (Table 1, III).

ACKNOWLEDGMENTS

This paper contains part of the Diplomarbeit of the first author. We thank Prof. U. Winkler for guidance and criticism as well as for giving us his conditional lethal mutants of Kappa (*ts* W50u, *ts*Z57u, all *sus* mutants). We appreciated the excellent technical assistance of Miss S. Schulte.

REFERENCES

1. EDGAR, R. S., and LIELAUSIS, I., *Genetics* **49**, 649–662 (1964).
2. WINKLER, U., KOPP-SCHOLZ, U., and HAUX, CH., *Mol. Gen. Genet.* **106**, 239–253 (1970).
3. EDGAR, R. S., DENHARDT, G. H., and EPSTEIN, R. H., *Genetics* **49**, 635–648 (1964).

WILFRIED WACKERNAGEL
ROSEMARIE WINKLER

University of Bochum
Department of Biology
Bochum, Germany 463
Accepted August 14, 1970