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Complementation Analysis of Measles Virus Temperature-Sensitive Mutants

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Two sets of independently isolated measles virus temperature-sensitive mutants were quantitatively tested for complementation. Analysis of the nine possible combinations of representative mutants indicated that only one pair of mutants is noncomplementing. Thus, the measles virus mutants studied to date define five complementation groups.

Two sets of temperature-sensitive (ts) mutants of measles virus have been isolated and partially characterized (1, 6). Bergholz et al. (1) divided their mutants into three complementation groups (A, B, and C); similarly, Haspel et al. (6) classified their mutants into three complementation groups (I, II, and III). To further our understanding of the gene products involved in measles virus replication, a collaborative complementation study was undertaken. Our results indicate that five complementation groups are represented among the two sets of mutants.

Two different methods to test for complementation were used in this study. Method A basically followed the procedure of Haspel et al. (6). The mutants to be tested were incubated at 25°C for 30 min in the presence of 3 μg of poly-L-ornithine per ml (molecular weight, >165,000; Sigma Chemical Co.) either in various combinations or singly. The samples of treated virus were then inoculated onto monolayers of BSC-1 cells (originally obtained from R. Dulbecco, Salk Institute, La Jolla, Calif.) in 35-mm plastic petri dishes at input multiplicities of 0.1 to 1.0 PFU/cell. The infected cells were incubated at 39°C and observed for the development of measles virus cytopathic effect. At 48 to 72 h, the infected cells were scraped off the plates, and the suspension was sonicated. The infectious virus was then assayed by the plaque method (6) at 33.5 and 39°C. The index of complementation (4) was calculated as follows: [yield $(x + y)$ 33.5°C − yield $(x + y)$ 39°C]/[yield (x) 33.5°C + yield (y) 33.5°C]. A complementation index greater than one was considered to be positive.

Method B was that of Bergholz et al. (1). Samples of virus, individual mutants, or various pairs of mutants were inoculated onto monolayers of Vero cells (originally obtained from the Naval Biological Research Laboratory, Oakland, Calif.) in small (well area, 12 mm^2) Leighton tubes at a multiplicity of 0.2 to 2.0 PFU/cell. After a 2-h adsorption period at 33°C, the cell monolayers were rinsed with balanced salt solution, growth medium was added, and the cultures were incubated at 33°C for 5 h. Subsequent incubation was at 39°C. When doubly infected cells showed 75% measles virus cytopathic effect, they were frozen and thawed along with their corresponding singly infected cultures. Virus yields were determined by plaque assay in Vero cells at 33 and 39°C. The index of complementation was calculated as in method A.

One representative mutant was used from each complementation group previously identified independently by our laboratories. A total of nine possible combinations was analyzed (Table 1). To simplify the nomenclature used to identify mutants in this study, numerical designations (101-124) were substituted for the alphabetical designations (A-X) used by Haspel et al. (6). As previously, designations 1 through 9 were used for mutants isolated by Bergholz et al. (1).

Complementation was observed between ts-120 and both ts-1 and ts-9. As indicated by complementation indexes of less than one, ts-120 and ts-4 definitely did not complement. Thus, group I mutants of Haspel et al. (6) and group B mutants of Bergholz et al. (1) belong to the same complementation group. This is consistent with the similarities in the phenotypic properties of these mutants (1, 6). With one exception (i.e., ts-8 of Bergholz et al. [1]), mutants in this complementation group (designated group B) are defective in virus-specific RNA and antigen synthesis at 39°C (Table 2).

For reasons presently unknown, an unusually large proportion (28/33) of the ts mutants isolated to date belong to this complementation group.

ts-107 clearly complemented all three mutants against which it was tested. ts-107 is currently the only available mutant in this complementation group, which we have designated group C. Like group B mutants, ts-107 is defective in virus-specific RNA and antigen synthesis at 39°C; however, ts-107 shows no increased thermolability (Breschkin and Rapp, unpublished observations) and retains significant neurovirulence (2, 7).

TABLE 1. *Complementation analysis of measles virus mutants*

Mutant combinations[a]	Complementation index[b]	
	Method A	Method B
ts-120		
ts-1	4.5	>490
ts-4	<0.1	<0.5
ts-9	1.0	3.0
ts-103		
ts-1	0.3	4
ts-4	0.7	54
ts-9	0.7	18
ts-107		
ts-1	25.0	>870
ts-4	380.0	>1,600
ts-9	200.0	>4,000

[a] Representative mutants (ts-120, ts-103, and ts-107) of complementation groups I, II, and III from Haspel et al. (6). Representative mutants (ts-1, ts-4, and ts-9) of complementation groups A, B, and C from Bergholz et al. (1).

[b] Complementation indexes were calculated from data obtained using the two methods described in the text.

Difficulty was encountered in testing ts-103 for complementation, particularly with method A. This difficulty may have been due to the characteristically low titers of virus obtained when growing stocks of ts-103. Preliminary results with method B indicate that ts-103 complements ts-4, ts-9, and probably ts-1. These results are consistent with the obvious phenotypic differences between ts-103 and the other mutants (Table 2). Therefore, we have tentatively assigned ts-103 to complementation group E.

ts-1 and ts-9 (1) both complemented all three mutants against which they were tested, although the complementation indexes in two cases (ts-1 versus ts-103 and ts-9 versus ts-120) were rather low. For the present, we have assigned ts-1 to complementation group A and ts-9 to complementation group D. Group A is a third RNA- and antigen-negative complementation group (Table 2). The group D mutant is apparently defective in a function required for virus-induced hemadsorption.

ts mutants of two other paramyxoviruses, Sendai virus and Newcastle disease virus, have previously been isolated and characterized (3, 10, 12). Complementation tests divided the Sendai virus mutants into seven groups. Two independently isolated sets of Newcastle disease virus mutants were divided into nine (10) and five (12) complementation groups. The genomes of Sendai virus, Newcastle disease virus, and measles virus have molecular weights of approximately 6×10^6 (5, 8, 9, 11) and may contain enough information for eight or ten polypeptides. Thus, results of this investigation indicate that the two sets of measles virus mutants studied represent only five of a possible total of eight or ten complementation groups.

TABLE 2. *Characteristics of mutant complementation groups*

Complementation group[a]	Representative mutants	Thermolability (°C)	In BSC-1 or Vero cells at 39°C					Neurovirulence in newborn hamsters
			Virus-specific RNA	Nucleocapsid antigen	Hemadsorption	Hemolysin antigen	Syncytia	
A (A)	ts-1[b]	+ (41)	−	−	−	NT[c]	−	−
B (B) (I)[d]	ts-4[b]	+ (41)	−	−	−	NT	−	−
	ts-120[e]	+ (50)	−	−	−	−	−	−
C (III)	ts-107[e]	− (50)	−	−	−	−	−	Decreased with hydrocephalus
D (C)	ts-9[b]	+ (41)	+	+	−	NT	±	−
E (II)	ts-103[e]	+ (50)	+	+	+	−	−	+

[a] Former designations used by Bergholz et al. (1) and Haspel et al. (6) are indicated in parentheses.

[b] From Bergholz et al. (1)

[c] NT, Not tested.

[d] ts-8 of Bergholz et al. (1) appears to belong to group B, although it produces virus-specific RNA and antigen at 39°C.

[e] From Haspel et al. (6).

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