Replication of herpes simplex virus type 1 DNA is inhibited in a temperature-sensitive mutant of BHK-21 cells lacking RCC1 (regulator of chromosome condensation) and virus DNA remains linear

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tsBN2, a temperature-sensitive (ts) growth mutant of the hamster cell line BHK-21, has a point mutation in the RCC1 (regulator of chromosome condensation) gene, and prematurely enters mitosis at 39.5 °C, a nonpermissive temperature. In this mutant at 39.5 °C infectious progeny of herpes simplex virus type 1 (HSV-1) was not produced and replication of HSV-1 DNA was inhibited. HSV-1 DNA from virus particles is normally circularized upon infection, and circularized HSV-1 DNA molecules can serve as template for DNA replication. In tsBN2 at 39.5 °C, HSV-1 DNA appeared to remain linear after infection, suggesting the obstruction of HSV-1 DNA circularization, which could account for failure of HSV-1 DNA replication. In transient replication assays performed in tsBN2 at 39.5 °C, through superinfection with HSV-1 helper virus, there was no evidence of replication of circular DNA of the hybrid plasmid containing the HSV-1 replication origin. Production of mRNAs of HSV-1 early genes required for HSV-1 DNA replication was decreased in tsBN2 at 39.5 °C. Therefore, RCC1 was assumed to be involved in the formation of an HSV-1 DNA configuration suitable for replication (that is circularization) and the supply of proteins required for replication of the circularized HSV-1 DNAs.

Introduction

The herpes simplex virus type 1 (HSV-1) genome is a linear double-stranded DNA of about 152 kb. The DNA consists of two covalently linked components, L and S, and each component consists of unique sequences (UL and US) flanked by inverted repeat sequences (TRL, IRL, IRS and TRS) (Fig. 1a) (Roizman, 1979). Because the L and S components independently invert, extracted HSV-1 DNA consists of four isomers. The short sequence a is repeated directly at both ends of the genome and is present in inverse orientation at the L–S junction (Fig. 1a) (Mocarski & Roizman, 1982). One to several copies of the a sequence are present at the end of the L component and at the L–S junction, but only one copy is present at the end of the S component. The a sequence encodes the cis-acting site for circularization of viral DNA after infection and cleavage of unit-length HSV-1 DNA from concatemeric forms.

The prevailing model for HSV-1 DNA replication proposes that (i) the termini of each input viral genome fuse immediately after infection, giving rise to circular DNA molecules (endless DNA), (ii) the viral genome replicates as a concatemer, by a rolling-circle mechanism, and (iii) unit-length genome is cleaved and packaged from the concatemeric DNA (Roizman, 1979). The HSV-1 genome has three known origins of DNA replication during lytic infection, one in UL (OriL) and a diploid origin in the short inverted repeats (OriS) (Stow & McMonagle, 1983; Stow, 1992). A set of seven HSV-1 genes together encode all the viral protein required for replication of origin-containing plasmids in transient transfection assays (Wu et al., 1988). Additional functions of the host cell needed for HSV-1 DNA replication remain to be elucidated.

HSV-1 genes are grouped into three temporal classes, immediate-early (IE), early and late, which are sequentially expressed in a cascade fashion (Wagner, 1985). Shortly after infection of a cell by HSV-1, the tegument protein Vmw65 (also designed VP16 or ζTIF) is released from the virion and induces transcription of immediate-early genes (Dalrymple et al., 1985; Pellett et al., 1985). The immediate-early proteins...
then induce expression of early and late genes. Early genes are activated prior to the onset of viral DNA replication, while late genes require DNA replication for maximal expression.

Temperature-sensitive (ts) conditional lethal mutants can serve as important tools for investigation of biological phenomena. We isolated ts mutants in cell proliferation from the BHK-21 cell line and identified genes that complement these ts mutants (Nishimoto & Basilico, 1978). A ts mutant from the BHK-21 cell line, tsBN2, showed premature chromosome condensation at the nonpermissive temperature of 39.5 °C and had a point mutation in the RCC1 (regulator of chromosome condensation) gene, which resulted in the instability of RCC1 protein at the nonpermissive temperature (Nishimoto et al., 1978; Uchida et al., 1990). The RCC1 protein is a nuclear-localized DNA binding protein (Ohtsubo et al., 1989). RCC1 homologues were isolated from various organisms, and many complement across species (Dasso, 1993). These RCC1 homologues are involved in numerous nuclear events, including DNA replication, the cell cycle, maintenance of nuclear structure, protein import, and RNA transcription, processing and export, although no consensus has been reached on the primary role of RCC1 protein (Dasso, 1993; Schlenstedt et al., 1995). The RCC1 homologue protein forms a complex with Ran (Ras-related protein)/TC4, and functions as a guanine nucleotide release protein for Ran/TC4 (Dasso et al., 1994; Yokoyama et al., 1995).

Viral DNA synthesis and infectious progeny virus production by adenovirus 2 and HSV-1 were previously studied using ts growth mutants of the BHK-21 cell line (Nishimoto et al., 1975; Yanagi et al., 1978). At a nonpermissive temperature, 39 or 39.5 °C, both events were inhibited in several ts mutant cell lines. These cell lines were little characterized at that time and the mechanism of inhibition of virus replication was not determined. In the present study, we examined the mechanism of inhibition of HSV-1 DNA replication in tsBN2 cells at 39.5 °C. HSV-1 DNA appeared to remain linear in tsBN2 at 39.5 °C, which suggested the possibility of obstruction of circularization of linear HSV-1 DNA from viral particles. The replication of circular DNA of a hybrid plasmid containing HSV-1 OriF was not detected and production of mRNAs of HSV-1 early genes essential for HSV-1 DNA replication decreased in tsBN2 at 39.5 °C. Therefore, we propose that RCC1 was likely to be involved in the formation of DNA configuration, such as transition from linear to circular HSV-1 DNA, in addition to the supply of proteins required for replication of the circularized HSV-1 DNA.

**Methods**

- **BHK-21 cell lines and culture conditions.** BHK-21 is a continuous cell line of Syrian hamster fibroblasts (Nishimoto & Basilico, 1978). Temperature-sensitive (ts) mutants of the BHK-21 cell line, used in the present study, were tsBN2 (RCCI gene mutated) (Uchida et al., 1990), tsBN7 (DAD1 gene mutated) (Nakashima et al., 1993), tsBN63 (CCG2/RPS4X gene mutated) (Watanabe et al., 1991), tsBN75 (gene encoding ubiquitin-activating enzyme E1 mutated) (Nishitani et al., 1992) and tsBN462 (CCG1 gene mutated) (Sekiguchi et al., 1988). These ts mutant cell lines grow normally at a permissive temperature of 33.5 °C, but cease to grow at a nonpermissive temperature of 39.5 °C. The RCC1 gene is mutated in the tsBN2 cell line, and human RCC1 cDNA clones can complement the tsBN2 mutation (Dasso, 1993; Uchida et al., 1990). BN2-A1 is a ts + transformant of tsBN2, which was transfected with human RCC1 cDNA. Cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) in a humidified atmosphere containing 10% CO₂. Cultures of wild-type BHK-21 and ts mutant cell lines were maintained at 33.5 °C, and that of BN2-A1 at 39.5 °C.

- **Viruses.** The HSV-1 strain GN28 used for the infection of BHK-21 cell lines was described previously (Umene, 1993; Umene & Yoshida, 1993).
For infection of wild-type BHK-21 and ts mutant cell lines at 39.5 °C, cultures were shifted from 33.5 °C to 39.5 °C 16 h prior to infection. For infection of BN2-A1 at 33.5 °C, the culture was shifted from 39.5 °C to 33.5 °C 16 h prior to infection. BHK-21 cell monolayers in Petri dishes were infected with GN28 at an m.o.i. of 10 p.f.u. per cell. After 2 h adsorption at 33.5 or 39.5 °C, the cells were washed three times with DMEM and overlaid with DMEM containing 10% FBS. Incubation at 33.5 or 39.5 °C was continued until the infected cells were harvested. The nomenclature of EcoRI fragments of HSV-1 is from Skare & Summers (1977), and that of BamHI, KpnI and Sall fragments of HSV-1 from Locker & Frenkel (1979).

Hybridization. This was carried out on a Biodyne B transfer membrane (Pall Ultrafine Corp.) as described previously (Umene, 1985a, 1994). The DNA fragments used as the probe were prepared from hybrid phages, plasmids and cosmids.

Phages, plasmids and cosmids. Hybrid phages and plasmids used for the preparation of probe DNA were described previously (Umene, 1985a; Umene & Enquist, 1981; Umene & Yoshida, 1993). Sets of cosmids whose inserts overlap and represent the entire HSV-1 genome were constructed by Cunningham & Davison (1993), and sent to us. The hybrid plasmid containing Ori of HSV-1, pUK386, was constructed by inserting the 0.54 kb SmalI fragment containing Ori of HSV-1 strain Patton into the BamHI site of plasmid vector pUC19 (Stow & Monenagle, 1983; Umene & Enquist, 1981).

Results

Replication of HSV-1 in ts mutant BHK-21 cell lines

We examined replication of HSV-1 in five ts mutant BHK-21 cell lines (Fig. 2b–f). Infectious progeny viruses were not propagated in the three mutant cell lines, tsBN2, tsBN7 and tsBN462, at the nonpermissive temperature of 39.5 °C (Fig. 2b, c, f). Replication of HSV-1 DNA in BHK-21 cell lines was also examined (Fig. 3). Of three mutant cell lines in which infectious progeny viruses were not propagated, HSV-1 DNA was synthesized in cell lines tsBN7 and tsBN462 at 39.5 °C, albeit decreased in comparison with that in the wild-type cell line (Fig. 3a, c, f). HSV-1 DNA synthesis was not detected in tsBN2 at 39.5 °C (Fig. 3b). The mechanism involved in the inhibition of HSV-1 DNA synthesis was examined in the present study.

The RCC1 gene is mutated in the tsBN2 cell line, and human RCC1 cDNA clones can complement the tsBN2 mutation (Dasso, 1993; Uchida et al., 1990). BN2-A1 is a ts+ transformant of tsBN2, which was transfected with human RCC1 cDNA. In BN2-A1 at 39.5 °C, infectious HSV-1 progeny was produced (Fig. 2g) and HSV-1 DNA was replicated (Fig. 3g). Thus, mutation in the RCC1 gene is apparently the cause of failure of HSV-1 DNA replication in tsBN2 at 39.5 °C.

α sequences after HSV-1 infection into BHK-21 cell lines

To investigate the shape of HSV-1 DNA after infection into cells, whole cell DNA of BHK-21 cell lines infected with HSV-1 was extracted, cleaved with Dral, and analysed using Southern hybridization with the α sequence as a DNA probe (Fig. 4). A pair of Dral fragments corresponding to the α sequence was generated from the linear HSV-1 DNA (Fig. 1b); one of the fragments is the unit-length α sequence (0.34 kb fragment in case of HSV-1 strain GN28), and the other is shorter by 16.5 bp, the result of authentic cleavage on DR1 by the cleavage-packaging system (0.32 kb fragment in the case of GN28) (Fig. 4e) (Mocarski & Roizman, 1982; Umene, 1993). The shorter fragment derives from the end of the L component of linear HSV-1 DNA and is not generated from circular HSV-1 DNA. Therefore, the presence of a shorter Dral fragment indicates the presence of linear HSV-1 DNA.

The HSV-1 DNA fragments in samples prepared under permissive conditions (wild-type BHK-21 infected at 33.5 and 39.5 °C and tsBN2 infected at 33.5 °C) increased as infection progressed, as HSV-1 DNA was replicated (Fig. 4a–c). Under nonpermissive conditions (tsBN2 infected at 39.5 °C) there was no increase in fragments as infection progressed, indicating inhibition of HSV-1 DNA replication (Fig. 4d). Linear HSV-1 DNA is thought to be circularized after entry into the cell;
Fig. 3. Detection of HSV-1 DNA by Southern hybridization after infection into ts mutant cells. After cultivation at 33.5 °C (lanes 1–3) or 39.5 °C (lanes 4–6) for 16 h, wild-type (a), tsBN2 (b), tsBN7 (c), tsBN63 (d), tsBN75 (e), tsBN462 (f) and BN2-A1 (g) cells were infected with HSV-1 strain GN28 at an m.o.i. of 10 p.f.u. per cell. After adsorption for 2 h at 33.5 °C (lanes 1–3) or 39.5 °C (lanes 4–6), the monolayers were washed three times with DMEM, overlaid with DMEM containing 10% FBS, and incubated at 33.5 °C (lanes 1–3) or 39.5 °C (lanes 4–6). Cultures were harvested at 0 h (lanes 1 and 4), 12 h (lanes 2 and 5) and 24 h (lanes 3 and 6) post-adsorption and HSV-1 DNA was extracted by the Hirt method (Umene, 1985b). HSV-1 DNAs were cleaved with BsmHI, electrophoresed in a 0.8% agarose gel, transferred to a nylon membrane, and hybridized with 32P-labelled BsmHI-Q fragment of HSV-1. The position of the BsmHI-Q fragment (3.4 kb) is indicated by an arrow.

Fig. 4. Southern blot analysis of the HSV-1 α sequence after HSV-1 infection into BHK-21 cells. After cultivation at 33.5 °C (a, c) or 39.5 °C (b, d) for 16 h, wild-type BHK-21 (a, b) and tsBN2 (c, d) cells were infected with HSV-1 strain GN28 at an m.o.i. of 10 p.f.u. per cell. After adsorption at 33.5 °C (a, c) or 39.5 °C (b, d) for 2 h, the monolayers were washed three times with DMEM, overlaid with DMEM containing 10% FBS, and incubated at 33.5 °C (a, c) or 39.5 °C (b, d). Cultures were harvested at 2 h (lane 1), 4 h (lane 2), 6 h (lane 3) and 8 h (lane 4) post-adsorption, and infected cells were collected by low-speed centrifugation. The pellet was lysed with solution containing 0.02 N-Tris-HCl (pH 8.0), 0.1 M-EDTA, 0.5% SDS and 0.05 mg/ml proteinase K (Sakaoka et al., 1994). The lysate was maintained overnight at 37 °C, and extracted once with phenol and once with phenol/chloroform (1:1). HSV-1 strain GN28 viral particles were prepared after digestion with DNaseI and DNA was extracted from the particles, as described (Umene, 1993). Whole cell DNA of BHK-21 cell lines infected with HSV-1 (a–d) and HSV-1 viral particle DNA (e) were cleaved with Drel, electrophoresed in a 5% acrylamide gel, transferred to nylon membrane, and hybridized with a 32P-labelled 0.175 kb Smal fragment of pUk340, containing almost all the α sequence region of HSV-1 clone TW14 (Umene, 1994). Two fragments of 0.34 and 0.32 kb are indicated (lane M) (Mocarski & Roizman, 1982; Umene, 1994). The 0.34 kb fragment is the unit-length α sequence, and derives from the regions containing two or more copies of the α sequence. The 0.32 kb fragment is shorter by 16.5 bp than the unit-length α sequence as a result of authentic cleavage on DR1 by the cleavage-packaging system, and derives from the terminus of the L component (Fig. 1b).
hence, the amount of 0.32 kb fragment (derived from the end of linear HSV-1 DNA) would be expected to decrease early after infection under permissive conditions. With respect to DNA samples recovered at 2 h post-adsorption, the amount of 0.32 kb fragment obtained from tsBN2 at 39.5 °C appeared to be higher than that obtained under permissive conditions (lane 1, Fig. 4a–d). Therefore, we considered that circularization of linear HSV-1 DNA might be inhibited in tsBN2 at 39.5 °C.

Ends of the L component and the L–S junction of HSV-1 DNA after infection into BHK-21 cell lines

DNA fragments from the ends of the L component [SalI-(I + F) (12.6 kb) and SalI-(l + C) (14.8 kb)] can be distinguished from those from the L–S junction [SalI-(l + F + J) (18.0 kb) and SalI-(l + C + J) (20.2 kb)] (Fig. 1a). Extracted whole cell DNA of BHK-21 cell lines infected with HSV-1 was cleaved with SalI, and analysed by Southern hybridization using a DNA probe derived from the inverted repeat of the L component (Fig. 5). The amount of SalI fragments did not increase as infection progressed in tsBN2 at 39.5 °C, because of inhibition of HSV-1 DNA replication (Fig. 5a). For DNA samples recovered at 2 h post-adsorption, the proportion of SalI fragments derived from the ends of linear HSV-1 DNA (12.6 and 14.8 kb) decreased under permissive conditions, in comparison with that under nonpermissive conditions, while the proportion of fragments derived from the L–S junction (18.0 and 20.2 kb) under permissive and nonpermissive conditions seemed to be similar (lane 1, Fig. 5a–d). These results support the hypothesis that circularization of HSV-1 DNA was inhibited in tsBN2 at 39.5 °C, the DNA remaining linear, in agreement with the results of Fig. 4.

Shape of HSV-1 DNA after infection into BHK-21 cell lines analysed using ATP-dependent deoxyribonuclease

To analyse the shape of HSV-1 DNA present in tsBN2 at 39.5 °C, we used ATP-dependent deoxyribonuclease (ATP-DNase), which acts upon linear DNA molecules and single-stranded circular molecules, hydrolysing them into small, acid-soluble fragments. However, this enzyme does not act on double-stranded circular DNA molecules (Anai et al., 1970; Yamagishi et al., 1983).

The HSV-1 genome is large and would be sheared if its DNA was extracted by shaking. To prepare intact HSV-1 DNA, HSV-1 infected cells and HSV-1 viral particles were embedded in low-melting point agarose and lysed with proteinase K in the agarose block (Zhang et al., 1994). The blocks were then treated with ATP-DNase (Toyobo Inc., Osaka, Japan), and the HSV-1 DNA was extracted with phenol after incubation at 65 °C for 10 min to melt the agarose. Extracted HSV-1 DNA was digested with SalI and analysed by Southern hybridization, using a DNA probe derived from the inverted repeat of the L component (Fig. 6).

We first verified the action of ATP-DNase (Fig. 6a, b). BHK-21 cells infected with HSV-1 for 24 h were assumed to contain both circular and linear HSV-1 DNA, because HSV-1 DNA replicated by a rolling-circle mechanism would generate the circle plus tail molecule and unit-length linear HSV-1 molecule (Fig. 6a) (Severini et al., 1994; Zhang et al., 1994). Two SalI fragments of 18.0 and 20.2 kb derived from the both ends of the L component of linear HSV-1 DNAs were expected to be degraded by ATP-DNase. Two other SalI fragments of 12.6 and 14.8 kb derived from both ends of the L component of linear HSV-1 DNAs were expected to be degraded by ATP-DNase. After treatment with ATP-DNase, SalI fragments disappeared (lanes 2–5, Fig. 6a). Unit-length linear HSV-1 DNA from viral particles was expected to be degraded by ATP-DNase. After treatment with ATP-DNase, SalI fragments disappeared (lanes 2–5, Fig. 6a). Thus, ATP-DNase apparently degraded unit-length linear HSV-1 DNA. This degradation seemed to be specific, because of the presence of two SalI fragments of 18.0 and 20.2 kb in the replicated HSV-1 DNAs after treatment with ATP-DNase (Fig. 6a).

Although it was evident that HSV-1 DNA remained linear in tsBN2 at 39.5 °C, as shown in Figs 4 and 5, the question of whether circular HSV-1 DNA was present or not under nonpermissive conditions remained to be addressed. SalI fragments detected in DNA samples recovered from tsBN2 infected at 39.5 °C (lanes 4–6, Fig. 6c) became undetectable after treatment with ATP-DNase (lanes 1–3, Fig. 6c), thereby indicating the degradation of HSV-1 DNA. Therefore, the amount of circular HSV-1 DNA, which is resistant to ATP-DNase and would yield two SalI fragments of 18.0 and 20.2 kb, seemed to be a minority, if any, of the DNA samples under nonpermissive conditions. Most HSV-1 SalI fragments detected in samples from tsBN2 at 39.5 °C were assumed to derive from linear HSV-1 DNA molecules which would be degraded with ATP-DNase, thereby supporting the hypothesis that circularization of linear HSV-1 DNA was inhibited under nonpermissive conditions and that linear HSV-1 DNA was retained.

Replication of a hybrid plasmid containing HSV-1 Ori5 in BHK-21 cell lines

The circular DNA of hybrid plasmid containing HSV-1 Ori5 can be replicated in cells superinfected with HSV-1 helper virus (Stow & McMonagle, 1983). We examined the replication of a plasmid containing HSV-1 Ori5 (pUK386) in BHK-21 cell lines (Fig. 7). The replicated pUK386 DNA of length 3.2 kb (linearized with KpnI and resistant to DpnI digestion) was detected in samples from wild-type BHK-21 infected at 33.5 and 39.5 °C and tsBN2 infected at 33.5 °C (permissive conditions) (lanes 2–4, Fig. 7), but not in samples from tsBN2 at 39.5 °C (nonpermissive conditions) and wild-type BHK-21 mock-infected at 33.5 °C (lanes 1 and 5, Fig. 7). Thus,
Fig. 5. Southern blot analysis of genomic termini of the L component and L–S junction of HSV-1 DNA after HSV-1 infection into BHK-21 cells. HSV-1 DNAs analysed were the same as those prepared for Fig. 4. Wild-type BHK-21 (a, b) and tsBN2 (c, d) cells were infected with HSV-1 strain GN28 at 33.5 °C (a, c) or 39.5 °C (b, d), as indicated in the legend to Fig. 4. Cultures were harvested at 2 h (lane 1), 4 h (lane 2), 6 h (lane 3) and 8 h (lane 4) post-adsorption, and whole cell DNA of BHK-21 cell lines infected with HSV-1 was extracted. Extracted DNAs were cleaved with SstI, electrophoresed in a 0.8% agarose gel, transferred to a nylon membrane, and hybridized with 32P-labelled 2.35 kb KpnI–SaiI fragment from the KpnI–R fragment on the inverted repeat of the L component (Perry & McGeoch, 1988). The fragments SstI–(I + F) (12.6 kb) and SstI–(I + C) (14.8 kb) derived from both ends of the L component, and fragments SstI–(I + F + J) (18.0 kb) and SstI–(I + C + J) (20.2 kb) derived from the L–S junction, are indicated (lane IV) (Fig. 1 o).

Fig. 6. Southern blot analysis of HSV-1 DNAs prepared in agarose blocks after digestion with ATP-dependent deoxyribonuclease (ATP-DNase). (a, b) Digestion of HSV-1 DNAs with various amounts of ATP-DNase to verify the action of the enzyme. Wild-type BHK-21 cells were infected with HSV-1 strain GN28 and incubated for 24 h at 33.5 °C. Infected cells were incorporated into blocks of 0.6% low-melting point agarose. HSV-1 strain GN28 viral particles were prepared after digestion with Dnase I, as described (Umene, 1993), and were included in the 0.6% low-melting point agarose. Agarose blocks containing HSV-1 infected cells (a) and HSV-1 viral particles (b) were incubated with proteinase K and washed in 10 m M-Tris (pH 8.0) and 1 m M-EDTA (Severini et al., 1994). Agarose plugs were washed with ATP-DNase buffer (66.7 m M-glycine–NaOH, pH 9.4, 30 m M-MgCl₂, 8 m M-DTT, 0.5 m M-ATP) (Ana et al., 1970) and digested with ATP-DNase (Toyob Inc., Osaka) at 37 °C for 20 h: lane 1, 0 units; lane 2, 4.8 units; lane 3, 0.75 units; lane 4, 0.125 units; lane 5, 0.02 units; lane 6, 0.0035 units. The agarose blocks were then melted by incubation at 65 °C for 10 min, and extracted with phenol. The DNAs were precipitated with ethanol, digested with SstI, electrophoresed on an 0.8% agarose gel, transferred to nylon membrane, and hybridized with 32P-labelled 2.35 kb KpnI–SaiI fragment from the KpnI–R fragment on the inverted repeat of the L component (Perry & McGeoch, 1988). (c) Status of HSV-1 DNA after HSV-1 infection into tsBN2 cells at 39.5 °C. tsBN2 cells were infected with HSV-1 strain GN28 at 39.5 °C, and cultures were harvested at 1 h (lanes 1 and 4), 4 h (lanes 2 and 5) and 8 h (lanes 3 and 6) post-adsorption. Infected cells were incorporated into blocks of 0.6% low-melting point agarose. These blocks were incubated with proteinase K and washed in 10 m M-Tris (pH 8.0) and 1 m M-EDTA, and then washed in ATP-
replification of the hybrid plasmid containing Ori₅ was inhibited in tsBN2 at 39.5 °C. This inhibition of Ori₅ replication was considered to be due to a deficiency of trans-acting factors encoded by genes of HSV-1 and BHK-21, an event which could lead to failure of HSV-1 DNA replication.

Production of HSV-1 mRNA in BHK-21 cell lines infected with HSV-1

Production of HSV-1 mRNA in tsBN2 and wild-type BHK-21 cell lines infected with HSV-1 at both 33.5 and 39.5 °C was analysed by Northern hybridization after selection of poly(A)⁺ RNA (Fig. 8). Synthesis of mRNAs of three HSV-1 immediate-early genes (IE175, IE63 and IE110) in tsBN2 at 39.5 °C was comparable to that under permissive conditions, although detailed transcription profiles of these genes may differ under different conditions (Fig. 8a–c). The synthesis of mRNAs of two immediate-early genes (IE68 and IE12) and two early genes (UL29 and UL30) in tsBN2 at 39.5 °C was reduced in comparison with that under permissive conditions (Fig. 8d–g). UL29 and UL30 are essential for HSV-1 DNA replication (Wu et al., 1988).

Discussion

HSV-1 DNA remains linear in tsBN2 at the nonpermissive temperature

At the nonpermissive temperature of 39.5 °C, infectious HSV-1 progeny production and HSV-1 DNA replication were not detected in tsBN2 (Figs 2b and 3b). It has been proposed that parental linear HSV-1 DNA is circularized shortly after entry into the host cell, and that replication of HSV-1 DNA occurs via a rolling-circle mechanism (Roizman, 1979). If this is indeed the case, then circularization of HSV-1 DNA is a prerequisite for HSV-1 DNA replication, and inhibition of circularization will result in failure of HSV-1 DNA replication. In tsBN2 at 39.5 °C (nonpermissive condition), HSV-1 DNAs appeared to remain linear (Figs 4–6), which would contribute to the inhibition of HSV-1 DNA replication. Circular DNA of a hybrid plasmid containing Ori₅ was not replicated in tsBN2 at 39.5 °C, implying a deficiency in trans-acting factors required for HSV-1 DNA replication (Fig. 7). Thus, the failure of HSV-1 DNA replication in tsBN2 at 39.5 °C is probably due to the inhibition of circularization and the lack of trans-acting factors to replicate circularized DNAs.

Circularization of the HSV-1 genome is independent of protein synthesis after HSV-1 infection (Garber et al., 1993; Jamieson et al., 1995). Vmw65 protein, which trans-activates immediate-early genes and is a major constituent in the tegument of the virion, was assumed to be imported into the nucleus and to activate immediate-early genes under nonpermissive conditions, because of the comparable transcription of three immediate-early genes (Dalrymple et al., 1985; Pellett et al., 1985) (Fig. 8a–c). If functions of constituents of the HSV-1 virion are maintained in nonpermissive conditions, as is the case with Vmw65, inhibition of circularization of HSV-1 DNA would relate to defects in cellular factors. The nuclear DNA-binding protein RCC1 is absent from tsBN2 at the nonpermissive temperature, a situation in which HSV-1 DNA appears to remain linear (Ohtsubo et al., 1989). Therefore, RCC1 might be involved in the circularization of HSV-1 DNA, as a result of pleiotropic RCC1 functions or perhaps by directly binding to HSV-1 DNA (Dasso, 1993; Dasso et al., 1994).

Effect of RCC1 on HSV-1 transcription

Upon entry of HSV-1 DNA into the nucleus, the five immediate-early genes (IE12, IE63, IE68, IE110 and IE175) are transcriptionally activated by Vmw65, a protein present in infecting virus particles (Dalrymple et al., 1985; Pellett et al., 1985).
IE63 and IE175 genes, but not IE12 and IE68 genes, are essential for virus replication (DeLuca et al., 1985; McCarthy et al., 1989; Sears et al., 1985; Umene, 1986). Although the IE110 gene is not absolutely required for replication, it is necessary for efficient expression of early and late genes (Sacks & Schaffer, 1987). Members of the RCC1 protein family are assumed to be implicated in a variety of cellular processes, e.g., protein import, and mRNA transcription, splicing, 3' -end formation and export (Dasso, 1993; Schlenstedt et al., 1995; Yokoyama et al., 1995). Synthesis of the mRNAs of IE63, IE110 and IE175 genes under the nonpermissive condition was comparable to that seen under permissive conditions (wild-type BHK-21 at 33.5 °C and tsBN2 at 33.5 °C) (Fig. 8a–c), indicating that Vmw65 protein was imported into the nucleus and that it activated transcription of immediate-early genes, in the absence of RCC1 (Tachibana et al., 1994). Transcripts of IE12, IE68 and IE110 genes are spliced (Perry & McGeoch, 1988; Rixon & McGeoch, 1984; Sacks & Schaffer, 1987; Watson et al., 1981). The decrease in mRNA synthesis of IE12 and IE68 genes cannot be attributed solely to defects in splicing, because IE110 gene mRNA synthesis was not impaired. mRNA synthesis from the two early genes essential for HSV-1 DNA replication (UL29 and UL30) decreased under the nonpermissive condition (Fig. 8f, g) (Wu et al., 1988), which is assumed to have inhibitory effects on replication of HSV-1 and OriS-plasmid DNA (Figs 3 and 7). Therefore, RCC1 appears to be involved in the supply of proteins required for replication of the circularized HSV-1 DNA, including those proteins encoded by the UL29 and UL30 genes, in addition to the circularization of linear HSV-1 DNA.

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References


1 kb BamHI-KpnI-V/P fragment (f; early gene UL29, encoding major DNA binding protein essential for HSV-1 DNA replication) (Quinn & McGeoch, 1985; Wu et al., 1988); 3 kb BamHI-Q fragment (g; early gene UL30, encoding replicative DNA polymerase essential for HSV-1 DNA replication) (Quinn & McGeoch, 1985; Wu et al., 1988). The position of each mRNA detected is indicated by an arrow.


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