COLD-SENSITIVE MUTANTS OF BACTERIOPHAGE λ

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ON the chromosome of bacteriophage λ, more than thirty cistrons have been identified mostly by the analysis of suppressor-sensitive *(sus)* and also of temperature-sensitive *(ts)* mutants. A reasonably dense genetic map has been constructed and some knowledge has been accumulated on the functions of individual genes (reviewed by DOVE 1968).

We have begun to isolate and characterize cold-sensitive *(cs)* mutants of phage λ mainly because we speculated that such mutants might be encountered preferentially or exclusively in a very limited set of genes. Here we present data to show that this is the case. On this basis one might hope that if independent information is obtained on the character of cold-sensitive mutants in general, it will help to elucidate the function of the particular genes involved; conversely, further information on the function of these genes might help to understand the nature of cold-sensitive mutations. A relatively high incidence of cold-sensitive mutations has also been shown for certain genes of phage T4 (SCOTTI 1968).

MATERIALS AND METHODS

Bacterial and phage strains: Stocks of λ and related phages used in this work were produced from lysogenic bacterial strains listed below, with the exception of i^{434}*sus* N_7 (obtained from M. PTASHNE). *E. coli* bacterial strains used were: C600, *pm*+, λ-sensitive; W3350, *pm*⁻, λ-sensitive; W3101 (λ-wild), *pm*⁻; W3101 (434*hy*), *pm*⁻ (KAISER and JACOB 1957); all obtained from A. D. KAISER; W3110, *pm*⁻, λ-sensitive, obtained from P. HOFSCHNEIDER; Hfr43, *pm*⁻, λ-sensitive, obtained from W. VIELMETTER; *pm*+ lysogens of various *sus* mutants isolated by CAMPBELL (1961), namely C600 lysogenic for, respectively, λ*sus* A_{11}; B_1; C_{39}; D_{123}; E_4; G_9; H_{12}; I_2; J_6; K_{24}; N_7; N_{53}; O_8; O_{29}; P_3; Q_{73}; Q_{117}; R_5, and R_{54}; all obtained from E. CALEF.

Media: Tryptone broth: 1% Bacto-Tryptone (Difco), 0.8% NaCl; soft agar: 1% Bacto-Tryptone, 0.8% NaCl, 0.75% Bacto-Agar (Difco); tryptone agar: 1% Bacto-Tryptone, 0.8% NaCl, 1% Bacto-Agar; Tris-Mg: 10^{-2}M Tris pH 7.1, 10^{-2}M $MgCl_2$; infection medium: 10^{-2}M Tris pH 7.1, 6×10^{-5}M $MgCl_2$, 5×10^{-4}M $(NH_4)_2SO_4$, 4×10^{-10}M $FeSO_4$.

Ultraviolet lamp: Osram HN ofr.

N-methyl-N'-nitro-N-nitrosoguanidine (NNG) was purchased from Koch-Light Laboratories Ltd., Colnbrook, England.

UV-induced lysates: Cultures of lysogenic bacteria were grown with aeration in tryptone broth at 37°C until a cell titer of about 4×10^8/ml was reached. The cultures were centrifuged and resuspended in an equal volume of cold Tris-Mg. These suspensions were irradiated with an optimal dose of UV light (a 4 mm layer of the suspension exposed to the Osram HN ofr lamp for 40 sec at a distance of 40 cm). After adding an equal volume of tryptone broth they were aerated at either 37°C or 29°C. In the former cases, lysis occurred after about 2 hr, at which time the lysates were chloroformed. Lysis in most of the latter cases occurred after about 3 hr, at which time the cultures were chloroformed.

Phage crosses: Cultures of strain C600, grown in tryptone broth at 37°C with aeration to a cell titer of 4×10^8/ml, were centrifuged, resuspended in infection medium, incubated at 37°C for 10 min, chilled in ice and infected with phage at a multiplicity of 2 for each of the two genotypes to be used in a cross, or of 4 of the one genotype to be used in a control. After 10 min, the mixture was transferred to 37°C for 6 min, diluted 2×10^3-fold into tryptone broth, aerated at 37°C for 2 hr, and chloroformed. Total phage and recombinant titers from these lysates were assayed as described in legends of Tables 2 and 3.

RESULTS

*Isolation of cold-sensitive (cs) mutants from phages λ and 434*hy*:* 29°C was chosen as selection temperature for cold-sensitive mutants because it was found to be the lowest temperature at which λ and λ*CI* mutants would form plaques with an efficiency (eop) close to 100% on host strains C600, W3110, W3350, Hfr43, and W3101 (434 *hy*) (reference: at 37°C on C600). These plaques, though they remain smaller than the ones formed at 37°C, are recognizable as either turbid or clear on plates incubated for 16 hr.

Mutagenized lysates of λ and 434*hy* phages were prepared by a procedure similar to the one used by GOTTESMAN and YARMOLINSKY (1968):

E. coli C600 was aerated in tryptone broth + 1% maltose at 37°C, obtaining a cell titer of 4×10^8/ml. Phage was added at a multiplicity of 0.5 and the mixture was incubated at 37°C for 15 min. NNG was added to a concentration of 40 μg/ml and gentle aeration started; after 10 min, the mixture was diluted 10^3-fold into tryptone broth + 1% maltose and samples of the dilution were aerated at 37°C for 90 min. The resulting lysates were treated with chloroform. About 10% of the phages in these lysates were clear mutants.

The mutagenized lysates were used to lysogenize strain C600 at 37°C in spots on tryptone-agar plates. Bacteria grown in these spots were spread to yield colonies on tryptone-agar plates incubated at 29°C. These were replicated in duplicate to plates seeeded with C600, to be incubated at 37°C and 29°C, respectively. Lysogens of prophages with a cold-sensitive mutation were recognized by the absense of a zone of lysis around their 29°C replicas, while on the 37°C control replicas such a zone was present. The cold-sensitive characters discovered in this way may be due to a block in prophage excision, or in early stages of infection, or in vegetative phage multiplication including phage maturation. Among 50,000 colonies tested, 30 turned out to be of this type. Each one has been derived from a different mutagenized lysate, thus their prophages are independent mutants.

Phenotypic characterization of cold-sensitive mutants: The phages released from each of the 30 *cs* lysogens mentioned are cold sensitive in the sense that their eop on C600 is an order of magnitude lower at 29°C than at 37°C. This was tested with lysates produced at 37°C after UV-induction from the *cs* lysogens. This avoids some of the accumulation of cs^+ revertants that always occurs if phage are allowed to undergo several cycles of infection. The results are given in Table 1, column 2, for 17 of the 30 mutants. The other mutants have been excluded from further study because they appeared too leaky and/or too easily

TABLE 1

Survey of cold-sensitive mutants

Mutant	Derived from	eop at 29°C	Titer at 29°C	Cistron	Linkage	Remarks
cs_1	λ	1.4×10^{-3}	.075	*A*	+	many *CI* derived from it are *cs*+
cs_2	λ	1.5×10^{-4}	.068	*G*	*csi*⁴*6*	not cold sensitive on W3110 : *ts*
cs_3	λ	1.1×10^{-3}	.074	*A*	+	....
cs_4	λ	1.3×10^{-4}	.059	*N*	..	*ts*
cs_5	λ	1.1×10^{-3}	.071	*A*	+	....
cs_6	λ	1.2×10^{-3}	.071	*J*	cs_8, csi^43O	....
cs_7	λ	5.0×10^{-6}	.047	*A*	cs_{16}, csi^43	tail donor
cs_8	λ	1.2×10^{-4}	.050	*J*	cs_6, csi^43O	no excess of tails, *ts*
cs_9	λ	1.4×10^{-3}	.077	*A*	+	....
cs_{10}	λ	1.0×10^{-3}	.071	*A*	+	....
cs_{12}	λ	6.0×10^{-4}	.067	*G*	csi^45	....
cs_{16}	λ	1.0×10^{-5}	.050	*A*	cs_7, csi^43	tail donor
csi^43	434*hy*	2.5×10^{-4}	.050	*A*	cs_7, cs_{16}	*ts*
csi^45	434*hy*	2.1×10^{-4}	.050	*G*	cs_{12}	*ts*
csi^46	434*hy*	2.0×10^{-4}	.050	*G*	cs_2	*ts*
csi^410	434*hy*	2.2×10^{-4}	.060	*A*	+	....
csi^430	434*hy*	1.4×10^{-3}	.060	*J*	cs_6, cs_8	....
λ-wild			.340			

eop at 29°C: Number of plaques obtained on strain C600 at 29°C, divided by the number of plaques obtained at 37°C on this strain, from UV-induced lysate of the mutant produced at 37°C.
Titer at 29°C: Titer of UV-induced lysate of the mutant produced at 29°C, divided by the titer of such lysate produced at 37°C. Data from the experiment in which the highest values of these ratios were obtained.
Cistron: Cistron on the λ map (CAMPBELL 1961) to which the mutant is assigned on the basis of mapping and complementation data.
Linkage: Mutants with which less than .005% *cs*+ recombinants were scored in two-factor crosses. + : group of mutants associated with each other and the cs_7 group through a frequency of *cs*+ recombinants in two-factor crosses not exceeding .4%, about the frequency of revertants.

reverting (eop on C600 at 29°C between 1×10^{-2} and 7.5×10^{-2}). The titers of UV-induced lysates produced at 29°C from the remaining 17 *cs* lysogens (Table 1, column 3) prove that their cold-sensitive defects all concern steps in the vegetative cycle of phage multiplication rather than the ability of mature phage to infect at low temperatures. For the mutants described in Table 1, values determined for the eop at 29°C from different lysates in general agreed within a factor of 2. For cs_1, one lysate was obtained with an eop at 29°C of 10^{-4}.

For each of the 17 mutants a few of the rare plaques at 29°C were sampled for the proportion of *cs* and cs^+ phage they contained by replating them on C600 with subsequent incubation at 37°C and at 29°C. In every case the eop at 29°C was close to one which means that the plaques seen at 29°C contained mostly or exclusively cs^+ revertants.

Some of the *cs* mutants, when tested for plaque formation at 30°C rather than 29°C, showed minute plaques in variable numbers. At 33°C, the cold-sensitive character is not revealed by any of the mutants; plaques are as large as they

TABLE 2

Recombination between cold-sensitive mutants

	cs_1	cs_2	cs_3	cs_4	cs_5	cs_6	cs_7	cs_8	cs_9	cs_{10}	cs_{12}	cs_{16}	$csi^{4}3$	$csi^{4}5$	$csi^{4}6$	$csi^{4}10$	$csi^{4}30$
cs_1	0.4	2.0	0.8	6.0	0.42	4.8	0.7	3.2	0.52	0.5	1.8	0.38	0.6	2.1	2.3	0.56	5.6
	cs_2	≦.002	1.4	2.0	1.6	0.31	1.45	0.2	1.02	1.2	0.3	0.9	1.5	0.3	≦.003	1.2	0.8
		cs_3	0.4	6.0	0.5	4.0	0.5	4.0	0.35	0.45	3.8	0.4	0.5	4.0	2.3	0.5	4.3
			cs_4	≦.003	3.0	2.0	3.0	1.7	3.8	4.0	2.7	3.2	4.5	2.5	2.5	4.1	3.0
				cs_5	≦.005	2.3	0.3	1.8	0.51	0.3	2.0	≦.005	0.08	1.8	1.5	0.1	2.0
					cs_6	≦.002	2.0	≦.002	1.45	1.6	0.3	2.5	2.8	0.3	0.3	2.9	0.5
						cs_7	≦.002	1.5	0.48	0.2	1.6	≦.002	≦.003	1.2	1.5	0.09	1.8
							cs_8	≦.003	1.25	2.1	0.3	1.3	1.9	0.2	0.21	2.6	0.4
								cs_9	0.2	0.68	0.98	0.46	0.32	1.1	1.0	0.41	1.3
									cs_{10}	0.2	1.7	0.21	0.3	2.0	1.7	0.31	2.7
										cs_{12}	≦.002	1.0	1.8	≦.002	0.2	2.0	0.6
											cs_{16}	≦.002	≦.003	1.2	1.0	0.09	2.2
												$csi^{4}3$	≦.003	1.6	1.7	0.1	2.3
													$csi^{4}5$	≦.003	0.3	2.2	0.9
														$csi^{4}6$	≦.003	1.5	0.7
															$csi^{4}10$	0.02	2.8
																$csi^{4}30$	0.42

cs+ recombinants were scored as plaques on strain C600 at 29°C formed by lysates from 2-factor crosses. Titers of these lysates were assayed on C600 at 37°C. Numbers represent percent of *cs*+ recombinants.

are upon 37°C incubation, in most cases equal in size to λ wild-type plaques. The 12 mutants derived from λ have also been tested for their *cs* character on strains W3110, W3350, Hfr43, and W3101 (434*hy*). Their plating properties on each of these strains were not different from those on C600 with one exception: cs_2 is not cold sensitive on W3110.

Two-factor crosses of cs *and* sus *mutants:* To map the 17 *cs* mutants, they were crossed pairwise against each other and against reference markers, namely, the immunity region of λ and some of the mutants of CAMPBELL (1961). The frequencies of cs^+, cs^+i^λ and cs^+ sus^+ recombinants and the respective frequencies of revertants in the controls are given in Tables 2 and 3.

The results of all pairwise crosses of the *cs* mutants define groups of *cs* mutants as follows: within a group, recombination frequencies are too low to be determined (< .005%), between groups, they are at least 0.2%. A prerequisite for assigning a mutant to one particular group is a sufficiently low frequency of revertants in the self-cross (control). In a way as yet unexplained, these frequencies were much lower for several mutants in the lysate produced by the cross than in the one entering it. These mutants are cs_2, cs_4, cs_5, cs_6, cs_8, cs_{12}, csi^43, csi^45, csi^46. The condition is not fulfilled with mutants cs_1, cs_3, cs_{10}, and csi^430; they and csi^410 can be associated with the group cs_7–cs_{16}–csi^43 on account of their relatively low recombination frequencies with the latter (Table 2; Table 1, column headed Linkage).

The recombination frequencies given in Table 3 were obtained for each *cs* mutant in crosses with all the available *sus* mutants using the same bacterial culture. This has eliminated one possible source of quantitative variability encountered in λ crosses. We did not make an attempt to quantitatively validate the results given in Table 3 by a statistics over repeated crosses. *sus* A_{11} was not used in our crosses because in self-crosses it would always produce more than 1% revertants. Self-crosses of all the other *sus* mutants yielded less than 10^{-3}% revertants.

Complementation tests of cs mutants: The mapping data given above determine in general terms the location of each *cs* mutant on the genetic map of λ, but in no case are they sufficient to decide whether a particular *cs* mutant belongs to a particular known cistron or not. Attempts to obtain this knowledge by the complementation test of CAMPBELL (1961), adapted to score *sus* against *cs* mutants by observing mixed spots on the nonpermissive host W3350 incubated at the nonpermissive temperature 29°C, mostly failed because of cs^+ revertant phages overgrowing the spots. It could only be shown that mutants cs_7 and cs_{16}, the *cs* mutants with by far the lowest reversion rates, would not complement *sus* A_{11} (nor each other) but all the other *sus* phages tested (it was verified that our lysate of *sus* A_{11} would complement *sus* B_1 and *sus* J_6). Thus mutants cs_7 and cs_{16} belong to cistron *A*.

A different complementation test was found adequate to assign the other *cs* mutants to known cistrons: Double lysogens of two *cs* mutants, or a *cs* and a *sus* mutant in a pm^- host were constructed. If such a double lysogen is able to yield substantially higher phage titers in a UV-induced lysate prepared at 29°C than

TABLE 3

Recombination of cold-sensitive mutants with suppressor-sensitive mutants and the i^{434} *region*

	Self-cross	B_1	C_{39}	D_{123}	E_4	G_9	H_{12}	K_{24}	I_2	J_6	N_7	O_8	P_3	Q_{73}	R_5	i^{434}
cs_1	0.40	0.73	1.08	1.98	2.00	2.28	2.70	2.00	2.90	2.0	2.32	7.45	7.6	10.0	11.4	10.50
cs_2	$\leqq$.002	0.20	0.15	0.12	0.10	0.09	0.02	0.08	0.10	0.14	0.63	0.64	0.66	0.71	0.74	1.30
cs_3	0.40	0.45	0.53	0.81	2.00	2.94	7.2	5.90	6.00	5.90	6.90	7.10	9.00	15.00	16.5	4.50
cs_4	$\leqq$.003	1.40	1.50	1.50	1.60	1.47	1.14	0.69	0.60	0.86	0.05	0.31	0.37	1.00	1.10	0.10
cs_5	$\leqq$.005	0.54	1.06	1.45	1.70	2.14	3.90	4.10	2.40	2.60	3.20	5.00	6.90	6.20	5.00	2.00
cs_6	$\leqq$.002	1.87	2.37	2.87	2.60	2.36	1.14	1.50	0.82	1.04	1.96	3.22	3.08	2.50	3.50	1.30
cs_7	$\leqq$.002	0.21	0.22	0.40	0.42	0.42	0.69	0.60	0.83	1.06	0.81	2.15	3.70	1.15	2.20	1.40
cs_8	$\leqq$.003	0.93	1.00	1.05	0.80	0.69	0.39	0.20	0.05	0.04	0.81	2.10	1.80	2.00	1.50	2.40
cs_9	0.20	0.37	0.56	2.00	3.00	4.00	13.00	11.00	12.00	18.30	13.00	8.20	8.00	5.20	7.30	11.20
cs_{10}	0.20	0.37	0.39	0.42	0.75	1.05	1.08	1.46	1.17	1.35	1.17	2.00	3.60	2.40	2.34	1.70
cs_{12}	$\leqq$.002	0.55	0.35	0.32	0.76	0.05	0.94	0.63	0.51	1.05	0.51	0.52	0.52	1.20	0.93	0.67
cs_{16}	$\leqq$.002	0.70	0.70	0.75	0.80	0.86	1.15	1.00	1.20	2.30	1.70	3.10	1.50	1.75	3.60	1.70
csi4 3	$\leqq$.002	0.66	1.74	1.79	2.00	3.55	3.53	2.80	2.94	3.07	8.44	13.00	10.60	12.30	19.4	...
csi4 5	$\leqq$.003	0.23	0.58	0.38	0.20	0.03	0.22	0.32	0.50	0.40	1.40	1.30	1.20	1.16	1.08	...
csi4 6	$\leqq$.003	0.51	0.37	0.60	0.45	0.11	0.09	0.35	0.50	0.69	2.00	2.10	2.20	3.60	4.00	...
csi4 10	0.02	0.17	0.29	0.27	0.30	0.28	0.37	0.47	0.60	0.72	1.57	1.90	2.10	1.23	1.36	...
csi4 30	0.42	1.20	1.10	1.00	0.90	0.80	0.75	0.69	0.60	0.49	2.63	4.20	4.76	2.84	3.13	...

Columns B_1 to R_5 : *sus* mutants used in cross (see MATERIALS AND METHODS). Numbers represent percentage of sus^+cs^+ recombinants obtained in 2-factor crosses (titer on W3350 at 29°C, divided by titer on C600 at 37°C) × 100.

Column i^{434} : Cross with 434*hy*. Numbers represent percentage of cs^+i λ recombinants (titer on W3101 (434*hy*) at 29°C, divided by titer on C600 at 37°C) × 100.

either corresponding single lysogen, complementation between the prophages is positive. Table 4 shows the results of such experiments.

As expected, complementation is always positive between well-separated mutants as shown by the mapping data, and often negative between closely linked mutants. That positive complementation where it is observed is genuine, rather than a result of recombination in the prophage or during vegetative development, is shown by the fact that at least 99% of the phages contained in the lysate are cold sensitive. The data of Table 4 show that all our *cs* mutants fail to complement either one *sus* mutant each, or a *cs* mutant already assigned to a complementation group. Lysates from several *sus* mutants in the nonpermissive host W3350 were examined and the resulting phage titers were found to be much lower than those of the best *cs* mutants. For example, a W3350 (*sus* B_1) lysate at 29°C showed a titer of 7.0×10^2/ml. Identifying our complementation groups with the cistrons defined by CAMPBELL (1961), the data taken together show that our *cs* mutants belong to cistrons *A*, *G*, *J* and *N* according to Table 1, column headed Cistron.

In earlier experiments, we had used lysates of cs_7, cs_{16}, and cs_8 in an *in vitro* complementation test according to WEIGLE (1966). It was found that cs_7 and cs_{16} are defective in head formation, cs_8 defective in tail formation. As this only confirms the more precise information given above, we do not present details of these experiments.

Mutants simultaneously cold and temperature sensitive, or cold and suppressor sensitive: CAMPBELL has found 19 out of his 149 suppressor-sensitive mutants to be temperature sensitive in a permissive host (CAMPBELL 1961). We have tested the *sus* mutants used in this work for their ability to form plaques at 29°C on C600. The results are given in Table 5. The cold-sensitive *sus* mutants revert to cs^+ with low frequency, comparable to the reversion rate to sus^+. Also, all the cs^+ revertants checked turned out to be sus^+. Each one of the 30 *cs* mutants isolated according to the replication pattern of their C600 lysogens on C600 also plates on W3110. Thus cold-sensitive *sus* mutants, which could have been detected by our procedure, seem to occur much less frequently than other *cs* mutants.

Our cold-sensitive mutants were tested for their ability to form plaques at 42°C on W3110. Surprisingly, 6 out of 17 turned out to be temperature sensitive (Table 1, column 6). From these, revertants were selected at 29°C and 42°C (Table 6). The fact that, for each of the *ts–cs* mutants, revertants are found that are phenotypically ts^+cs^+ proves the *ts* and the *cs* characters were due to the same mutation. For cs_8, csi^43, csi^45, and cs_6 there are also revertants with respect to one sensitivity only. These must be due to a suppressor mutation.

DISCUSSION

The screening procedure used in isolating our cold-sensitive mutants of phage λ appears to be nonselective: All mutations leading to a cold-sensitive character in functions necessary for prophage excision or required in the infective cycle of

TABLE 4

Complementation in double lysogens

Strain	Lysate produced at	Titer on : (× 10^{-5})			Complementation
		C600	C600 (λ)	C600 (434*hy*)	
C600 (λ wild)	37°C	63,000	0	53,000	. .
	29°C	2,500	0	2,400	. .
C600 (cs_1)	37°C	39,000	. .	. .	. .
	29°C	26	. .	. .	. .
W3350 (cs_1)	37°C	2,700	. .	. .	. .
	29°C	4.4	. .	. .	. .
W3350 (cs_2)	29°C	12	. .	. .	. .
C600 (cs_3)	37°C	39,000	. .	. .	. .
	29°C	38	. .	. .	. .
C600 (cs_4)	37°C	39,000	. .	. .	. .
	29°C	3.0	. .	. .	. .
W3110 (cs_4)	29°C	1.0	. .	. .	. .
C600 (cs_5)	37°C	46,000	. .	. .	. .
	29°C	8.7	. .	. .	. .
W3350 (cs_6)	29°C	30	. .	. .	. .
C600 (cs_7)	37°C	40,000	. .	. .	. .
	29°C	0.29	. .	. .	. .
C600 (cs_8)	37°C	20,000	. .	. .	. .
	29°C	1.9	. .	. .	. .
W3350 (cs_8)	29°C	9.6	. .	. .	. .
C600 (cs_9)	37°C	41,000	. .	. .	. .
	29°C	58	. .	. .	. .
C600 (cs_{10})	37°C	37,000	. .	. .	. .
	29°C	56	. .	. .	. .
W3350 (cs_{12})	29°C	17	. .	. .	. .
C600 (cs_{16})	37°C	42,000	. .	. .	. .
	29°C	0.88	. .	. .	. .
C600(*csi43*)	37°C	19,000	21,000	0	. .
	29°C	2.9	2.8	0	. .
W3350 (*csi45*)	29°C	4.5	. .	. .	. .
W3350 (*csi46*)	29°C	0.9	. .	. .	. .
C600 (*csi410*)	37°C	32,000	. .	. .	. .
	29°C	6.0	5.6	0	. .
W3350 (*csi430*)	29°C	5.5	. .	. .	. .
C600 (*csi43*, cs_1)	37°C	54,000	18,000	22,000	. .
	29°C	8.0	3.1	5.5	—
C600 (*csi43*, cs_3)	37°C	81,000	22,000	56,000	. .
	29°C	18	6.0	12	—
C600 (*csi43*, cs_4)	37°C	67,000	20,000	44,000	. .
	29°C	440	220	280	+
C600 (*csi43*, cs_5)	37°C	48,000	15,000	44,000	. .
	29°C	5.9	1.4	4.5	—
C600 (*csi43*, cs_7)	29°C	4.6	2.4	2.0	—
C600 (*csi43*, cs_8)	37°C	17,000	1,000	13,000	. .
	29°C	240	72	150	+
C600 (*csi43*, cs_9)	37°C	50,000	13,000	32,000	. .
	29°C	15	3.5	8.5	—

TABLE 4—Continued

Complementation in double lysogens

Strain	Lysate produced at	Titer on : (× 10^{-5}) C600	C600 (λ)	C600 (434*hy*)	Complementation
C600 (*csi*434, cs_{10})	29°C	8.0	6.9	1.6	—
C600 (*csi*434, cs_{16})	29°C	3.6	1.2	1.7	—
C600 (*csi*434, λ wild)	37°C	22,000	10,000	12,000	. .
	29°C	1,700	3,600	1,100	+
C600 (*csi*410, cs_7)	29°C	2.0	.95	1.0	—
C600 (*csi*410, cs_8)	29°C	120	52	98	+
W3350 (*csi*45, cs_2)	29°C	7.3	3.2	2.6	—
W3350 (*csi*45, cs_{12})	29°C	4.7	2.2	2 8	—
W3350 (*csi*45, cs_8)	29°C	330	130	210	+
W3350 (*csi*45, *sus* G_9)	29°C	5.5	3.2	1.4	—
W3350 (*csi*45, *sus* H_{12})	29°C	45	31	18	+
W3350 (*csi*45, *sus* I_2)	29°C	160	91	100	+
W3350 (*csi*45, *sus* J_6)	29°C	120	71	52	+
W3350 (*csi*45, *sus* K_{24})	29°C	200	100	120	+
W3350 (*csi*46, cs_1)	29°C	512	276	288	+
W3350 (*csi*46, cs_2)	29°C	3.0	1.5	2.2	—
W3350 (*csi*46, cs_{12})	29°C	4.2	1.9	2.2	—
W3350 (*csi*46, cs_8)	29°C	400	120	200	+
W3350 (*csi*46, *sus* G_9)	29°C	2.3	1.7	1.1	—
W3350 (*csi*46, *sus* H_{12})	29°C	28	20	12	+
W3350 (*csi*46, *sus* I_2)	29°C	94	42	51	+
W3350 (*csi*46, *sus* J_6)	29°C	240	170	90	+
W3350 (*csi*46, *sus* K_{24})	29°C	83	36	42	+
W3350 (*csi*430, cs_6)	29°C	9.5	4.5	4.3	—
W3350 (*csi*430, cs_8)	29°C	6.2	1.6	3.8	—
W3350 (*csi*430, cs_{12})	29°C	290	100	150	+
W3350 (*csi*430, *sus* G_9)	29°C	220	150	98	+
W3350 (*csi*430, *sus* H_{12})	29°C	200	110	89	+
W3350 (*csi*430, *sus* I_2)	29°C	41	28	13	+
W3350 (*csi*430, *sus* J_6)	29°C	5.1	2.5	3.1	—
W3350 (*csi*430, *sus* K_{24})	29°C	87	36	49	+
W3110 (i^{434} *sus* N_7)	29°C	0.01	. .	. .	. .
W3110 (*sus* N_7)	29°C	0.008	. .	. .	. .
W3110 (*sus* N_{53})	29°C	0.01	. .	. .	. .
W3110 (cs_4, i^{434} *sus* N_7)	29°C	2.0	0.7	1.2	—
W3110 (cs_4, *sus* N_7)	29°C	1.2	. .	. .	—
W3110 (cs_4, *sus* N_{53})	29°C	2.1	. .	. .	—
W3350 (cs_1, *sus* B_1)	29°C	98.0	. .	. .	+

the phage should have an equal chance to be detected. A conceivable exception would concern mutations which, as a side effect, would have a low probability of being established as prophage at 37°C or of being propagated as prophage (should such mutations unexpectedly exist). Out of the mutants originally de-

TABLE 5

Cold-sensitive sus *mutants*

	37°C	29°C
sus A_{11}	+	—
sus B_{1}	+	—
sus C_{39}	+	—
sus D_{123}	+	+
sus E_{4}	+	+
sus G_{9}	+	+
sus H_{12}	+	—
sus I_{2}	+	—
sus J_{6}	+	(+)
sus K_{24}	+	—
sus N_{7}	+	(+)
sus N_{53}	+	(+)
sus O_{8}	+	(+)
sus O_{29}	+	(+)
sus P_{3}	+	+
sus Q_{73}	+	—
sus Q_{117}	+	—
sus R_{5}	+	+
sus R_{54}	+	+

Formation of plaques on C600
+ : formation of normal size plaques
(+) : formation of minute plaques
— : no plaque formation

tected, 13 were eliminated on the basis of their high leakiness only. The 17 mutants that were studied should therefore be an unbiased sample of the spectrum of cold-sensitive mutants.

These 17 mutants belong to only 4 cistrons, all of which had been defined previously as suppressor-sensitive and some also as temperature-sensitive mutants (CAMPBELL 1961; BROWN and ARBER 1964; PARKINSON 1968): 9 mutants belong to cistron *A* required for head formation; among them, at least 4 are different from each other because $csi^{4}10$ has been separated from cs_7 by recombination, and because some of the mutants (e.g., cs_3) possess a much higher reversion rate than these two, while cs_1 shows the unique property of being suppressed by *cI* mutants. This shows that the high incidence of cold-sensitive mutants in cistron *A* cannot be due to a single mutational hot spot.

It is likely that the accumulation of mutants in this cistron reflects different possible alterations in the *A* gene product that render its function cold sensitive, though mutants belonging to the same cistron as defined by the complementation test do not necessarily lie in the same structural gene. A *cis*-dominant defect in the synthesis of the same gene product could be present instead. Four mutants belong to cistron *G*, involved in tail formation. Here also at least two different sites are represented because $csi^{4}5$ and $csi^{4}6$ have been separated by recombination;

TABLE 6

Cold-sensitive–temperature-sensitive mutants

				cs^+ : selection at 29°C			ts^+ : selection at 42°C	
cs–ts mutant	eop at 29°C	eop at 42°C	4	eop at 29°C	eop at 42°C	7	eop at 29°C	eop at 42°C
cs_2	1.5×10^{-4}	4.9×10^{-3}	1	0.30	0.97	3	0.83	0.86
			2	0.32	0.73	4	1.10	0.94
cs_4	1.3×10^{-4}	1.4×10^{-3}	1	0.76	0.84	3	1.06	1.03
			2	0.81	0.87	4	1.04	0.97
cs_8	1.2×10^{-4}	3.7×10^{-3}	1	0.79	0.006	3	0.50	0.31
			2	0.80	0.011	4	0.63	0.16
csi⁴3	2.5×10^{-4}	5.3×10^{-4}	1	0.95	0.035	3	1.00	0.10
			2	0.86	0.43	4	1.10	0.18
csi⁴5	2.1×10^{-4}	6.2×10^{-5}	1	0.30	0.25	3	0.027	0.61
			2	0.66	0.60	4	0.004	0.60
						5	0.008	0.43
						6+	≤0.005	0.19
						7+	≤0.001	0.21
						8+	≤0.001	0.99
csi⁴6	2.0×10^{-4}	6.4×10^{-6}	1	0.66	0.68	3	0.15	0.94
			2	1.00	0.73	4	0.072	0.74
						5	0.065	0.74
						6+	0.061	0.25
						7+	0.12	0.031
						8+	0.14	0.89

All platings on C600, eop at 29°C and at 42°C (column 2 and 3): number of plaques formed at these temperatures, relative to the titer determined at 37°C, by phages from UV-induced lysates produced at 37°C.

Columns 4 and 7: Isolation numbers of $cs+$ and $ts+$ revertants, respectively, from *cs* mutants listed in column 1. Columns 5, 6, 8, 9: eop's determined as described above but with phage from a $cs+$ or $ts+$ revertant plaque.

+ : revertants forming small plaques on C600 at 37°C.

moreover cs_2 is different from both, being cs^+ on W3110. Three mutants belong to cistron *J*, also involved in tail formation and known to code for a structural component of the mature phage particle (DOVE 1966). One mutant belongs to cistron *N*, involved in early control of λ.

Thus nine mutants belong to one cistron concerning head formation while at least six others are not represented (see PARKINSON 1968; DOVE 1968). No gene mapping to the right of the immunity region is represented, nor are the *int A* and *xis* genes. *cs* mutants in these latter genes should have been detected by the replication procedure of the lysogens because both the *int A* function and the *xis* function are required on prophage excision (GINGERY and ECHOLS 1967; GOTTESMAN and YARMOLINSKY 1968; GUARNEROS and ECHOLS 1970).

The finding that 6 out of 17 *cs* mutants were also temperature sensitive was unexpected. It presents difficulties for an assumption that temperature sensitivity is generally due to changes that deprive the mutant protein of conformational stability, while cold sensitivity would mean the inability of the mutant protein to undergo certain conformational changes. The latter assumption would have appeared plausible not only from the work of O'DONOVAN and INGRAHAM (1965), showing that the cold-sensitive character of an *E. coli* mutant (requirement for histidine at 20°C) is due to an enhanced feedback inhibition of its phosphoribosyl-ATP-pyrophosphorylase by histidine, but also from the recent findings of GUTHRIE, NASHIMOTO and NOMURA (1969) concerning ribosome assembly in cold-sensitive mutants to *E. coli*.

That certain mutants form plaques only in a narrow interval around an optimum growth temperature may turn out not to depend on particular physicochemical properties of the gene product involved but rather on the rate of synthesis of this product in relation to other gene products. If the gene product in question is made in relative abundance in the optimum temperature interval, plaque formation in that temperature interval may still be possible in spite of an inferior product or a reduction in its rate of synthesis. Some *sus* mutants in particular could be cold sensitive, not because the gene product may be altered (amino acid substitution, according to the amber suppressor present), but because suppression becomes quantitatively insufficient at lower temperature. There is no reason to assume that the occurrence of *cs–ts* mutants is restricted to phage λ. With the selection employed in other cases [e.g., of phage T4 (SCOTTI 1968; EDGAR and LIELAUSIS 1964)], they would not have been detected.

In discussing the high frequencies of revertants observed with *cs* mutants, a technical problem has to be considered first. If the block of phage formation in a cold-sensitive mutant is not absolute, not only revertants already present in the phage suspension plated but also some arising after infection in the plate would form plaques. The majority of revertant plaques observed could be due to this mechanism only if plaques of all sizes down to the smallest detectable ones were present in comparable numbers, which is not the case. In the case of the cold-sensitive *sus* mutants, there is direct evidence that the formation of cs^+ revertants on the plate (in a permissive host at 29°C) does not interfere with the determination of the frequency of revertants: The number of cs^+ revertants scored at 29°C does not exceed the number of sus^+ revertants at 37°C. As there does not seem to be a major artifact involved in the determination of high reversion rates concerning plaque formation at 29°C, and as there is no reason to assume a greatly enhanced rate of true reversion specifically for cold-sensitive mutants, the explanation for the high apparent reversion rate must be found in a high probability of compensating further mutations, so-called suppressor mutations. Cold-sensitive mutants of bacteriophage φX 174 have also been observed to revert with high frequency (DOWELL 1967).

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SUMMARY

Cold-sensitive mutants of bacteriophage λ isolated by a nonselective procedure were found clustered in cistrons *A*, *G*, and *J*. One mutant was found in cistron *N*.

About one-third of the mutants are simultaneously cold and temperature sensitive. Mutants suppressing cold-sensitive phenotypes seem to occur with high frequency.

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