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# The Replication of Parvovirus KBSH DNA in the Embryonic Swine Kidney Cells

**Lee, S.W., Y.S. Kim and H.S. Kang**
*Department of Microbiology, College of Natural Science, Seoul National University*

# 돼지 태아 신장세포에 있어 Parvovirus KBSH 의 DNA 복제과정에 관하여

**이성욱 · 김연수 · 강현삼**
서울대학교 자연과학대학 미생물학과

**ABSTRACT: To study the replication process of the single-stranded DNA parvovirus KBSH -isolated from normal human cell cultures-in actively dividing embryonic swine kidney cells, amount of the synthesized viral hemagglutinating(HA) antigen and the overall rate of viral double-stranded replicative form(RF) DNA synthesis were examined. The initiation of viral RF DNA synthesis and the decrease of host DNA synthesis rate in viral infected cells occurred almost same time at 15-16 hour post infection(PI). And the release of viral HA antigen to media followed at 24 hour PI, concurrently the overall rate of viral RF DNA synthesis reaching its maximum. Evidence is presented which indicates that successful performance of viral RF DNA replication requires proteins synthesized in viral infected cells at 10-14 hour PI.**

**KEY WORDS □ parvovirus KBSH, embryonic swine kidney cells, viral hemagglutinating antigen, viral double-stranded replicative form DNA.**

KBSH virus which was isolated from human cells and found to be antigenically related to porcine parvovirus(PPV) by hemagglutination(HA) inhibition(Hallauer *et al.*, 1971) is a member of the autonomous parvovirus group, small and nonenveloped viruses containing a minus single-stranded DNA genome(Siegl, 1972). The molecular and antigenic features of KBSH and PPV appeared very similar but not identical(Molitor *et al.*, 1985). Although KBSH is readily propagated in swine testis(ST) cells in culture, it shows no signs of pathogenicity in swine(Molitor *et al.*, 1985) which PPV causes reproductive failure to(Mengeling and Cutlip, 1975 ; Joo *et al.*, 1976).

Investigations of the parvovirus replication have been performed mainly in case of hamster(H-1) parvovirus and minute virus of mice(MVM). These autonomous parvoviruses replicate only in growing cells and have a requirement for the S phase of the cell cycle(Rhode, 1973). During the replication, the virion single-stranded DNA is converted to the virion single-stranded DNA is converted to a linear double-stranded replicative form(RF) DNA (Rhode, 1974a; Rhode, 1974b) which serves as a template for viral RNA synthesis and the synthesis of single-stranded minus strand DNA that becomes encapsidated into progeny virions(Rhode, 1974b; Wolter *et*

*al.*, 1980). And the genome contains two large open reading frames, 5′half for capsid proteins and 3′half for nonstructural proteins(Rhode and Paradiso, 1983) which maybe are responsible for RF DNA replication(Rhode, 1982). But very little is known of the replication process of KBSH as well as PPV.

In this paper, we study growth features, viral RF DNA kinetics and correlation between protein synthesis and viral RF DNA replication of the parvovirus KBSH in actively dividing embryonic swine kidney(ESK) cells which PPV is capable of infecting besides ST cells. Based on these studies, more comparative molecular analyses of KBSH with PPV may help elucidate the molecular requirements associated with parvovirus replication and pathogensis in infected host animals.

## MATERIALS AND METHODS

**Cell culture**

ST and ESK cells which readily support PPV growth(Pirtle, 1974) were cultured for the multiplication of parvovirus KBSH. Both were kindly provided by Dr. Ahn, S.H.(Institute of Veterinary Research, Anyang) and grown in Eagle minimum essential medium(Flow Lab., USA) containing 5 to 10% fetal bovine serum(FBS, Hyclon Lab., USA), 100 units/m$l$ of penicillin G(Sigma, USA), 100 $\mu$g/m$l$ of streptomycin sulfate(Sigma, USA) and 0.22% $NaHCO_3$ at 37℃ in a 5% $CO_2$ atmosphere.

**Virus infection**

Parvovirus KBSH was obtained also from Dr. Ahn, S.H.. Prior to the studies described here, the KBSH virus has been passaged three times in ST cells and subsequently passaged two times in ESK cells.

Virus(0.1m$l$ of 1,024 HA units per 50 $\mu l$) was inoculated onto 50 to 70% confluent monolayers of both cells in 60 mm petri-dishes. After adsorption for 60 min at 37℃, virus inocula were removed, the cells were washed two times with fresh medium and refed with fresh medium containing FBS. Then, the infection was allowed to continue for various periods of time as described in the individual experiments. After infection, high titer virus stocks were prepared as described by Green, *et al.* (1979). Viral titration was determined by HA assay.

**HA assay**

As mentioned above, the antigenic features of the KBSH is very similar to those of PPV. Therefore, HA assay of KBSH antigen was performed with 0.6% guinea pig RBC which could hemagglutinate PPV antigen as noted by Mengeling(1972). HA titers are expressed as the reciprocal of the maximal dilution of virus that caused complete or incomplete HA.

**DNA labeling and isolation of viral RF DNA**

Viral infected ESK cells and mock infected ESK cells were labeled with $^3$H-thymidine (TdR) at a final concentration of $2.5\times10^{-7}$ M (20 Ci/mmol) for 60 min in the presence of 5-fluoro-2′-deoxyribose(FUDR) at 5 $\mu$g/m$l$ to inhibit endogeneous TdR synthesis(Rhode, 1974b), which meant the specific activity of the $^3$H-TdR incorporated was that of the TdR of the medium and uncontrolled effects were avoided.

Viral RF DNA was extracted by a modification of the Hirt method(Hirt, 1967). Labeled cells were washed three times with 150 mM NaCl, 20 mM Tris-HCl, pH 7.5, 1 mM EDTA and lysed with 0.6% SDS in 50 mM Tris(pH 7.5)-10 mM EDTA for 15 min at 37℃. Then the lysed solution was collected in tube, where proteinase K(Sigma, USA) was added at 2.5 mg/m$l$. After one hour(hr) at 37℃, a solution of 3 M NaCl was added dropwise to a final concentration of 1 M and gently mixed with the lysate. After incubated overnight at 4℃, the lysates were centrifuged at 25,000×g for 50 min. Then samples were taken of the supernatant fraction which contained viral RF DNA and pellet for cellular DNA which was dissolved in 0.1 N NaOH for trichloroacetic acid precipitate and measurements of radioactivity were followed as noted by Rhode(1974a).

**Chemicals**

Cycloheximide(Sigma, USA) was used at a

concentration of 50 $\mu$g/m$l$ and actinomycin D(Calbiochem, USA) at 1 $\mu$g/m$l$. $^3$H-TdR was obtained from NEN, USA and FUDR from Sigma, USA.

## RESULT AND DISCUSSION

**Growth features of KBSH virus in ESK cells**

ESK cells as well as ST cells infected with KBSH began to show cytopathic effects, condensation of cytoplasm and a frequent occurrence of rounding of cells, at 72 hr postinfection(PI) and most cells had detached into medium by 120 hr PI. These are the same phenomena as observed by Mengeling(1972), namely in ESK cells infected with PPV.

To study the growth cycle of KBSH in ESK cells, the amount of HA antigen synthesized at individual time after infection was determined by HA assay. KBSH antigen extracted from KBSH infected ESK cells could hemagglutinate guinea pig RBC as in Fig.1. As shown in Fig. 2, viral HA antigen synthesis first occurred at 15 to 18 hr PI and no HA antigen was detected in the medium until 21 to 24 hr PI but subsequent HA antigen in medium had only a small amount of the total up to 96 hr PI, which was because viral progeny accumulated within cells so that for much of the rise period the amount of cell-associated virus greatly exceeded that of released virus and the release of virus occurred only when cells broke down at the end of rise period. HA assay of cell-associated virus

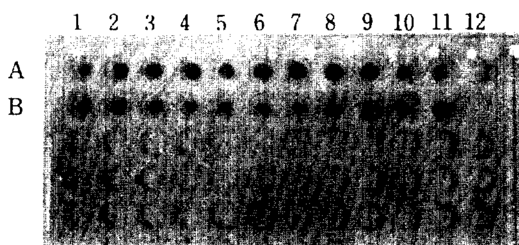


**Fig.1.** *HA assay of parvovirus KBSH with 0.6% guinea pig RBC.*

A-2~A-10 & B-2~B-10, serial two fold diluted KBSH solution plus RBC solution; A-1, A-11, B-1 & B-11, only RBC; A-12, only KBSH; B-12, only phosphated-buffered saline solution(pH 7.2).

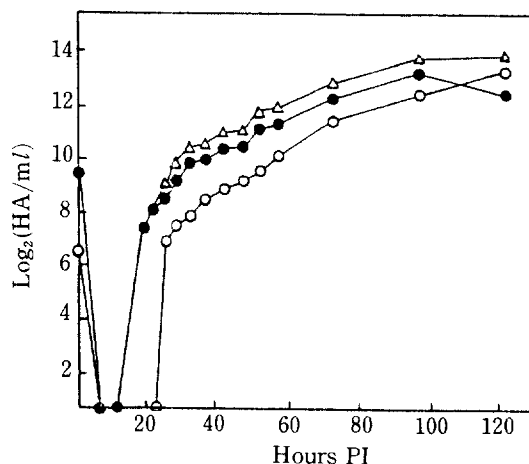


**Fig.2.** *Production of parvovirus KBSH HA antigen in ESK cells (●) and medium (○). Total HA production (△) was indicated.*

was performed as described by Rhode(1973). But the important parameter determining HA antigen production is the developed phase of infected cells rather than time for postinfection(Rhode, 1973).

**RF DNA kinetics of KBSH parvovirus**

To examine KBSH RF DNA and host DNA synthesis rate in infected ESK cells, $^3$H-TdR incorporation rate into acid insoluble precipitate was determined. Mock infected host DNA synthesis rate(the amount of incorporated $^3$H-TdR detected in supernatant plus pellet extracted from mock infected cells), viral infected host DNA synthesis rate(that detected in supernatant from mock infected cells plus pellet from viral infected cells) and viral RF DNA synthesis rate(that detected in supernatant from viral infected cells minus supernatant from mock infected cells) were shown in Fig.3. As the amount of incorporated $^3$H-TdR is detected somewhat in supernatant from mock infected cells, host DNA may be contaminated in supernatant from viral infected cells. Therefore viral RF DNA synthesis rate is considered as mentioned above in parentheses. We are detecting the amout of incorporated $^3$H-TdR in hydridized DNA after hybridization between viral DNA and supernatant from viral infected

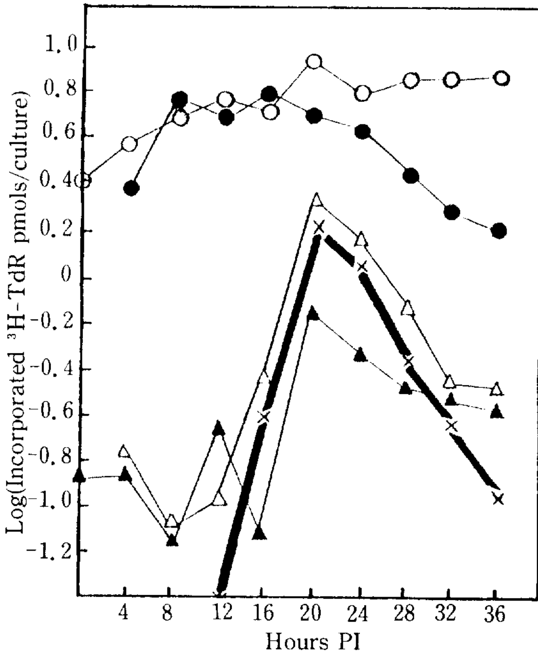


**Fig.3.** *Kinetics of parvovirus KBSH RF DNA synthesis in ESK cells*

○, mock infected host DNA synthesis rate; ●, viral infected host DNA synthesis rate; △, supernatant extracted from viral infected cells; ▲, supernatnat extracted from mock infected cells; × ●×, viral RF DNA synthesis rate.

cells to examine viral RF DNA kinetics more accurately.

Viral RF DNA began to be synthesized from 15 to 16 hr PI and almost simultaneously viral infected host DNA synthesis rate began to decrease. This result showed that viral infected host DNA synthesis was prevented by beginning of viral RF DNA synthesis. And viral RF DNA synthesis rate reached its maximum and began to decrease at 20 hr PI when viral HA antigen was first released to media, which suggested that $^3$H-TdR should be incorporated not into viral RF DNA but into single stranded viral genomic strand.

**Correlation between protein synthesis and KBSH RF DNA synthesis**

To determine if the synthesis of proteins was required for synthesis of viral RF DNA, the effects of a translation inhibitor, cycloheximide, on the incorporation of $^3$H-TdR into

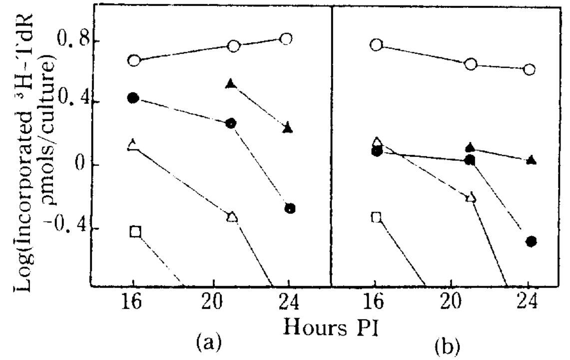


**Fig.4.** *Effects of cycloheximide on mock infected host DNA synthesis rate(a) and parvovirus KBSH infected host DNA synthesis rate(b)*

Drug was added from 5 hr PI(□), 10 hr PI(△), 14 hr PI(●), 19 hr PI(▲) and not added(○).

viral RF DNA were examined. After the drug was added to culture media, the rate of $^3$H-TdR incorporation into mock infected host DNA, viral infected host DNA(Fig.4) and viral RF DNA(Fig.5) was measured. As shown in Fig.4, when drug was added from 5 hr PI and 10 hr PI the inhibited degree of

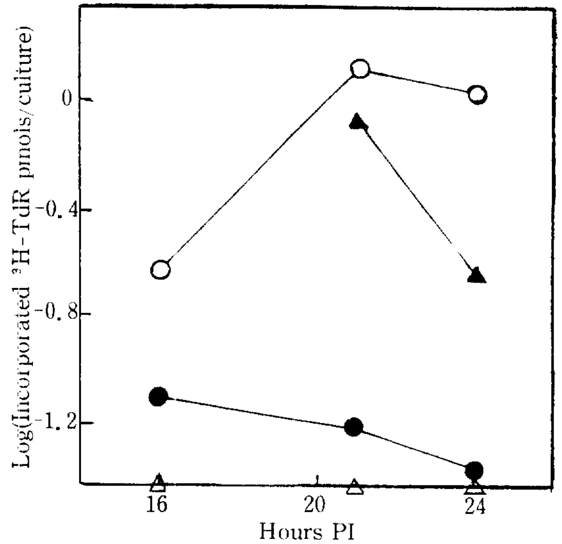


**Fig.5.** *Effects of cycloheximide on the RF DNA synthesis rate of parvovirus KBSH in ESK cells.*

Drug was added from 5, 10 hr PI(△), 14 hr PI(●), 19 hr PI(▲) and not added(○).

viral infected host DNA synthesis rate was similar to that of mock infected host DNA synthesis rate. But when drug was added from 14 hr PI and 19 hr PI, the synthesis rate of viral infected host DNA at 15 to 16 hr PI and 20 to 21 hr PI was much more inhibited respectively. As shown in Fig.5, the amount of incorporated $^3$H-TdR in viral RF DNA was not nearly detected when drug was added from 5 hr PI and 10 hr PI but was detected a little when added from 14 hr PI and 19 hr PI, which showed that if proteins were synthesized at 10 to 14 hr PI, the KBSH replicated its RF DNA. $^3$H-TdR was incorporated into viral RF DNA at 15 to 16 hr PI when drug was added from 14 hr PI, so that the synthesis rate of viral infected host DNA was much inhibited in this case.

Proteins which can be synthesized at 10 to 14 hr PI and induce viral RF DNA replication may be cellular DNA polymerase $\alpha$, $\gamma$ (Kolleck *et al.*, 1982; Pritchard *et al.*, 1978) and non-structural polypeptide synthesized by the KBSH(Molitor *et al.*, 1985).

## 적 요

활발히 성장하고 있는 돼지 태아 신장세포에서, 정상적인 human 세포 배양으로부터 분리된 single-stranded DNA parvovirus KBSH의 초기 증식특성을 알아보기 위해, 합성되는 virus의 hemagglutinating(HA) antigen 양과 virus의 double-stranded replicative form(RF) DNA 합성 속도를 조사하였다. virus의 RF DNA 합성이 시작되는 감염 후 15-16시간 때와 거의 동시에 virus에 감염된 숙주세포의 DNA 합성 속도가 감소하기 시작하였으며, virus의 RF DNA 합성속도가 최대에 달한 후 감소하기 시작하는 감염 후 24시간 때부터 virus의 HA antigen이 배지상으로 방출되기 시작하였다. 그리고 virus의 RF DNA 복제에는 virus에 감염된 세포에서 감염 후 10-14시간 때에 형성되는 단백질들이 관여함을 알았다.

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