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An Intracellular Interaction Between a Temperature-sensitive Mutant and the Original Wild-type HVJ (Sendai Virus) is Responsible for the Establishment and Maintenance of HVJ Persistent Infection

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SUMMARY

In order to understand the selective survival of temperature-sensitive (*ts*) mutants in persistent infection by HVJ (Sendai virus), an intracellular interaction between a *ts* clone (HVJ cl.14) isolated from HVJ carrier G2 cells and the original wild-type virus (HVJo) was studied. HVJ cl.14 differed from HVJo mainly in its *ts* property at 39 °C, weak cytopathogenicity and faster electrophoretic mobility of P protein (P_{77K}), but showed similar trypsin-activated growth to that of HVJo.

When LLCMK2 cells were simultaneously infected with HVJo and HVJ cl.14 at 32 °C, synthesis of HVJo-derived P protein (P_{79K}) was inhibited with concomitant reduction of cytopathic effect (c.p.e.) and more dominant growth of HVJ cl.14 was observed. For the analysis of progeny viruses in these mixed infections, another mutant of HVJo designated HVJe which formed plaques activated only by elastase was isolated and employed instead of HVJo. At 39 °C, HVJ cl.14 was rescued by coinfected HVJe at about 900- to 13 000-fold over single infection. This recovery was also shown by sequential synthesis of HVJ cl.14-derived P protein (P_{77K}) following the earlier synthesis of HVJo-derived P polypeptide (P_{79K}) in the mixed infection at 39 °C. However, the u.v. inactivation of HVJe or HVJ cl.14 resulted in a loss of their activity on rescue or on c.p.e. reduction, suggesting the necessity of protein synthesis by opposite viruses for these interactions. The mechanisms involved in the predominant growth of the *ts* mutant and concomitant reduction of c.p.e. seemed to provide a general explanation for the preferable persistence of the *ts* mutant in the HVJ carrier cells.

INTRODUCTION

Previous reports from this and other laboratories have shown that temperature-sensitive (*ts*) mutants are spontaneously selected in several cell lines persistently infected with HVJ (Sendai virus) (Nagata *et al.*, 1972; Tanaka *et al.*, 1976), NDV (Preble & Youngner, 1973*a*, *b*), VSV (Youngner *et al.*, 1976) and Sindbis virus (Stollar *et al.*, 1974). Specific interference between wild-type NDVo and clones of *ts* mutants spontaneously selected during persistent infection initiated with NDVo was reported by Preble & Youngner (1973*a*, *b*). Youngner & Quagliana (1976) interpreted VSV persistence in L cells as being due to an interfering activity of the *ts* mutant B virion against wild-type virus, and discussed the role of the *ts* mutant in the establishment and maintenance of persistent infection.

Recently, two types of *ts* mutants, HVJ cl.14 and HVJ cl.151, from HVJ carrier G2 and BHK cells respectively, were isolated and characterized (Ogura *et al.*, 1981; Yoshida *et al.*, 1979). HVJ cl.14 was temperature-sensitive at the level of RNA synthesis and had a P

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polypeptide with faster electrophoretic mobility (P_{77K}) than that of HVJo wild-type (P_{79K}), while HVJ cl.151 was temperature-sensitive in HN and M protein synthesis and had an M protein with slower mobility than that of HVJo (M_{34K}).

A protease activation mutant of HVJo (designated as HVJe), which forms plaques with elastase but not with trypsin in overlay medium, was isolated for the assay of progeny viruses released from LLCMK2 cells coinfected with HVJo and HVJ cl.14 or HVJ cl.151. Thus, each strain has characteristic markers: HVJ cl.14 is temperature-sensitive, is activated by trypsin but not by elastase and has P protein of mol. wt. 77000, M protein of mol. wt. 34000 and weak cytopathogenicity (*ts*$^-$, try$^+$, ela$^-$, P_{77K}, M_{34K}, c.p.e.$^-$); HVJ cl.151 (*ts*$^-$, try$^+$, ela$^-$, P_{79K}, M_{36K}, c.p.e.$^-$); HVJo (*ts*$^+$, try$^+$, ela$^-$, P_{79K}, M_{34K}, c.p.e.$^+$); and HVJe (*ts*$^+$, try$^-$, ela$^+$, P_{79K}, M_{34K}, c.p.e.$^+$). In an attempt to explain how *ts* mutants are selected spontaneously in cells persistently infected with HVJ, intracellular interaction between HVJo or HVJe and HVJ cl.14 or HVJ cl.151 was examined using the criteria described above.

METHODS

Viruses and cell culture. HVJ Nagoya 1-60 strain and HVJ cl.14 were prepared as described previously (Ogura *et al.*, 1981). LLCMK2 cells were grown in Eagle's minimal essential medium (MEM) with 5% calf serum. Isolation of the HVJo mutant activated by elastase was carried out as follows. Confluent monolayers of LLCMK2 cells were infected with HVJo at a multiplicity of infection (m.o.i.) of 0·1 and incubated in the presence of nitrosoguanidine (5 μg/ml) and elastase (5 μg/ml) (both from Sigma) at 32 °C for 4 days. The harvest from this culture was used to infect LLCMK2 cell monolayers and these were incubated at 32 °C for 5 days with an agar overlay as described previously (Ogura *et al.*, 1981), except that trypsin was replaced by 5 μg/ml elastase. Following three successive clonings in the presence of elastase, a clone which formed plaques with elastase (5 μg/ml) but not with *N*-acetyltrypsin (1 μg/ml) in the overlay was propagated in LLCMK2 cells in the presence of elastase and was designated as HVJe. HVJ cl.151 was a kind gift from Dr T. Yoshida of Nagoya University.

Single or mixed infection with HVJo, HVJe, HVJ cl.14 and HVJ cl.151. Confluent monolayers of LLCMK2 cells on 35 mm plastic dishes were infected with 0·2 ml of a virus or mixtures containing two of these strains at the m.o.i. indicated. After an adsorption period of 90 min at 32 °C, the inoculum was removed. The monolayers were washed twice with warm MEM, 1 ml of warm MEM was added, and then they were incubated at 32 °C or 39 °C. The maintenance medium was harvested every 24 h for assay of infectivity.

Other procedures. Plaque assays, labelling of virus particles, polyacrylamide gel electrophoresis and autoradiography were carried out as described previously (Ogura *et al.*, 1981). Immunoprecipitation was performed by the method of Wechsler *et al.* (1979). Densitometer tracing of the autoradiogram was done by a Shimazu dual beam Chromoscanner model GS-900.

RESULTS

Electrophoretic analysis of polypeptides of progeny virus released from HVJ cl.14 × HVJo coinfected cells

In Fig. 1, progeny viruses produced at 32 °C and 39 °C by single and mixed infection with the two viruses at a variety of m.o.i. are compared. Polypeptides of HVJ are indicated according to the convention of Scheid & Choppin (1974). No detectable amount of [^{35}S]methionine-labelled particles were produced by single infection with HVJ cl.14 at 39 °C. However, synthesis of P polypeptide derived from HVJ cl.14 (P_{77K}) was observed at 39 °C when infected together with HVJo (Fig. 1*b*, tracks 1 to 3). The appearance of HVJ cl.14 P

polypeptide (P_{77K}) and loss of HVJo P polypeptide (P_{79K}) shown in Fig. 1 (*a*, *b*) was quantified by densitometer scanning of the autoradiogram. As shown in Fig. 1 (*c*) it was found that the amounts of HVJ cl.14 P protein (P_{77K}) synthesized increased significantly with a concomitant loss or decrease of HVJo-derived P protein (P_{79K}) at both 32 °C and 39 °C. The loss of HVJo-derived P polypeptide (P_{79K}) synthesis by coinfection with HVJ cl.14 was more dramatic at 32 °C, the permissive temperature for the *ts* mutant, and was accompanied by a reduction of c.p.e. by HVJo. The loss of P_{79K} synthesis and c.p.e. reduction, may be one expression of the intracellular interaction between HVJ cl.14 and HVJo. However, u.v.-inactivated HVJ cl. 14 could not reduce synthesis of HVJo-derived P_{79K} or c.p.e. by HVJo.

Immunoprecipitation of intracellular viral proteins

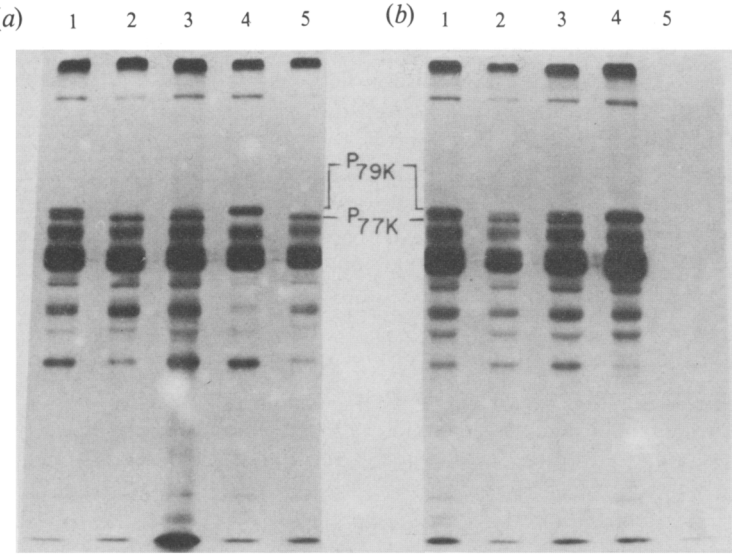
Viral polypeptides synthesized in HVJ-infected cells were precipitated by anti-HVJ serum plus protein A–Sepharose and then examined by SDS–polyacrylamide gel electrophoresis and autoradiography (Fig. 2). No detectable virus proteins were produced by single infection with *ts* mutant HVJ cl.14 at 39 °C (Fig. 2, track 7). However, when HVJ cl.14 was coinfected with HVJo at 39 °C, both P polypeptides of HVJ cl.14 (P_{77K}) and HVJo(P_{79K}) were clearly observed (Fig. 2, tracks 3 and 5). The ratio of P_{77K} to P_{79K} recovered from the coinfected cells at 39 °C by immunoprecipitation was quite consistent with that from virus particles released into culture medium from the coinfected cells at 39 °C (Fig. 1 *b*, tracks 2 and 3). These data indicate that dominance of HVJ cl.14-derived P protein detectable in progeny viruses from HVJ cl.14 × HVJo coinfected cells, reflects also the dominant intracellular synthesis of HVJ cl.14 P polypeptide.

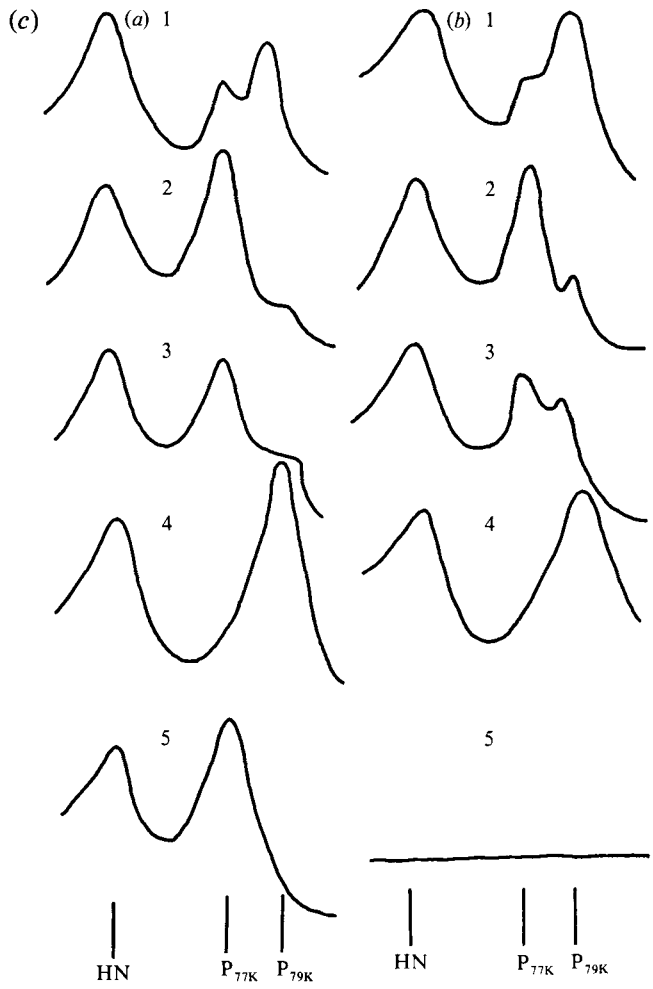
Kinetic analysis of progeny viruses from HVJe × HVJ cl.14 mixed infection

As HVJo and HVJ cl.14 plaques could not be distinguished from each other by plaque size, a protease activation mutant of HVJo was isolated as described in Methods for the assay of mixed progeny viruses from coinfected cells. This mutant designated as HVJe was no longer activated by trypsin, but was able to undergo multiple-cycle replication in the presence of elastase at both 32 °C and 39 °C. In contrast, HVJ cl.14 was activated only by trypsin but not by elastase.

The analysis of infectivity of progeny viruses produced by single and mixed infection with these two viruses is shown in Table 1. The rescue phenomenon of HVJ cl.14 in mixed infection was dramatic at 39 °C, showing an increased infectivity of more than about 900 to 13 000 times the one from single infection with this mutant at 39 °C. At this temperature, the ratio of HVJ cl.14 to HVJe (infectivity titre assayed in the presence of trypsin to that of elastase) in progeny from this mixed infection became gradually larger. At the permissive temperature (32 °C), the more rapidly dominant growth of the *ts* mutant in coinfected cells was observed. However, net virus production by mixed infection was not altered significantly at either 32 °C or 39 °C.

In order to examine the accuracy of the protease-selection method for scoring of progeny viruses, viruses were isolated from plaques formed in trypsin- or elastase-containing plates inoculated with terminal dilution of the progeny viruses resulting from HVJ cl.14 × HVJe mixed infection at 39 °C and tested for temperature sensitivity and mobility of P polypeptide on acrylamide gel. All viruses in these plaques from trypsin-agar overlay produced haemagglutinin without clear c.p.e. in the presence of trypsin at 32 °C but not at 39 °C. The P polypeptide of these viruses migrated to the position of HVJ cl.14 P protein (P_{77K}) on SDS–polyacrylamide gel (data not shown). In contrast, viruses in the plaques from elastase-agar overlay produced haemagglutinin and complete cell destruction at both 32 °C and 39 °C. The P proteins of these viruses were identical with that of HVJe(P_{79K}) as judged


(a) 1 2 3 4 5
(b) 1 2 3 4 5
P79K
P77K


(c)
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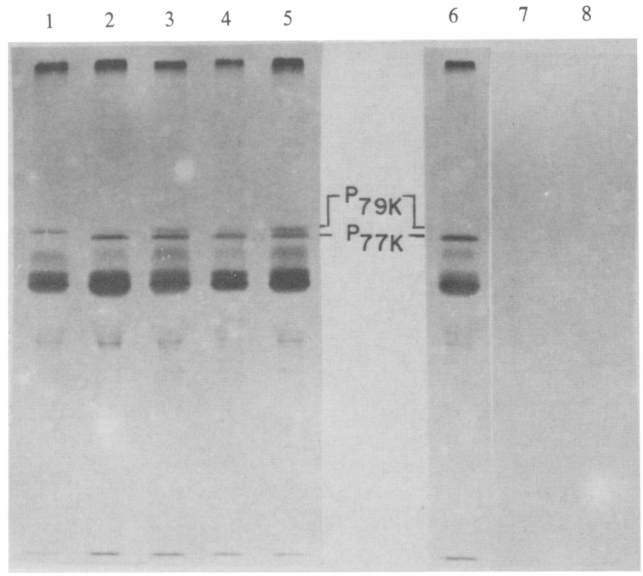

Fig. 2. Immunoprecipitation of LLCMK2 cells infected with HVJo or HVJ cl.14 separately or with both of these two strains. Track 1, LLCMK2 cells infected with HVJo (m.o.i. = 1) at 39 °C; track 2, HVJ cl.14 × HVJo (m.o.i. = 5 × 1) at 32 °C, track 3, at 39 °C; track 4, HVJ cl.14 × HVJo (m.o.i. = 5 × 5) at 32 °C, track 5, at 39 °C; track 6, HVJ cl.14 (m.o.i. = 5) at 32 °C, track 7, at 39 °C; track 8, uninfected LLCMK2 cells were labelled at 24 h post-infection, solubilized and precipitated by anti-HVJ serum plus protein A–Sepharose as described in Methods.

by SDS electrophoresis. Therefore, it is clear that all viruses from the trypsin-containing overlay dish showed markers of HVJ cl.14 such as temperature sensitivity (ts^-), a small P protein (P_{77K}), and weak cytopathogenicity (c.p.e.$^-$), while viruses from the elastase-containing agar-overlay dish had markers of HVJe (ts^+, P_{79K}, c.p.e.$^+$).

Interaction between HVJ cl.151 and HVJe or HVJ cl.14

To assess the selective advantage by dominant growth of the *ts* mutant of HVJ isolated from persistently infected cells, another type of *ts* mutant, HVJ cl.151, was examined which has been well characterized by Yoshida *et al.* (1979). The analysis of progeny from single and mixed infections with HVJ cl.151 and HVJe is shown in Table 2. The progeny of this mixed infection at 39 °C contained 114-fold higher concentrations of HVJ cl.151 virus than those from single infection with the same *ts* mutant, showing the rescue of the *ts* mutant by wild-type virus, HVJe. At 32 °C the number of *ts* mutant progeny did not differ between single and mixed infections. However, there was a significant decrease of around 1/30 to 1/170, in the number of wild-type progeny viruses (HVJe) produced in mixed infections at both 32 °C and 39 °C, as an expression of homologous interference induced by the *ts* mutant, HVJ cl.151. The infectivity ratio of the *ts* mutant (HVJ cl.151) to wild-type virus (HVJe) in the present mixed infection at both 32 °C and 39 °C showed a tendency similar to

Fig. 1. Autoradiograph of acrylamide gel electrophoresis of [^{35}S]methionine-labelled progeny virus released from LLCMK2 cells infected with HVJo and HVJ cl.14 separately or with mixtures of these two viruses. Multiplicity of infection per cell (HVJo/HVJ cl.14) was track 1, 5/1; track 2, 1/5; track 3, 5/5; track 4, 1/0; track 5, 0/5. Infected cells were incubated at either (*a*) 32 °C or (*b*) 39 °C and labelled with [^{35}S]methionine at 24 h after infection as described in Methods. Densitometer scans of the autoradiographs of the P and HN regions of (*a*) and (*b*) are shown in (*c*).

Table 1. *Analysis of progeny viruses produced by single and mixed infection of LLCMK2 cells with HVJe and HVJ cl.14 at 32 °C and 39 °C*

Virus infected*	Incubation temperature (°C)	Protease added†	Infectivity titres (p.f.u./ml)† 24 h	48 h	72 h
HVJe	39	Elastase	$1{\cdot}1 \times 10^8$	$3{\cdot}5 \times 10^8$	$2{\cdot}0 \times 10^6$‡
		Trypsin	$<10^4$	NT§	NT
	32	Elastase	$9{\cdot}5 \times 10^7$	$1{\cdot}3 \times 10^8$	$3{\cdot}9 \times 10^7$‡
		Trypsin	$<10^4$	NT	NT
HVJ cl.14	39	Elastase	$<10^4$	NT	NT
		Trypsin	$1{\cdot}5 \times 10^3$	$9{\cdot}0 \times 10^3$	$2{\cdot}5 \times 10^4$
	32	Elastase	$<10^4$	NT	NT
		Trypsin	$1{\cdot}1 \times 10^7$	$4{\cdot}0 \times 10^7$	$8{\cdot}3 \times 10^7$
HVJe + HVJ cl.14	39	Elastase	$1{\cdot}6 \times 10^8$	$7{\cdot}7 \times 10^7$	$1{\cdot}2 \times 10^7$
		Trypsin	$2{\cdot}0 \times 10^7$ (0·13)‖	$4{\cdot}6 \times 10^7$ (0·60)	$2{\cdot}2 \times 10^7$ (1·8)
	32	Elastase	$3{\cdot}5 \times 10^7$	$3{\cdot}8 \times 10^7$	$3{\cdot}0 \times 10^6$
		Trypsin	$5{\cdot}8 \times 10^7$ (1·7)	$2{\cdot}8 \times 10^8$ (7·4)	$2{\cdot}5 \times 10^8$ (83)

* LLCMK2 cells were infected with HVJe (m.o.i. = 1) and HVJ cl.14 (m.o.i. = 5) alone or mixedly at 39 °C or 32 °C. HVJe (*ts*$^+$, try$^-$, ela$^+$, c.p.e.$^+$); HVJ cl. 14 (*ts*$^-$, try$^+$, ela$^-$, c.p.e.$^-$).

† Plaque assays were carried out at 32 °C with trypsin (1 μg/ml) or elastase (5 μg/ml) in overlay medium.

‡ Loss of virus production due to cell destruction.

§ NT, Not tested.

‖ The ratio of HVJ cl.14 to HVJe (infectivity titre assayed in the presence of trypsin to that of elastase).

Table 2. *Analysis of progeny viruses produced by single and mixed infection of LLCMK2 cells with HVJe, HVJ cl.151 and HVJ cl.14 at 32 °C and 39 °C*

Virus infected*	Incubation temperature (°C)	Protease added†	Infectivity titres (p.f.u./ml) 24 h	48 h
HVJ cl.151	39	Elastase	$<10^4$	NT‡
		Trypsin	$1{\cdot}4 \times 10^4$	$8{\cdot}2 \times 10^4$
	32	Elastase	$<10^4$	NT
		Trypsin	$4{\cdot}0 \times 10^6$	$2{\cdot}6 \times 10^7$
HVJ cl.151 + HVJe	39	Elastase	$6{\cdot}5 \times 10^5$	NT
		Trypsin	$1{\cdot}6 \times 10^6$ (2·5)§	NT
	32	Elastase	$3{\cdot}1 \times 10^6$	$4{\cdot}2 \times 10^6$
		Trypsin	$5{\cdot}5 \times 10^6$ (1·7)	$2{\cdot}6 \times 10^7$ (6·2)
HVJ cl.151 + HVJ cl.14	39	Trypsin	$7{\cdot}0 \times 10^5$	$2{\cdot}2 \times 10^6$
	32	Trypsin	$6{\cdot}6 \times 10^6$	$2{\cdot}8 \times 10^7$

* LLCMK2 cells were infected with HVJe (m.o.i. = 1), HVJ cl.151 (m.o.i. = 5) and HVJ cl.14 (m.o.i. = 5) alone or mixedly at 39 °C or 32 °C. HVJ cl.151 (*ts*$^-$, try$^+$, ela$^-$, c.p.e.$^-$); HVJe (*ts*$^+$, try$^-$, ela$^+$, c.p.e.$^+$); HVJ cl.14 (*ts*$^-$, try$^+$, ela$^-$, c.p.e.$^-$).

† Plaque assays were carried out at 32 °C with trypsin (1 μg/ml) or elastase (5 μg/ml) in overlay medium.

‡ NT, Not tested.

§ The ratio of HVJ cl.151 to HVJe (infectivity titre assayed in the presence of trypsin to that of elastase).

those shown in Table 1. The M protein of progeny viruses from the coinfected cells was examined by SDS electrophoresis and autoradiography. As shown in Fig. 3 (track 3), the progeny viruses from these coinfections at 32 °C contained predominantly M polypeptide of HVJ cl.151 (M_{36K}). Thus, most of the results obtained in this type of mixed infection using

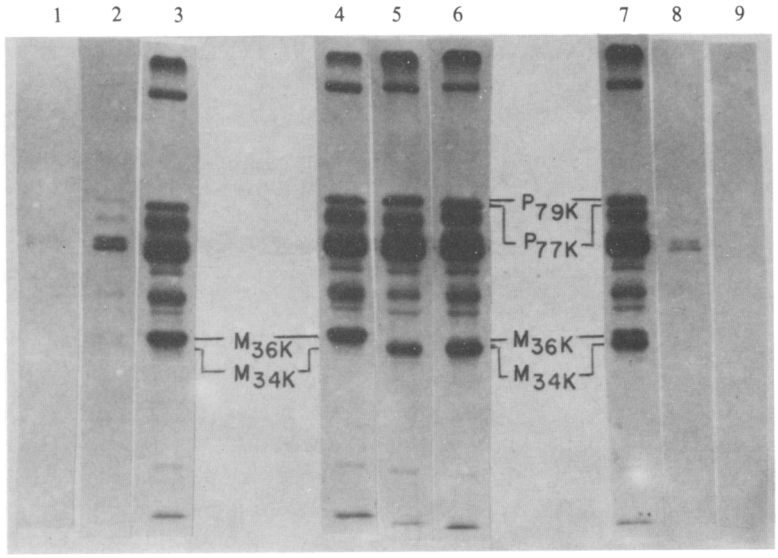

Fig. 3. Autoradiograph of polyacrylamide gel electrophoresis of progeny from single and mixed infections with HVJ cl.151, HVJe and HVJ cl.14. LLCMK2 cells were infected with track 1, HVJ cl.151 (m.o.i. = 5) at 39 °C; track 2, HVJ cl.151 × HVJe (m.o.i. = 5 × 1) at 39 °C, track 3, at 32 °C; track 4, HVJ cl.151 (m.o.i. = 5) at 32 °C; track 5, HVJe (m.o.i. = 1) at 32 °C; track 6, HVJ cl.14 (m.o.i. = 5) at 32 °C; track 7, HVJ cl.151 × HVJ cl.14 (m.o.i. = 5 × 5) at 32 °C, track 8, at 39 °C; track 9, HVJ cl.14 (m.o.i. = 5) at 39 °C. At 24 h post-infection, cells were labelled with [^{35}S]methionine as described in Methods.

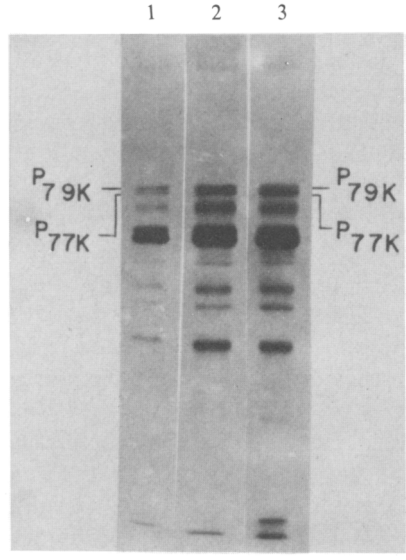

Fig. 4. Kinetic analysis of pulse–chase experiments. LLCMK2 cells were infected with HVJo and HVJ cl.14 at an m.o.i. of 1 and 5 respectively. At 16, 20 and 24 h post-infection at 39 °C, cells were labelled with [^{35}S]methionine and then chased with MEM containing a fivefold excess of cold methionine (tracks 1, 2 and 3 respectively). At 22 h after the chase, virus released in the medium was collected and subjected to electrophoresis and autoradiography.

HVJ cl.151 instead of HVJ cl.14 appeared to be nearly the same as those described above (Table 1, Fig. 1, 2), although some parts differed slightly.

A different pattern of progeny virus production was observed when the mixed infections were done using two *ts* mutants, HVJ cl.151 and HVJ cl.14. The progeny viruses of this mixed infection at 32 °C exhibited M and P polypeptides of both strains (M_{34K}, M_{36K}) (P_{79K}, P_{77K}) with almost equal amounts (Fig. 3, track 7). This means that homologous interference between these two *ts* mutants does not occur. However, this mixed infection at 39 °C caused a significant complementary replication between these two *ts* mutants as indicated in Table 1 and 2.

Kinetic study of pulse–chase experiments

To investigate how the *ts* mutant was rescued by HVJo, a kinetic study of pulse–chase experiments with HVJ cl.14 × HVJo coinfected cells was carried out at 39 °C. As seen in Fig. 4, the P polypeptide first detected at 16 h in infected cells was shown to be coded for by HVJo(P_{79K}). At 20 h post-infection, P polypeptide derived from HVJ cl.14(P_{77K}) was synthesized in an amount almost equal to that from HVJo. Synthesis of HVJ cl.14-derived P protein (P_{77K}) then became dominant over HVJo-derived P protein (P_{79K}) after 24 h, even at this non-permissive temperature (39 °C). This finding indicates that primary transcription of the HVJo genome and subsequent HVJo protein synthesis occur in the coinfected cells prior to protein synthesis coded by HVJ cl.14, possibly being followed by successive rescue of the *ts* mutant at 39 °C.

DISCUSSION

In an attempt to examine the intracellular interaction between HVJ cl.14 and HVJo, progeny viruses from mixed infection with HVJ cl.14 and the protease activation mutant of HVJo (HVJe), activated only by elastase were analysed. The kinetic analysis of the progeny viruses demonstrated the preferential replication of the *ts* mutant HVJ cl.14 at permissive temperature (32 °C). It was also shown that the synthesis of P polypeptide (P_{79K}) and occurrence of c.p.e. by HVJe were concomitantly inhibited in spite of detection of infectious HVJe by plaque assay. This discrepancy seems to require more detailed analysis in the future, suggesting some inconsistency between the genotypes and phenotypes of progeny viruses produced from coinfected cells. In addition, at the non-permissive temperature the *ts* mutant HVJ cl.14 was rescued by coinfected HVJe of around 900- to 13 000-fold over that found for single infection with this *ts* mutant. The results of pulse–chase experiments showed that the P polypeptide synthesized first in HVJo × HVJ cl.14-coinfected cells at 39 °C were coded for by HVJo(P_{79K}) followed by HVJ cl.14-coded P protein (P_{77K}) synthesis, and then HVJ cl.14-derived P polypeptide (P_{77K}) synthesis became dominant. This finding and the fact that u.v.-inactivated wild-type virus (HVJe) cannot rescue HVJ cl.14 suggests that newly synthesized HVJo RNA polymerase may transcribe and replicate HVJ cl.14 genomes more efficiently than, and in preference to the HVJo genome, although the reason is unknown at present. A similar situation exists in the case of HVJe × HVJ cl.151 mixed infection at 32 °C where progeny viruses contain predominantly M protein derived from HVJ cl.151(M_{36K}) rather than from HVJe(M_{34K}), and replication of HVJe is considerably inhibited. Considering the intracellular interaction described previously, the evidence observed here for dominant growth or rescue of the *ts* mutant and, conversely, growth inhibition of wild-type virus with reduced c.p.e., appeared to be favourable factors to the survival of HVJ persistent infection. However, the complementation phenomenon between the two *ts* mutants, HVJ cl.14 and HVJ cl.151, suggests that a specific temperature-sensitive lesion required for establishment of persistent infection does not exist, or that temperature sensitivity of the virus is not always necesssary for it.

Kimura *et al.* (1976) described three factors involved in the intracellular interference: (i) interferon-mediated interference, (ii) production of proteins coded by interfering virus and (iii) competition for enzymes substrates and replication sites. However, selective growth of HVJ cl.14 was observed even in coinfected Vero cells (data not shown) which are known to be genetically defective in interferon production (Desmyter *et al.*, 1968), showing no causative relation of interferon production to our present case. Other new proteins coded by HVJ cl.14 have not yet been detected, except for those previously reported as HVJ-derived proteins. The third factor, particularly competition for replication sites, seems to provide a possible explanation for our present case. HVJ cl.14 genomes may stay for relatively longer periods in the replication site, controlling subsequent production of the *ts* mutant virus. However, HVJo genomes may be rapidly incorporated into progeny particles, as shown in the P polypeptide synthesis of mixed infection and pulse–chase experiments (Fig. 1, track 4), and gradually decreased within the cells. If this explanation is true, it also seems to be quite useful for the general interpretation of establishment and maintenance of HVJ persistent infection, particularly with the *ts* mutant, although detailed mechanisms need to be made clearer.

Portner & Kingsbury (1971) reported homologous interference by defective-interfering particles (DIP) of HVJ, and Roux & Holland (1979) easily established HVJ-carrier cells with standard virus and a large amount of DIP. However, the possibility of involvement of this DIP activity in our present system may be eliminated by the facts that net virus production was not significantly altered in mixed infection from single infection, and RNA synthesized in HVJ cl.14-infected cells was found to contain the same proportion of 18S and 50S RNA synthesized in HVJo-infected cells (data not shown).

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