Interference between Virulent and Avirulent Strains of Sendai Virus

By KENJI SUGITA
Shionogi Research Laboratories, Shionogi & Co. Ltd., Fukushima-ku, Osaka 553, Japan

(Accepted 2 February 1981)

SUMMARY

Interference between a virulent and an avirulent strain of Sendai virus was studied by plaque morphology and analysis of viral protein and RNA. On simultaneous infection a virulent, large plaque-forming strain (RL) was inhibited by an avirulent, small plaque-forming strain (RS). This interference was dose-dependent and decreased with u.v. irradiation of RS. One infectious particle was sufficient to induce the interference. SDS–polyacrylamide gel electrophoresis showed that interference was detectable in the synthesis of viral P protein; this was abolished when RS was u.v.-irradiated. Both growth and P protein synthesis of RL was restricted by superinfection with RS when this was done within 4 h after infection of RL, but the interference decreased gradually after this period and was not detectable after 8 h. Cycloheximide prolonged the period susceptible to superinfection by RS.

INTRODUCTION

When a virus which is virulent to a certain animal is passaged in embryonated eggs or tissue cultures, it often loses virulence to the original host animal due to the appearance of defective-interfering (DI) particles or the addition of an avirulent strain. Autointerference by DI particles has been studied by many workers (Kingsbury & Portner, 1970; Johnston et al., 1975; Roman & Simon, 1976; Igarashi et al., 1977; Khan & Lazzarini, 1977; King et al., 1979; Kolakofsky, 1979; Logan, 1979). Although several reports have described the interference of an avirulent, non-plaque-forming mutant (Bratt & Rubin, 1968; Noronha-Blob & Schulze, 1976 b), or a conditional lethal mutant (Roizman, 1965; Zebovitz & Brown, 1968; Kimura & Norrby, 1976; Keränen, 1977), there have been few papers on the interference induced by an avirulent strain which replicates as well as a virulent one. In our study we have found that the interference between strains of Sendai virus belongs to the latter category. We used mainly a virulent, large plaque-forming strain (RL) and an avirulent, small plaque-forming strain (RS). The P proteins of these strains have been found to move at different rates on gel electrophoresis (K. Sugita, unpublished results). The present study made use of these strain characteristics in an examination of the mechanism of the interference.

METHODS

Cells. LLCMK\(_2\) cells (provided by Dr T. Kurimura, Osaka Prefectural Institute of Public Health), were grown as described previously (Sugita et al., 1974). MDBK cells (a kind gift from Dr Y. Nagai, Nagoya University) were grown in minimal essential medium (MEM) supplemented with 10% foetal calf serum. Primary chick embryo fibroblast (CEF) and chick embryo lung (CEL) cells were prepared from 11-day-old and 18-day-old chick embryos respectively and cultured in the growth medium. These cells were used as monolayer cultures grown in 25 cm\(^2\) Falcon plastic flasks for virus production and plaque titration.

Viruses. Four strains of Sendai virus were used. (i) RL (a virulent, large plaque former) and (ii) RS (an avirulent, small plaque former) were isolated in this laboratory. RS is a variant of
RL obtained after 50 passages in embryonated eggs, the characteristics of which are published elsewhere in detail (Sugita et al., 1981). The other (avirulent) strains (iii) Fushimi and (iv) Z, were gifts from Professor N. Ishida, Tohoku University and from Dr Y. Hosaka, Microbiological Institute of Osaka University respectively. Plaques of RS on a plate were clearly distinguishable from those of the other three strains in which plaque morphology was identical. Newcastle disease virus (NDV, Miyadera strain) and influenza virus (Ao/WSN strain) were provided by Professor N. Ishida. All viruses were grown in embryonated eggs and stored at -70 °C.

Infection. The cells were infected with the viruses (unless otherwise stated) at an m.o.i. of 10. Superinfection, when carried out, was at the same m.o.i. After 1 h of adsorption, free virus was removed, cells were washed once with Hanks' solution and 5 ml MEM was added; the infected cells were then incubated at 37 °C for various periods. The cells were lysed by freezing and thawing and the cell debris was removed by centrifugation at 800 g for 15 min and p.f.u. in the supernatant were determined by plaque assay as described previously (Sugita et al., 1974).

Ultraviolet irradiation of virus. Two ml virus suspension (10⁷ p.f.u./ml in Hanks' solution placed in a 6 cm glass Petri dish) were irradiated with a Toshiba u.v. germicidal lamp (15 W) at a distance of 20 cm. The sample was occasionally shaken to allow even distribution.

Interfering activity of u.v.-irradiated virus. LLCMK₂ cells were co-infected with RL at an m.o.i. of 10 and with u.v.-irradiated RS also at an m.o.i. of 10 (Buv). Two other cultures were prepared: one was infected with RL only (A) and another co-infected with RL and non-irradiated RS (B). Interfering activity was calculated from RL yields after 24 h incubation using the formula: (A-Buv)/(A-B).

Analysis of proteins by slab polyacrylamide gel electrophoresis. This procedure was carried out with the modifications described by Lamb et al. (1976). LLCMK₂ cells were infected and incubated in 2 ml MEM at 37 °C. Actinomycin D (1 µg/ml, Sigma) was added at 1 h after infection to stop cellular protein synthesis. For isotopic labelling, MEM was changed to MEM deficient in methionine and supplemented with actinomycin D (1 µg/ml), and [³⁵S]methionine (10 µCi/ml, 780 Ci/mm; The Radiochemical Centre, Amersham) was added. After 2 h incubation, the labelled cells were scraped with a rubber policeman, centrifuged at 800 g for 15 min, and resuspended in 0.25 ml phosphate-buffered saline free from calcium and magnesium; cells were lysed by the addition of an equal volume of SDS (10%) and 1/25 vol. β-mercaptoethanol. The cell lysates were heated at 100 °C for 2 min, and then mixed with 1/6 vol. 60% sucrose and 1/100 vol. 0.5% bromophenol blue as a marker. Samples were loaded on a slab gel (17 cm × 15 cm × 2 mm) composed of a 12.5% acrylamide resolving gel and 5% acrylamide stacking gel. Preparation of polyacrylamide gel and electrophoresis buffer was according to the method of Laemmli (1970). The resolving gel was composed of final concentrations of 12.5% acrylamide, 0.3% N₂,N'₂-methylenebisacrylamide, 0.1% SDS, 0.07% N₂,N₁,N₁',N₁'-tetramethylethylenediamine (TEMED) and 0.07% ammonium persulphate in 0.375 M-tris–HCl buffer pH 8.8. The stacking gel was composed of 5% acrylamide, 0.1% N₂,N'₂-methylenebisacrylamide, 0.1% SDS, 0.15% TEMED and 0.045% ammonium persulphate in 0.375 M-tris–HCl buffer pH 6.8. The electrophoresis buffer was composed of 25 mM-tris, 0.192 M-glycine and 0.1% SDS. Electrophoresis was carried out at room temperature for 16 h at a constant current of 20 mA. The slab gel was stained with a mixture of 50% methanol, 7% acetic acid and 0.2% Coomassie Brilliant Blue R-250 for 15 min and destained with several changes of 10% methanol, 7% acetic acid, and dried under vacuum on to chromatographic paper. Dried gels were exposed to Fuji X-ray film overnight.

Measurement of synthesis and analysis of viral RNA by slab agarose gel electrophoresis. LLCMK₂ cells were infected with RL or RS, incubated at 37 °C, and labelled with
Interference between Sendai virus strains

[3H]uridine (50 μCi/ml, 40 Ci/mmol; The Radiochemical Centre, Amersham) for 2 h at various times. Actinomycin D (20 μg/ml) was added to the culture 1 h before labelling to stop host RNA synthesis. After labelling, the cells were scraped with a rubber policeman and centrifuged at 1500 rev/min for 15 min, with a washing of the flask with culture medium, and suspended in 2.5 ml NET buffer pH 7.4 (0.1 M-NaCl, 0.01 M-EDTA, 0.01 M tris-HCl). A 20 μl portion of the suspension was sampled and put on a filter paper disc (Toyo No. 2). Discs were dried, washed three times with 0.3 M-trichloroacetic acid and once with acetone, and the radioactivity of samples was then counted.

SDS (1%) was added to the remaining cell suspension. Immediately after the turbidity disappeared an equal volume of hot phenol (60°C) was added. The mixture was then incubated at 60°C for 30 min and mixed gently in a rolling drum at 4°C overnight. The aqueous fraction was separated by centrifugation at 10000 g for 10 min and RNA was precipitated by the addition of 2.5 vol. cold ethanol (−20°C). The precipitate was dissolved in 0.2 ml NET buffer supplemented with 1% SDS and heated at 70°C for 30 s. To this solution was added 40 μl of a mixture containing 60% sucrose and 0.025% bromophenol blue. Agarose horizontal slab gels (Seakem grade II, 15 cm x 15 cm x 3 mm) were prepared at a concentration of 0.7% in 40 mM-tris-acetate pH 7.3, 5 mM-sodium acetate and 1 mM-EDTA. A 40 μl sample of the RNA solution (containing 4 x 10^4 ct/min) was applied to the gel and electrophoresed at room temperature for 5 h at a constant current of 30 mA in the same buffer. For fluorography gels were treated at room temperature, twice with a mixture of 80% ethanol and 20% dioxan for 1 h each and once with the same mixture containing 10% 2,5-diphenyloxazole for 2 h, and then dried under vacuum on chromatographic paper. Dried gels were exposed to Fuji X-ray film at −70°C.

RESULTS

Interference between two plaque variants

Virus growth in LLCMK₂ cells either singly infected with RL or RS, or in co-infected cells was compared at various times after infection. On single infection both strains grew well and there was almost no difference in the growth curves (Fig. 1). However, in the co-infected culture RL grew at a reduced rate and ceased to grow at an earlier period; also, the maximum yield was a small percent of that in the singly infected culture. On the other hand, replication of RS was neither reduced nor enhanced by co-infection with RL.

Host effect on the interference

Previous investigators have demonstrated that virus interference is influenced by the host cells (Choppin, 1969; Huang & Baltimore, 1970; Noronha-Blob & Schulze, 1976a), e.g. two plaque variants of influenza virus caused interference in MDBK cells but did not in CEF cells (Noronha-Blob & Schulze, 1976a). We performed interference experiments using different host cells (LLCMK₂, MDBK, CEF and CEL cells). Cells were singly infected with RL or RS, or co-infected with RL and RS; the virus yield was titrated 24 h after infection. The results showed that the virus yield of RL in co-infected cells was 16 to 19% of that in single infection on MDBK, CEF and CEL cells. This was further reduced to 2.4% when LLCMK₂ cells were used. Although interference appeared in all cell lines, LLCMK₂ cells were the best ones in which to detect it. Therefore, only these cells were used in later experiments.

Interference of RS on the growth of other strains

To ascertain whether RS interferes with RL specifically, interference between four strains of Sendai virus was examined. No interference was found in mixed infections of RS and Fushimi, or of RS and Z, although each virus yield was decreased to about half in the
Fig. 1. Interference between RL and RS strains of Sendai virus. LLCMK2 cells were infected with RL or RS, or mixedly infected with RL and RS, each with an m.o.i. of 10. The cells were incubated at 37 °C and lysed at various times; numbers of (a) RL and (b) RS were titrated by plaque assay. , Single virus infection; , mixed virus infection.

Fig. 2. Effect of time of RS infection on the interference. LLCMK2 cells were infected with RL (m.o.i. 10) at zero time, and challenge of RS (m.o.i. 10) was carried out at the times indicated on the abscissa. Infected cells were lysed at 24 h and both virus yields were titrated. , RL; , RS.

Fig. 3. Dose effect of RS on the interference. LLCMK2 cells were infected with RL at an m.o.i. of 10 and co-infected with RS at various multiplicities as indicated. The yield of RL was titrated after 24 h and is expressed as the relative yield of RL in the co-infected culture to the yield of RL in the culture infected with RL only. A broken line indicates the fraction of cells uninfected with RS which was calculated according to the Poisson distribution.

respective singly infected culture. Unfortunately, the interference between RL and Fushimi, RL and Z, and Fushimi and Z could not be observed by plaque assay because of the close resemblance of their plaque morphology. This result suggests that RS interferes specifically with RL. Multiplication of RL was not significantly restricted by influenza and Newcastle disease viruses; RS did not interfere with multiplication of these viruses (data not shown).

Effect of time of infection with RS on interference

LLCMK2 cells were infected with RS at various times before and after infection with RL. Cultures were lysed at 24 h after RL infection and both virus yields were titrated. As shown
Interference between Sendai virus strains

Fig. 4. Effect of u.v. irradiation on the interference. RS was u.v.-irradiated for various periods as described in Methods and the survival of infectivity was determined by plaque assay (●). LLCMK₂ cells were co-infected with RL and irradiated RS, and incubated for 24 h. Numbers of RL were titrated and survival of the interfering activity was calculated (○).

in Fig. 2, the yield of RL was reduced by RS superinfection up to 4 h after RL infection. After this period the interference was reduced and was almost completely abolished by superinfection at 8 h. This showed that the early phase of the growth cycle of RL was sensitive to infection of RS. On the other hand, the yield of RS was complementary to that of RL. It was almost unaffected when cells were infected with RS before RL infection and superinfected within 4 h after RL infection, but decreased when the superinfection of RS was carried out later than 4 h.

Dose effect of RS on the interference

LLCMK₂ cells were infected with RL at an m.o.i. of 10 and co-infected with RS at various multiplicities, and the titre of RL assayed at 24 h after infection. Fig. 3 shows the result and a theoretical curve (a broken line) indicating the fraction of cells not infected with infectious particles of RS; this was calculated from an assumption that particle adsorption obeys the Poisson distribution. As the multiplicity of RS increased the yield of RL decreased and declined to a plateau of a few percent at the maximum interference. At multiplicities lower than 2 the curve of RL yield is in good agreement with the one hit curve. Therefore, it is likely that infection by a single particle in the RS preparation is sufficient to restrict the growth of RL when both viruses infect simultaneously. It would then seem to be the case that RS particles are involved in the restriction, unless we assume that infectious RS particles do not restrict RL, but that other (e.g. DI) particles are really responsible for the interference and are present in the same quantity as the infectious RS particles.

Loss of interfering activity of RS by u.v. irradiation

If infectious RS is responsible for the interference, then interference should be as sensitive to u.v. irradiation as is infectivity. However, if the interference is induced by other particles which contain a smaller RNA genome fragment then interference should be less sensitive than virus infectivity (Johnston et al., 1974; Nayak et al., 1978; Bay & Reichmann, 1979; Kowal & Stollar, 1980), but it could also be possible that the interference is resistant to u.v. irradiation (Baluda, 1959; Noronha-Blob & Schulze, 1976b).

RS was u.v.-irradiated for various periods and its survival was calculated as described in Methods. To determine the interfering activity, LLCMK₂ cells were simultaneously infected with RL at an m.o.i. of 10 and irradiated RS also at an m.o.i. of 10. The cells were incubated for 24 h and numbers of RL were assayed after lysis. The interfering activity was calculated as described in Methods. Survivals of infectivity of RS and of the interfering activity after u.v. irradiation are shown in Fig. 4. The interference was reduced as rapidly as infectivity. This
indicates strongly that the entire viral genome is involved in the interference and that if defective particles are involved then their genome size must be very close to that of infectious RS particles. It is also suggested in the former case that replication of RS RNA is necessary for the interference, or at least that all genes of RS must be expressed. Similar results have recently been observed with a temperature-sensitive (ts) mutant of Sindbis virus in which the interfering capacity was as sensitive as viability to u.v. irradiation (Kowal & Stollar, 1980). However, another ts mutant of the same virus was reported in which a single infectious particle induced interference with superinfected wild-type and in which the target size of interfering activity to u.v. irradiation was one-fifth that of infectivity (Johnston et al., 1974). This discrepancy suggests that the interference may be induced by more than one mechanism.

**Interference in the synthesis of RL protein**

Cells were singly infected with one of the four virus strains and labelled with \[^{35}\text{S}\]methionine (10 µCi/ml) between 8 and 10 h after infection, and the lysates were prepared and subjected to electrophoresis as described in Methods. Proteins characteristic of Sendai virus were well separated and there was a strain-specific difference in the migration velocity of P and NP protein (Fig. 5a to d). P protein of RS (P-RS) migrated faster than P protein of the
Interference between Sendai virus strains

other three strains; the NP proteins of RL, Z and Fushimi had different mobilities but NP-RL and NP-RS moved at the same rate. The different mobilities of P and NP of these strains were confirmed by co-electrophoresis of a mixture of the labelled lysates.

When cells were co-infected with RL and RS, P-RL did not appear and only P-RS was detectable in the P protein region (Fig. 5e). Strong parallelism between the interference in
virus growth and in the strain-specific P protein synthesis was confirmed by further experiments. First, P-RL was not replaced by P-RS when cells were co-infected with RL and u.v.-irradiated RS (1 × 10⁻⁶ survival). Second, when cells were infected with RL and superinfected with RS at 0, 2, 4, 6 and 8 h after RL infection and labelled between 12 and 14 h, only P-RS was detectable in the first two cultures but both P-RL and P-RS were found when cells were superinfected at 4 h (Fig. 6). Later superinfection permitted synthesis of P-RL but reduced synthesis of P-RS. These results are in good agreement with the result shown in growth experiments (Fig. 2). This confirms that the complete interference of RS is induced by 4 h after RL infection. In another experiment, cells simultaneously infected with RL and RS were labelled for 2 h at various times after infection to see when P-RL synthesis is inhibited; the P-RL band was never observed. The migration of NP in cells co-infected with RL and Fushimi, or RL and Z was particularly interesting. However, interference of virus growth was not seen in these cells because of their similar plaque morphology. The band of NP-RL disappeared and only NP-F, or NP-Z, was detected (Fig. 5f, g) suggesting that RL was not only interfered with by RS but also by Fushimi and Z.

There was no interference in the growth experiment cells co-infected with RS and Fushimi or with RS and Z; there were two discrete bands in the P and NP protein regions (Fig. 5 h, i). However, since two bands were identified in the NP protein region in cells co-infected with Fushimi and Z (Fig. 5 j) it must not be assumed that these strains do not interfere.

It is unlikely that the synthesis of mRNAs for P and NP protein are specifically suppressed by the interfering virus since Glazier et al. (1977) found a single promoter for transcription of the Sendai virus genome. The above results may indicate that the interfering virus genome is preferentially transcribed. Another possibility, that translation of mRNA for P-RL and NP-RL is selectively inhibited, may be ruled out since our u.v. irradiation experiment suggested that all the genes of the interfering virus are necessary for induction of the interference.

**Effect of cycloheximide on interference**

RNA synthesis by paramyxoviruses proceeds in three sequential steps (Kingsbury, 1974): (i) mRNA synthesis from virion RNA as a template by the virion-associated polymerase (primary transcription), (ii) replication of complementary RNA from virion RNA and subsequent replication of genome RNA (genome replication) and (iii) mRNA synthesis after genome replication (secondary transcription). Since cycloheximide inhibits protein synthesis, only primary transcription by the virion-associated RNA polymerase proceeds in the presence of this drug; genome replication and secondary transcription do not. By using cycloheximide, we attempted to determine whether genome replication was involved in the interference. At various times after RL infection, cycloheximide (5 µg/ml) was added to the cultures and removed at 8 h to allow virus growth and the cultures superinfected with RS. Superinfection of RS at 8 h did not interfere with RL (Fig. 2). All cultures were further incubated for 16 h. The yield of RL in the cultures was titrated and expressed as a percentage of that in the control culture, which was not treated with cycloheximide and infected with RL only. As seen in Fig. 7, cycloheximide, when administered at 4 h, allowed the superinfected RS to interfere with RL growth; addition later than 4 h slightly lowered the interference at the beginning and strongly lowered it at the later period. Comparing Fig. 2 and Fig. 7, it is clear that cycloheximide stops the intracellular interference at the time when it is added. This experiment indicates that the interference is induced before RNA replication of RL begins, and predicts that this replication starts later than 4 h.

**RNA synthesis of RL and RS**

Cells singly infected with RL or RS were fed with [³H]uridine for 2 h at 2, 3, 4 and 5 h after infection in the presence of actinomycin D (20 µg/ml) to inhibit host RNA synthesis. Cells
Interference between Sendai virus strains

Fig. 7. Effect of cycloheximide added at various times on the interference. LLCMK₂ cells were infected with RL at zero time and cycloheximide (5 µg/ml) was added at the times indicated. At 8 h cycloheximide was removed, and cells washed twice with Hanks' solution and superinfected with RS. After adsorption for 1 h cells were fed with 5 ml MEM and incubated until 24 h after infection with RL. Yields of RL were titrated and expressed as a percentage of the yield of untreated cells which were infected with RL only.

Fig. 8. Kinetics of RNA synthesis of RL and RS. LLCMK₂ cells were infected with RL or RS and labelled with [³H]uridine for 2 h at 2, 3, 4 and 5 h post-infection. Actinomycin D (20 µg/ml) was added 1 h before labelling to inhibit host RNA synthesis (more than 95% of incorporation of radioactivity into acid-insoluble fraction was inhibited). At the end of the labelling period, cells were collected and resuspended in 2.5 ml NET buffer. A 20 µl sample of the suspension was put on a paper disc and radioactivity was measured after washing with 0.3 M-trichloroacetic acid and acetone. The labelled RNA was extracted from the remainder and a portion containing 40000 cts/min was electrophoresed (see also Fig. 9). To calculate the incorporation of [³H]uridine into acid-insoluble materials radioactivity of infected cells was corrected for radioactivity of uninfected cells and plotted against the midpoint of the 2 h pulse. ●, RL; ○, RS.

were collected, resuspended and lysed. A portion of the lysate was applied to filter paper discs which were dried and washed three times with 0.3 M-trichloroacetic acid and once with acetone to determine incorporation of [³H]uridine into acid-insoluble material as described in Methods. The incorporation started rapidly after infection at a higher rate into RS-infected cells. Conversely, its rate in RL-infected cells was low for the first 5 h but suddenly increased and finally became close to that in RS-infected cells (Fig. 8).

³H-labelled RNA was extracted and samples with a fixed radioactivity were subjected to slab agarose gel electrophoresis and fluorography. On electrophoresis, part of, or sometimes most of, the non-specific radioactivity migrated very rapidly; four virus-specific RNAs were found with sizes estimated as 14S, 18S, 35S and 57S from comparison with cellular 18S and 28S rRNA which are similar to the results of Blair & Robinson (1968). RNA from virions migrates at the same rate as the 57S species and the other three RNAs are mRNAs (Blair & Robinson, 1968; Glazier et al., 1977).

Viral RNAs appeared according to a definite time sequence. Most of the radioactivity in preparations from both uninfected cells and cells infected with RL labelled between 2 and 4 h migrated very rapidly and did not remain in the region of viral RNAs (Fig. 9 a, b). When cells were infected with RL only, the 18S RNA was weakly detected between 2 and 4 h. Between 3 and 5 h the 14S and 18S RNAs were distinctly visible and the 35S and 57S RNAs appeared between 4 and 6 h. The last finding supports the previous prediction that RL RNA replicates later than 4 h. There was no difference in mobility of RNAs and synthetic order between
RNAs of RL and RS but onset of RS RNAs started at an earlier period. In RS-infected cells all mRNAs were detected between 2 and 4 h, although there was little 35S RNA. The 57S RNA may be synthesized during this period but only became distinct between 3 and 5 h. Thus, synthesis of all RNAs of RS started earlier than the synthesis of the corresponding RNA of RL.

**DISCUSSION**

There have been many investigations carried out on the interference induced by viruses defective in infectivity: u.v.-inactivated virus (Baluda, 1959), a conditional lethal mutant (Pohjanpelto & Cooper, 1965; Roizman, 1965; Kimura & Norrby, 1976) and DI particles (Portner & Kingsbury, 1971; Cole & Baltimore, 1973; Huang, 1973; LaMontagne et al., 1975; Chow et al., 1977; Colonna et al., 1977; Stamminger & Lazzarini, 1977). Only a few investigations have been reported on the interference caused by infectious virus, e.g. Sindbis virus (Johnston et al., 1974; Igarishi et al., 1977; Kowal & Stoller, 1980) and herpes simplex virus (HSV) (Purifoy & Powell, 1977). Interference of Sindbis virus was found only when the cells were persistently infected by the interfering virus. Superinfecting virus, in the case of HSV, induced interference during the first 3 h after infection of the interfered virus, when the m.o.i. of the interfering virus was 10 times higher than that of the interfered virus.

The present investigations have strongly suggested that growth of RL is interfered with by infectious RS particles since (i) the yield of RL was reduced by increased multiplicities of co-infecting RS and 1 p.f.u. of RS was sufficient to induce the interference, and (ii) infectivity and the interfering activity of RS were similarly reduced by u.v. irradiation. Since Sendai virus induces production of interferon even when u.v.-irradiated (Henle et al., 1959), the
Interference between Sendai virus strains

present observation suggests that interferon is not involved in this interference. This is further supported by the fact that the interference in strain-specific protein synthesis was induced in the presence of actinomycin D, an inhibitor of interferon production (Fig. 5 e). Growth of RL and synthesis of strain-specific proteins have shown that RL was sensitive to challenge by superinfecting RS during the first 4 h after RL infection. This implies that maturation is not involved in the interference. Superinfection after this period allowed RL to grow and form RL-specific protein. Furthermore, later challenge of RS did not only affect RL, but also growth, and specific protein synthesis of RS was strongly reduced. Cycloheximide, a potent inhibitor of protein synthesis and of replication of viral RNA, prolonged the susceptible period when it was added within this critical time. When added later than 4 h, it tended to lose the ability to prolong the period. We confirmed that cycloheximide inhibited replication of RL RNA when added within 4 h, but did not inhibit replication when added after 5 h. The interference of RS occurred before replication of RL RNA started and was reduced after replication of RL RNA started. RNA synthesis of RS started earlier and at a higher rate than RNA synthesis of RL at an early phase of infection. What then is the cause of earlier onset of RNA synthesis by RS? Since both strains adsorbed to the cells at a similar rate (data not shown) it did not result from a difference in adsorption rate. At present, we have no knowledge about the mechanism of penetration and uncoating of these strains. However, it is interesting to note that P-RS migrated faster than P-RL by electrophoresis and that the NP of the other interfering strains, Fushimi and Z, had a different mobility from NP-RL, a product of the interfered strain. It is notable that these two proteins are components of nucleocapsids and that they were the only proteins found in a purified preparation which had RNA polymerase activity (Marx et al., 1974). Barry & Mahy (1979) have recently reported that genes of influenza virus which control the synthesis of capsid proteins, P1, P2, P3 and NP, are also involved in viral RNA synthesis. These findings may suggest that the altered P and NP proteins of the interfering strains are involved in the earlier initiation of the RNA synthesis. Comparative studies on in vitro RNA synthesis using these proteins are yet to be done.

Earlier initiation of primary transcription and genome replication of RS would lead to active secondary transcription and suppress transcription and replication of RL RNA. Since, when RS superinfected the RL-infected cells after the onset of RL growth the interfering activity and the yield of RS decreased, the interference is not simply unidirectional. It seems likely that the genome of the virus which has initiated active replication becomes dominant in RNA replication. This could be understood if we assume that a common factor(s) (host and/or viral) essential for and specific to the replication machinery of Sendai virus is limited in infected cells.

We thank Professor T. Minagawa, Dr S. Toyama, Dr E. Ryo for their critical discussions and Dr H. Fujisawa for his helpful advice. We also thank Dr K. Sato for his encouragement throughout this work.

REFERENCES


Interference between Sendai virus strains


(Received 14 August 1980)