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Genetic Variation During Persistent Reovirus Infection: Presence of Extragenically Suppressed Temperature-Sensitive Lesions in Wild-Type Virus Isolated from Persistently Infected L Cells

RAFI AHMED,[1] P. R. CHAKRABORTY,[1]† A. F. GRAHAM,[2] R. F. RAMIG,[1]‡ AND B. N. FIELDS[1,3]*

Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts 02115[1]; Department of Biochemistry, McGill University, Montreal, Quebec, Canada H3G 1Y6[2]; and Department of Medicine (Infectious Disease), Peter Bent Brigham Hospital, Boston, Massachusetts 02115[3]

Persistent reovirus infection of L cells was established with a serially passaged stock of temperature-sensitive (*ts*) mutant C(447) containing greater than 90% defective interfering particles. Within a month after establishment of the carrier culture, the *ts* mutant was replaced by virus that expressed the wild-type (ts^+) temperature phenotype (R. Ahmed and A. F. Graham, J. Virol. **23**:250-262, 1977). To determine whether the ts^+ phenotype of the virus was due to intragenic reversion or to the presence of an extragenic mutation suppressing the original *ts* defect, several clones were backcrossed to wild-type reovirus, and the progeny of each cross were screened for temperature sensitivity. The results indicated that the original *ts*C lesion had reverted. However, in two of the seven clones examined, new *ts* lesions were found. These new *ts* lesions appeared phenotypically as ts^+ due to the presence of extragenic suppressor mutations. Temperature-sensitive mutants representing three different groups were rescued from one suppressed clone, indicating that this ts^+ clone contained multiple *ts* lesions. Among the *ts* mutants rescued were the initial isolates of a new recombination group which we have designated H. Some of the *ts* mutants rescued from the suppressed clones are capable of interfering with the growth of wild-type reovirus and may play a role in maintaining the carrier state. The results of this study show that persistently infected L cells contain a genetically heterogeneous population of reovirus even though all virus clones express the ts^+ phenotype. It is thus critical to distinguish between genotype and phenotype when analyzing viruses that emerge during persistent infection.

Many animal viruses, including some that are highly cytocidal, can establish persistent infections in cell culture (9). DNA viruses, and RNA viruses containing reverse transcriptase, can persist either by integrating viral DNA into the host DNA, or by forming circular extrachromosomal plasmids. The mechanisms of persistence of virulent RNA viruses that multiply in the cytoplasm are not completely understood, but three factors have been implicated in regulating viral growth and allowing the carrier state to be maintained. These are (i) the generation of temperature-sensitive (*ts*) mutants that grow poorly at normal incubation temperature (16), (ii) the appearance of defective interfering particles that lack part of the viral genome and suppress growth of infectious virus (10), and (iii) production of interferon, which reduces the yield of infectious virus (20). These factors, separately or in association, play a role in maintaining a balance between viral and cellular growth.

Viruses selected during persistent infection often differ from the virus used to initiate the infection. Temperature-sensitive mutants have been recovered from carrier cultures initially infected with wild-type (ts^+) Newcastle disease virus, Sendai virus, Sindbis virus, measles virus, and vesicular stomatitis virus (7, 13, 15, 16, 22, 23). Small-plaque mutants have been selected in cells persistently infected with vesicular stomatitis virus, respiratory syncitial virus, and simian virus 40 (11, 14, 17). Holland et al. have shown by oligonucleotide mapping that the vesicular stomatitis virus genome changes extensively during a long-term persistent infection of BHK cells (8). Thus, there are numerous examples of *ts* and other mutants emerging during persistent infections. When persistent infections are estab-

† Present address: Liver Research Center, Albert Einstein College of Medicine, Yeshiva University, Bronx, NY 10461.
‡ Present address: Department of Virology and Epidemiology, Baylor College of Medicine, Houston, TX 77030.

lished with *ts* mutants of reovirus, the situation is unusual, since the virus acquires a wild-type (ts^+) phenotype (2). The presence of a ts^+ phenotype, however, does not prove that the genotype of the virus is wild type. A *ts* mutant can acquire wild-type phenotype by true reversion, i.e., reverse mutation at the same nucleotide as the original mutation, or by pseudoreversion. Pseudorevertants can arise as a result of a second mutation in the same gene as the original mutation (intragenic suppression), or a mutation in some other gene that suppresses the original *ts* lesion (extragenic suppression). Since the reovirus genome is segmented and recombination takes place by reassortment of genome segments (21), it is easy to detect the presence of extragenic suppressor mutations. However, it has not been possible to distinguish between intragenic suppression and "true" reversion by genetic analysis, since intragenic recombination has never been detected in reovirus (5, 21). Thus, we will use the term "intragenic reversion" to indicate both true reversion and intragenic suppression. Ramig and Fields have shown that extragenic suppression is the major way that *ts* mutants of reovirus revert to a ts^+ phenotype (18, 19). This finding prompted us to check whether the ts^+ phenotype of virus isolated from the persistently infected cells was due to the presence of extragenic suppressor mutations or due to intragenic reversion.

In the present study we have analyzed a number of ts^+ clones isolated from the carrier cells for the presence of extragenic suppressor mutations. The results indicate that although the original *ts*C lesion appears to have reverted, some of the ts^+ clones contained suppressed *ts* lesions. Among the *ts* mutants rescued from the suppressed clones were members of a new recombination group, which we have designated *ts*H. In addition we found that some of the *ts* mutants interfere with the growth of wild-type virus. (A preliminary report of this work was presented at the 1978 ICN-UCLA Symposia on Molecular and Cellular Biology [6].)

MATERIALS AND METHODS

Cells and virus. L cells were grown in suspension or as monolayers in Joklik's modified Eagle minimal essential medium supplemented with 5% fetal calf serum. The wild-type Dearing strain of reovirus type 3 and *ts* mutants were from the laboratory stock collection. The *ts* mutants were isolated and described by Fields and Joklik (5) and Cross and Fields (4). The following recombination group prototype mutants were used; group A, *ts*A(201); group B, *ts*B(352); group C, *ts*C(447); group D, *ts*D(357); group E, *ts*E(320); group F, *ts*F(556); and group G, *ts*G(453). All viruses were plaque purified and passaged twice on L-cell monolayers. Second-passage virus was used in all experiments unless otherwise indicated.

L cells persistently infected with reovirus. The carrier cell line used in this study was originally infected with a serially passaged stock (passage 9) of *ts*C(447) (2). These cells, designated L/C447, were grown as monolayers in Joklik's modified Eagle minimal essential medium supplemented with 5% fetal calf serum. The L/C447 cell line was propagated at 37°C for a period of 1.5 years. Subcultures were made by trypsinizing each culture and transferring half of the cells to a new dish. The doubling time of L/C447 cells was about 3 to 4 days, except when the cells were going through their occasional "crises."

Backcross of ts^+ virus to wild-type virus and screening of progeny clones. ts^+ virus isolated from L/C447 cells was backcrossed to wild-type reovirus by the following procedure. A total of 2.5×10^6 L cells in suspension were mixedly infected with each virus at a multiplicity of infection (MOI) of 10 PFU/cell. In control self-crosses, an MOI of 20 was used. After 48 h at 31°C, the samples were sonicated and titrated at 31 and 39°C on L-cell monolayers. The 39°C plates were scored on day 5, and the 31°C plates were scored on day 10 postinfection. Well-isolated plaques were picked from 31°C plates and passaged once in L-cell monolayers at 31°C. First-passage virus titer and efficiency of plating (EOP) (39°C titer/31°C titer) were determined by plating on L-cell monolayers at 39°C and 31°C. Clones with an EOP less than 5×10^{-2} were considered temperature sensitive. Under our assay conditions an EOP between 0.1 and 1.0 is characteristic of wild-type virus, whereas *ts* clones have EOPs between 5×10^{-2} and 1×10^{-5} (18).

Recombination grouping of *ts* progeny clones. Recombination grouping tests were performed as described by Ramig and Fields (18) except that the crosses were incubated for 72 h (instead of 48 h) at 31°C. The percentage of ts^+ recombinants was calculated by the following formula: Percentage of ts^+ recombinants = $\{(\text{titer A} \times \text{B})^{39} - [(\text{titer A})^{39} + (\text{titer B})^{39}]\} / (\text{Titer A} \times \text{B})^{31} \times 100$.

Interference of wild-type growth by *ts* mutants. To assay interference by *ts* mutants, L-cell monolayers (2×10^5 cells) in 2-dram (ca. 2.3-g), glass screw-cap vials were coinfected by *ts* mutants and wild-type virus at the indicated MOI. One set of vials was incubated at 37°C, and a second set was incubated at 39°C. The cells incubated at 39°C were harvested at 44 h postinfection, and the 37°C set were harvested at 56 h postinfection. The cells were frozen and thawed three times, sonicated, and titrated on L-cell monolayers at 39°C.

RESULTS

Temperature phenotype of virus produced in persistently infected cells. The persistently infected cell line used in this study was established by infecting L cells with a serially passaged stock (passage 9) of *ts*C(447) containing greater than 90% defective interfering particles. These carrier cells, designated L/C447, were maintained at 37°C for a period of 1.5 years. At various intervals the presence of virus was determined by titrating L/C447 cell lysates on L-cell monolayers at 31 and 39°C (2). The

phenotype of the virus being produced changed from *ts* to ts^+ within a month after establishment of the carrier culture and remained so for as long as the cells were maintained (2). After these cells had been maintained in culture for about 1 year (61 passages), the cell lysate was titrated at 31°C, and 42 well-isolated plaques were picked and passaged once in L cells at 31°C. These clones were titrated at 31 and 39°C, and their EOP (39°C titer/31°C titer) was determined. As controls, 25 plaques were picked from wild-type reovirus type 3, and 27 plaques were picked from the *ts*C(447) P9 stock that was used to establish the persistent infection. These plaques were passaged once in L cells at 31°C, and their EOP was determined by titration at permissive (31°C) and nonpermissive (39°C) temperatures (Fig. 1). Clones picked from wild-type virus had EOPs between 0.1 and 1.0 (Fig. 1A), whereas all virus clones from *ts*C(447) P9 stock were temperature sensitive with EOPs between 0.01 and 0.0002 (Fig. 1B). The majority of the clones from L/C447 cells (38 of 42) had EOPs characteristic of wild-type virus, whereas four clones had EOPs between 0.05 and 0.08 (Fig. 1C). The four clones with intermediate EOPs are being further characterized. However, it is clear that the vast majority of clones isolated from the persistently infected cells had a wild-type temperature phenotype and that during the persistent infection a transition from *ts* to ts^+ virus had occurred.

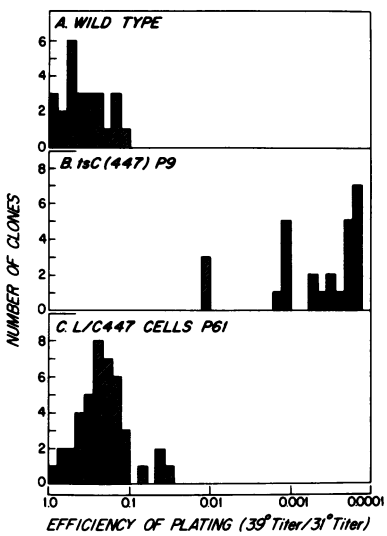

FIG. 1. *Temperature phenotype of virus clones picked from (A) wild-type reovirus type 3; (B) tsC(447) passage 9, the virus stock used to establish the persistent infection; and (C) L/C447 cells passage 61, about 1 year in culture. The plaques were picked from 31°C plates, passaged once in L-cell monolayers at 31°C, and titrated as described in the text.*

Presence of suppressed *ts* mutants among ts^+ clones from L/C447 cells. To determine whether the ts^+ phenotype of the virus isolated from the carrier cells was due to intragenic reversion or to the presence of a second extragenic mutation suppressing the original *ts* defect, we backcrossed the ts^+ revertants with wild-type reovirus and examined the progeny for temperature sensitivity. Since the reovirus genome is segmented and recombination takes place by reassortment of genome segments (21), the *ts* lesion separates easily from the suppressor mutation when it lies on a different double-stranded RNA segment. Thus the presence of *ts* mutants among the progeny of a cross between wild type and ts^+ revertant indicates that the revertant contained the *ts* lesion in a suppressed state.

Seven ts^+ clones isolated from L/C447 cells were examined for the presence of suppressed *ts* lesions. Each clone was backcrossed to wild-type virus at the permissive temperature (31°C), and the yield of each backcross was titrated at 31°C. As controls, each ts^+ clone and wild-type virus were self-crossed. Several progeny plaques were picked from each of the backcrosses and control self-crosses and passaged once in L-cell monolayers at 31°C. The EOP of first-passage virus was determined by plating on L-cell monolayers at the permissive (31°C) and nonpermissive temperatures (39°C). Five of the seven ts^+ clones from L/C447 cells appeared to be intragenic revertants, since there were no *ts* mutants found in the progeny derived from the backcrosses (Table 1). Two clones, 9 and 26, contained suppressed *ts* lesion(s), since *ts* mutants were present among the progeny clones obtained from the backcross. Thus, when clone 26 was crossed with wild-type reovirus, approximately 50% of the progeny were temperature sensitive. Also, three of the 20 progeny clones obtained from clone 9 × wild type cross were *ts*. The EOPs of *ts* mutants rescued from clone 9 and clone 26 are given in Table 2. All progeny clones from self-crosses of clone 9 and clone 26 had an EOP characteristic of wild-type virus (between 0.1 and 1.0). This indicates that these clones had a stable ts^+ phenotype and did not generate *ts* progeny at a frequency high enough to affect the results of the backcrosses. The wild-type virus used in the study also did not generate *ts* mutants at an appreciable frequency: all 50 progeny clones tested were ts^+. Thus the presence of *ts* mutants among the progeny of backcrosses between wild type and clone 9 or clone 26 indicated that both clone 9 and clone 26 contained suppressed *ts* lesion(s).

Recombination testing of *ts* mutants rescued from suppressed clones. When a viral

clone containing a suppressor mutation is backcrossed to wild-type virus, both the parental *ts* lesion and nonparental *ts* lesions can be found among the progeny of the cross (12, 18). In studies with phage P22, Jarvik and Botstein found that the nonparental *ts* was associated with suppressor activity (12). Nonparental *ts* lesions have also been rescued from suppressed clones of reovirus, but it is not known whether they are associated with suppressor activity (18). Recombination analysis was carried out to determine the number of mutant groups present among the *ts* mutants rescued from clone 9 and clone 26. When the three *ts* mutants rescued from clone 9 were crossed against each other, no wild-type recombinants were obtained (data not shown). Thus all these mutants had a common defect. A representative *ts* mutant (clone 9/15) was then crossed against the prototype reovirus *ts* mutants (groups A to G). Clone 9/15 showed significant recombination with all the mutants except *ts*G (Table 3). This indicated that the three *ts* mutants rescued from clone 9 belong to group G.

In a similar fashion, the seven *ts* mutants rescued from clone 26 were crossed against each other (Table 4). Clone 26/6 recombined with all the other mutants, as did clone 26/19. The remaining five clones (26/2, 26/5, 26/8, 26/11, and 26/14) showed no recombination with each other. Thus the *ts* mutants rescued from clone 26 fell into three recombination groups. These mutants were then crossed against the known prototype *ts* mutants (groups A to G) (Table 3). Clone 26/6 recombined with all the mutants except *ts*B, and 26/19 failed to recombine with *ts*G. Clones 26/5 and 26/8, used as representatives of the third group, recombined with all the existing prototype mutants, thus defining a new recombination group, designated H. The *ts*A(201) clone used in the study recombined inefficiently [0.1% ts^+ recombinants with *ts*B(352), 0.75% with *ts*C(447), and 0.66% with *ts*G(453)]. Therefore the low values (1.3 to 1.9% ts^+ recombinants) obtained in crosses with *ts*A(201) were scored as positive recomoination. The results of recombination testing indicate that *ts* mutants representing three different groups—B, G, and the new group, H—were rescued from clone 26.

Interference with the growth of wild-type reovirus by *ts* mutants rescued from suppressed clones. We have reported that certain *ts* mutants of reovirus inhibit the growth of wild-type virus at the nonpermissive temperature (3). To determine whether the *ts* mutants

TABLE 1. *Presence of suppressed ts mutants among ts^+ clones from persistently infected cells*

Virus inoculum[a]	No. of progeny clones checked[b]	No. of *ts* progeny[c]	Suppressed?
Clone 6 × wild type	16	0	No
Clone 7 × wild type	18	0	No
Clone 8 × wild type	16	0	No
Clone 9 × wild type	20	3	Yes
Clone 25 × wild type	17	0	No
Clone 26 × wild type	14	7	Yes
Clone 28 × wild type	20	0	No
Wild type × wild type	50	0	
Clone 9 × clone 9	20	0	
Clone 26 × clone 26	25	0	

[a] 2.5×10^6 L cells in suspension culture were mixedly infected with each virus at an MOI of 10 PFU/cell. In single infections an MOI of 20 PFU/cell was used. After 48 h at 31°C, the samples were sonicated and titrated at 31°C.

[b] Well-isolated plaques were picked from 31°C plates and passaged once in L-cell monolayers at 31°C. First-passage virus titer and EOP (39°C/31°C) were determined by plating on L-cell monolayers at 39 and 31°C.

[c] See Table 2 for EOPs of *ts* mutants rescued from clone 9 and clone 26.

TABLE 2. *EOP of ts mutants rescued from clone 9 and clone 26*

Virus	EOP (39°C titer/31°C titer)
ts 9/12	1.1×10^{-3}
ts 9/15	$<5 \times 10^{-5}$
ts 9/18	1.4×10^{-2}
ts 26/2	5.6×10^{-3}
ts 26/5	5.9×10^{-3}
ts 26/6	4.1×10^{-4}
ts 26/8	8.1×10^{-4}
ts 26/11	5×10^{-3}
ts 26/14	$<3.8 \times 10^{-4}$
ts 26/19	8.6×10^{-3}

TABLE 3. *Recombination testing of ts mutants rescued from clone 26 and clone 9*

ts mutant	Percentage of ts^+ recombinants when crossed with:						
	*ts*A(201)	*ts*B(352)	*ts*C(447)	*ts*D(357)	*ts*E(320)	*ts*F(556)	*ts*G(453)
ts 9/15	1.9	3.1	4.7	4.6	6.2	4.8	0
ts 26/5	1.5	7.4	14	15	8.5	10	10
ts 26/6	1.8	0	3.5	4.2	5.6	3.2	3.8
ts 26/8	1.3	15	4.5	17	4	10	12
ts 26/19	1.3	12.8	8.4	11.6	4.7	7.8	0

rescued from the suppressed clones isolated from L/C447 cells could interfere with the growth of wild-type virus, coinfections of *ts* mutants and wild-type virus were performed at 39 and 37°C. Interference was checked at 37°C as well as 39°C, since the persistently infected cells had been grown at 37°C. The results of these experiments are shown in Table 5. Among the mutants tested, *ts*G 9/15 interfered with the growth of wild-type virus at both 39 and 37°C. *ts*H 26/8 and *ts*B 26/6 interfered weakly, whereas *ts*H 26/5 and *ts*G 26/19 exhibited little or no interference at either temperature. In all interference experiments, early-passage virus (P1 or P2) was used. These stocks were analyzed for genome RNA and were found to contain normal ratios of the 10 double-stranded RNA segments (i.e., no deletion mutants were detected). Thus the interference observed was most probably due to the *ts* lesion. In one experiment, a passage 3 stock of *ts*H 26/8 was used. The P3 stock interfered strongly (>95% inhibition) with the growth of wild-type virus. Preliminary data indicate that *ts*H 26/8 and *ts*H 26/5 rapidly generate deletion mutants, and P3 and P4 lysates of these mutants interfere with growth of wild-type virus (data not shown). The other *ts* mutants rescued from the suppressed clones are also being checked for their ability to generate deletion mutants, and the results of this study will be published later.

TABLE 4. *Recombination between ts progeny clones rescued from suppressed clone 26*

ts mutant	Percentage of ts^+ recombinants when crossed with:						
	26/6	26/19	26/2	26/5	26/8	26/11	26/14
26/6	—	39	52	18	16	20	48
26/19		—	33	12	70	30	19
26/2			—	0	0	0	0
26/5				—	0	0	0
26/8					—	0	0
26/11						—	0
26/14							—

TABLE 5. *Interfering activity of ts mutants rescued from suppressed clones*

Virus inoculum	MOI	Yield after 44 h at 39°C (PFU/ml)[a]	% Inhibition of wild-type growth at 39°C	Yield after 56 h at 37°C (PFU/ml)[a]	% Inhibition of wild-type growth at 37°C
*ts*G9/15	2	$<5 \times 10^3$		4.3×10^4	
*ts*G9/15	10	$<5 \times 10^3$		4.2×10^4	
*ts*H26/5	2	$<5 \times 10^3$		$(1.5 \times 10^6)^b$	
*ts*H26/5	10	$<5 \times 10^3$		$(1 \times 10^7)^b$	
*ts*B26/6	2	$<5 \times 10^3$		1.5×10^5	
*ts*B26/6	10	$<5 \times 10^3$		2.1×10^5	
*ts*H26/8	2	4×10^4		5.3×10^5	
*ts*H26/8	10	3×10^4		6.8×10^5	
*ts*G26/19	2	$(1 \times 10^5)^b$		$(1 \times 10^6)^b$	
*ts*G26/19	10	$(1 \times 10^5)^b$		$(1.7 \times 10^6)^b$	
Wild type	2	2.7×10^7		5.4×10^7	
Wild type	10	4.8×10^7		6.7×10^7	
9/15 × wt[c]	2:2	1.1×10^7	60	4.2×10^7	23
9/15 × wt	10:2	3.5×10^6	88	1.5×10^7	73
26/5 × wt	2:2	3.8×10^7	0	5.6×10^7	0
26/5 × wt	10:2	2.2×10^7	19	5.8×10^7	0
26/6 × wt	2:2	2.2×10^7	19	6.4×10^7	0
26/6 × wt	10:2	1.5×10^7	45	2.8×10^7	49
26/8 × wt	2:2	1.5×10^7	45	5.3×10^7	0
26/8 × wt	10:2	1.3×10^7	52	3.3×10^7	39
26/19 × wt	2:2	2.8×10^7	0	4.3×10^7	20
26/19 × wt	10:2	2.0×10^7	26	4.7×10^7	13

[a] Yield was measured at 39°C on L-cell monolayers. Average titer from two experiments.
[b] Numbers in parentheses denote leak plaques.
[c] wt, Wild type.

DISCUSSION

Lytic RNA viruses undergo extensive mutation during persistent infection in tissue cultures (9). Our studies have been aimed at analyzing the genetic changes that occur in reovirus during persistent infection of L cells. The current study indicates the complexity of these genetic changes. Previous studies in our laboratories had indicated that, following the initiation of persistent infections with the *ts*C mutant and a large excess of defective interfering particles, the virus acquires a small-plaque character with a ts^+ phenotype (2). To determine whether these ts^+ clones were intragenic revertants or extragenically suppressed pseudorevertants, backcrosses to wild-type reovirus were performed, and the progeny of each cross were screened for temperature sensitivity. In no instance was the original *ts*C mutant recovered among the progeny. Thus it appears that either intragenic suppression or true reversion of the original *ts*C lesion had occurred in these seven clones. However, two of these apparent ts^+ revertants contained new *ts* lesions. The new *ts* mutants, including the initial isolates of *ts*H, were found in a "suppressed" form. Suppressor mutations have previously been noted to occur at high frequency in spontaneous ts^+ revertants of reovirus *ts* mutants (18). The extraordinary finding, that there are three distinct *ts* lesions in clone 26, indicates that multiple *ts* lesions can be present in a cloned virus with a ts^+ phenotype. Clone 26, in particular, illustrates the need to distinguish clearly between genotype and phenotype when analyzing viruses generated under circumstances where mutation may be favored (e.g., mutagenesis, serial passage at high multiplicity of infection, persistence).

In a parallel study, we have been examining the genetic changes that occur in reovirus during serial passage in L cells at high multiplicity of infection. We have found that high-passage stocks of wild-type reovirus contain both dele-

tion (defective interfering particles) and temperature-sensitive mutants (1). The presence of *ts* mutants in high-passage stocks of wild-type virus is of significance to the present study since a high-passage stock (P9) of *ts*C(447) was used to establish the persistent infection. It is possible that this P9 stock of *ts*C contains other *ts* mutants in addition to *ts*C and that the new *ts* mutants (*ts*B, G, and H) isolated from L/C447 cells were not generated but selected during the course of the persistent infection.

We have reported that reovirus *ts* mutants in groups A, B, and G interfere with the growth of wild-type virus, whereas *ts* mutants in groups D and E are noninterfering (3). The present study shows that interference is not necessarily group specific but may be a property of the individual mutant. Some of the *ts* mutants rescued from the suppressed ts^+ clones are capable of interfering with the growth of wild-type virus. The presence of interfering *ts* genes in the persistently infected cell may play a role in regulating viral growth and allowing the cell to survive. We have reported earlier that reovirus defective interfering particles are present in the persistently infected L cells (2). Preliminary data have indicated that two of the *ts* mutants (*ts*H 26/8 and *ts*H 26/5) isolated in this study rapidly generate deletion mutants. Thus it appears that the presence of *ts* mutants and defective interfering particles in carrier cells is interrelated and that both of these factors may be involved in maintaining a persistent reovirus infection in L cells.

These studies have thus helped clarify an unusual feature of cells persistently infected with reovirus. It is now clear that, similar to other carrier cultures, *ts* mutants of reovirus emerge during persistent infection. However, the presence of extragenic suppressor mutations makes it difficult to detect these *ts* mutants. The finding of extragenic suppressor mutations in viruses isolated from persistently infected cells raises the possibility that such mutations may be an additional important factor in the maintenance of persistent infection. Studies currently under way are aimed at determining more precisely the role of the different types of viral mutations in acute and persistent infections.

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LITERATURE CITED

1. **Ahmed, R., P. R. Chakraborty, and B. N. Fields.** 1980. Genetic variation during lytic reovirus infection: high-passage stocks of wild-type reovirus contain temperature-sensitive mutants. J. Virol. **34**:285–287.
2. **Ahmed, R., and A. F. Graham.** 1977. Persistent infections in L cells with temperature-sensitive mutants of reovirus. J. Virol. **23**:250–262.
3. **Chakraborty, P. R., R. Ahmed, and B. N. Fields.** 1979. Genetics of reovirus: the relationship of interference to complementation and reassortment of ts mutants at nonpermissive temperature. Virology **94**:119–127.
4. **Cross, R. K., and B. N. Fields.** 1972. Temperature-sensitive mutants of reovirus type 3: studies on the synthesis of viral RNA. Virology **50**:799–809.
5. **Fields, B. N., and W. K. Joklik.** 1969. Isolation and preliminary genetic and biochemical characterization of ts mutants of reovirus. Virology **37**:335–342.
6. **Fields, B. N., H. L. Weiner, R. F. Ramig, and R. Ahmed.** 1978. Genetics of reovirus: aspects related to virulence and viral persistence, p. 389–398. *In* J. G. Stevens, G. J. Todaro, and C. F. Fox (ed.), Persistent viruses. ICN-UCLA Symposia on Molecular and Cellular Biology, vol. XI. Academic Press Inc., New York.
7. **Gould, E. A., and P. E. Linton.** 1975. The production of a temperature-sensitive measles virus infection. J. Gen. Virol. **28**:21–28.
8. **Holland, J. J., E. A. Grabau, C. L. Jones, and B. L. Semler.** 1979. Evolution of multiple genome mutations during long-term persistent infection by VSV. Cell **16**: 495–504.
9. **Holland, J. J., and A. Levine.** 1978. Mechanisms of viral persistence. Cell **14**:447–452.
10. **Holland, J. J., and L. P. Villarreal.** 1974. Persistent noncytocidal VSV infections mediated by defective T particles that suppress virion transcriptase. Proc. Natl. Acad. Sci. U.S.A. **71**:2956–2960.
11. **Holland, J. J., L. P. Villarreal, R. M. Welsh, M. B. A. Oldstone, D. Kohne, R. Lazzarini, and E. Scolnick.** 1976. Long-term persistent VSV and rabies infection of cells *in vitro*. J. Gen. Virol. **33**:193–211.
12. **Jarvik, J., and D. Botstein.** 1975. Conditional lethal mutations that suppress genetic defects in morphogenesis by altering structural proteins. Proc. Natl. Acad. Sci. U.S.A. **72**:2738–2742.
13. **Kimura, Y., Y. Ito, K. Shimokata, Y. Nishiyama, I. Nagata, and J. Kitoh.** 1975. Temperature-sensitive virus derived from BHK cells persistently infected with HVJ (Sendai virus). J Virol. **15**:55–63.
14. **Norkin, L. C.** 1979. Small plaque variants of simian virus 40 from a persistent infection of Rhesus Monkey kidney cells. Virology **97**:201–206.
15. **Preble, O. T., and J. S. Youngner.** 1972. Temperature-sensitive mutants isolated from L cells persistently infected with Newcastle disease virus. J. Virol. **9**:200–206.
16. **Preble, O. T., and J. S. Youngner.** 1975. Temperature-sensitive viruses and the etiology of chronic and inapparent infections. J. Infect. Dis. **131**:467–473.
17. **Pringle, C. R., P. V. Shirodaria, P. Cash, D. J. Chiswell, and P. Malloy.** 1978. Initiation and maintenance of persistent infection by respiratory syncytial virus. J. Virol. **28**:199–211.
18. **Ramig, R. F., and B. N. Fields.** 1979. Revertants of ts mutants of reovirus: evidence for frequent extragenic suppression. Virology **92**:155–167.
19. **Ramig, R. F., R. M. White, and B. N. Fields.** 1977. Suppression of the ts phenotype of a mutant of reovirus type 3. Science **195**:406–407.
20. **Sekellick, M. J., and P. I. Marcus.** 1979. Persistent infection. II. Interferon-inducing ts mutants as mediators of cell sparing: possible role in persistent infection by VSV. Virology **95**:36–47.

21. **Sharpe, A. H., R. F. Ramig, T. A. Mustoe, and B. N. Fields.** 1978. A genetic map of reovirus. I. Correlation of genome RNAs between serotypes 1, 2 and 3. Virology **84**:63–74.
22. **Shenk, T. E., K. A. Koshelnyk, and V. Stollar.** 1974. Temperature-sensitive virus from *Aedes albopticus* cells chronically infected with Sindbis virus. J. Virol. **13**:439–447.
23. **Wechsler, S. L., R. Rustigian, K. C. Stallcup, K. B. Byers, S. H. Winston, and B. N. Fields.** 1979. Measles virus-specified polypeptide synthesis in two persistently infected HeLa cell lines. J. Virol. **31**:677–684.