

# Sequences Homologous to BAV Component of HL23V in Baboon Tissues

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HL23V-BAB, Sequence Homology, Competition Hybridization

The BAV component of HL23V virus (HL23V-BAB) hybridized to DNA from different Baboon tissues and appears to be an endogenous Baboon virus. Competition hybridization studies show that HL23V-BAB is closely related to viruses isolated from various Baboon species: *P. hamadrys*, *P. papio*, and *P. cynocephalus*. Competition hybridization studies also show that normal Baboon tissues contain more copies of proviral DNA than cells infected *in vitro*.

## Introduction

Baboon endogenous viruses have been isolated from placenta, lung, kidney, and other tissues of several Baboon species. M-7 was the first baboon virus isolated from the placenta of *Papio cynocephalus* [1]. The second Baboon virus was isolated from the lymph node of a lymphomatous *P. hamadrys*. This virus is referred to as BILN. Many strains of Baboon endogenous virus have been isolated from lung, kidney and testicular tissues of *Papio cynocephalus* (BAB 8–1 g, BAB 8K, BAB 455K), and from *Papio anubis* [3, 4]. The isolation of Baboon endogenous virus from the normal lung tissue of *P. papio* has also been reported. This virus is referred to as PP-1 Lu [5].

The virus HL23V isolated from the leukemic blood cells of a patient with acute myelogenous leukemia by Gallagher and Gallo [6] has been shown to be a mixture of Simian Sarcoma Associated Virus (SSAV) and Baboon endogenous Virus (BAV) [7, 8, 9, 10, 11]. The baboon component of HL23V (HL23V-BAB) was separated from SSAV and successfully propagated in human osteosarcoma cell lines [12]. Because HL23V-BAB was isolated from human leukemic cells and because it appears to be a new isolate of baboon virus, it was of interest to determine the sequence homology of HL23V-BAB in baboon tissues. In this paper, we show by competition molecular hybridization the homology that exists between HL23V-BAB and the endogenous virus from several Baboon species and that

many copies of HL23V-BAB are present in Baboon tissues.

## Materials and Methods

### Tissues and cell lines

Liver and kidney tissues from *P. hamadrys* and spleen and brain tissues from *P. papio* were kindly provided by Dr. R.C. Gallo (National Cancer Institute, Bethesda, Maryland). AS/HOS/A7573 is a dog thymus cell line transfected with DNA from a human osteosarcoma cell line (AS/HOS) infected with HL23V-BAB. M-7 (8155) is a line of dog thymus cells producing M-7 Baboon virus. Both cell lines were kindly provided by Dr. P.D. Markham, Litton Bionetics, Bethesda, Maryland.

### Buffer

NTE is 0.1 M NaCl, 0.01 M Tris HCl and 0.001 M EDTA. NETS is 0.1 M NaCl, 0.01 M Tris, 0.1 M EDTA and 0.1 % SDS.

### Preparation of <sup>3</sup>H-labeled viral RNA

Confluent AS/HOS/A7573 cells were cultured in RPMI 1640 which contained 80  $\mu$ Ci/ml of [<sup>3</sup>H]-Uridine (ICN, Irvine, California), specific activity 28 Ci/mM, and 80  $\mu$ Ci/ml of [<sup>3</sup>H]-Cytidine (ICN, Irvine, California), specific activity 26 Ci/mM and 10% dialyzed fetal calf serum. The cells were pre-labeled for 4 h with radioactive medium which was discarded and then refed with radioactive medium which was collected at 6 h intervals for 18 h.

### Viral RNA isolation

Virus was concentrated and partially purified from 16–20 liters of supernatant from AS/HOS/A7573 cells by centrifugation in a continuous flow

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rotor (CF-32, Beckman). Concentrated virus was purified by equilibrium sedimentation centrifugation in a SW 40 rotor (Beckman) through a 15–60% sucrose gradient in NTE, pH 7.4. The virus banded at a density of 1.16 g/ml. The viral RNA was extracted from purified virions with SDS and phenol, precipitated in presence of 20  $\mu$ g of yeast tRNA with two volumes of ethanol and stored at  $-20^{\circ}\text{C}$ . The viral RNA was pelleted, dissolved in 1.0 ml of NETS, pH 7.4 buffer, warmed at  $37^{\circ}\text{C}$  for 5 min and centrifugated on a gradient of 10–30% sucrose in NETS, pH 7.4 with 0.2 ml of 60% sucrose as cushion in a SW50.1 rotor (Beckman) at 40 K for 65 min at  $20^{\circ}\text{C}$ .  $\text{C}^{14}$ -labeled 28S rRNA was run in a parallel gradient. After sedimentation, the gradients were collected from the tube bottom. For unlabeled 60S RNA, OD at 260 was measured and fractions in the peak of 50–60S were pooled and precipitated with 2 volumes of ethanol and stored at  $-20^{\circ}\text{C}$ .

For  $^3\text{H}$ -labeled RNA, 10  $\mu$ l from each fraction were mixed with PCS scintillation fluid and counted in a Beckman scintillation counter. Fractions in the peak of 50–60S RNA were pooled, precipitated with 2 volumes of ethanol with yeast tRNA (20  $\mu$ g) as carrier, and stored at  $-20^{\circ}\text{C}$ . The next morning, the 60S BAV RNA was pelleted, dissolved in 1.0 ml of TE, pH 7.4 and warmed at  $37^{\circ}\text{C}$  for 5 min. The RNA was heated at  $80^{\circ}\text{C}$  for 3 min and cooled in ice water immediately. RNA was made to 0.1 M NaCl and 0.1% SDS added, layered on a gradient of 10–30% sucrose in pH 7.4 NETS and 0.2 ml of 60% sucrose as cushion. The gradient was centrifuged at 40 K for 130 min in a SW 50.1 rotor (Beckman) at  $20^{\circ}\text{C}$ .  $\text{C}^{14}$ -labeled 28S rRNA was run in parallel gradients. After velocity sedimentation, 25 fractions were collected from the tube bottom. 10  $\mu$ l from each fraction were used for counting radioactivity and fractions in the peak of 28–30S RNA were pooled and precipitated with 2 volumes of ethanol. RNA was stored at  $-20^{\circ}\text{C}$  until used.

#### *$I^{125}$ viral RNA*

$I^{125}$  labeled 60S HL23V-BAB RNA (specific activity:  $10^8$  cpm/ $\mu$ g) was kindly provided by Dr. R.G. Smith of the National Cancer Institute, Bethesda, Maryland).

#### *Preparation of cellular DNA*

The extraction and purification of cell DNA was as described by Baluda and Nayak [13].

#### *Competition hybridization*

The competition hybridization was performed as described [14–16]. The hybridization mixture contained 1 mg/250  $\mu$ l of DNA, 800–900 cpm of  $^3\text{H}$ -labeled BAV 30S viral RNA (specific activity approximately  $1 \times 10^6$  cpm/ $\mu$ g) and increasing concentrations ( $10^{-4}$  to 4  $\mu$ g) of unlabeled 60S viral RNA or yeast RNA, 0.001 M EDTA and 0.05% sodium dodecyl sulfate (SDS) in 0.4 M phosphate buffer (PB), pH 6.8. Mouse DNA was used to measure nonspecific binding of  $^3\text{H}$  viral RNA. The hybridization mixture was placed in tightly stoppered tubes, boiled at  $100^{\circ}\text{C}$  for 5 min and immediately transferred to a  $65^{\circ}\text{C}$  water bath. Hybridization was terminated after a Cot (mol of nucleotides  $\times$  seconds  $\times$  liter $^{-1}$ ) of 40,000 was reached. At the end of hybridization, samples were processed as described [17].

#### **Results**

In sucrose velocity gradients RNA isolated from purified HL23V-BAB had sedimentation values of 50–60 S. After heating 50–60 S viral RNA at  $80^{\circ}\text{C}$  for 3 min, 30 S subunits were obtained. These results are in agreement with the reported sedimentation value of baboon virus [18].

#### *Hybridization of $^3\text{H}$ 30S HL23V-BAB RNA to cellular DNA from various baboon species and from infected cells*

$^3\text{H}$  30S HL23V-BAB RNA was hybridized with a large excess of DNA from normal Baboon tissue or from BAV-infected dog thymus cell lines. The ratio of cellular DNA to viral RNA was  $10^6$ :1 on a mass basis and the ratio of proviral DNA to viral RNA was 10–15:1 assuming a single copy of proviral DNA per haploid cell genome.

The viral RNA was hybridized to a Cot of 40,000 molsec $^{-1}$ . As shown in Table 1,  $^3\text{H}$  30S HL23V-BAB RNA hybridized the most (64%) to DNA from *P. hamadrys* kidney or liver, 58% to DNA from *P. cynocephalus* lung, and an average of 52% to DNA from *P. papio* brain or spleen. There was 53% hybridization to DNA from AS/HOS/A7573 cells which produce HL23V-BAB and 51% hybridization to DNA from M-7 BAV.

Table I. Hybridization of  $^3\text{H}$  30S HL23V-BAB RNA to cellular DNA from various baboon species and from infected dog cells.

DNA Source	Percent Hybridization	
	Cot 40,000	Cot 0
Royal baboon kidney ( <i>P. hamadrys</i> )	64	7
Royal baboon liver ( <i>P. hamadrys</i> )	63	ND
Yellow baboon lung ( <i>P. cynocephalus</i> )	58	4
West african baboon brain ( <i>P. papio</i> )	54	ND
West african baboon spleen ( <i>P. papio</i> )	53	13
HL23V-BAB producing dog thymus cell line (AS/HOS/A7573)	54	4
M7 producing dog thymus cell line (M7/8155)	51	9
Uninfected dog thymus cell line	10	5
Mouse embryos	5	5

ND, not done.

Hybridization mixture contained 1 mg sonicated DNA, 800–1000 cpm of  $^3\text{H}$  30S HL23V-BAB RNA (specific activity approximately  $1 \times 10^6$  cpm/ $\mu\text{g}$ ), 0.001 M EDTA, 0.1% SDS in 125 or 250  $\mu\text{l}$  of 0.4 M phosphate buffer, pH 6.8. Hybridization mixture was boiled for 5 min, transferred to a 65 °C water bath and incubated to a Cot of  $4 \times 10^4$  mol sec  $^{-1}$ . At the end of hybridization, samples were chilled in ice water and diluted with  $2 \times \text{SSC}$  to a DNA concentration of 50  $\mu\text{g}/\text{ml}$  and divided into two fractions. One-half was treated with 15  $\mu\text{g}/\text{ml}$  of pancreatic RNase and 15 units/ml of  $T_1$  in  $2 \times \text{SSC}$  at 37 °C for 1 h and the other half served as control. Each sample was precipitated with 5% TCA, trapped on Whatman filters, washed, dried and radioactivity counted in a Beckman scintillation counter. The recovery of input radioactivity was 85–100%.

#### Kinetics of hybridization of $^3\text{H}$ 30S HL23V-BAB RNA to DNA from *P. hamadrys* liver or AS/HOS/A7573 cells

At a Cot of 40,000 mol sec  $^{-1}$ ,  $^3\text{H}$  30S HL23V-BAB RNA hybridized 63% to DNA from the liver of a normal *P. hamadrys* (Fig. 1). The  $\text{Cot}_{1/2}$  of hybridization was approximately  $2 \times 10^3$  mol sec  $^{-1}$ . The hybridization curve shows two components, 25% of the viral RNA hybridized by Cot of  $10^2$ , and a second transition begins at Cot of  $10^3$ . The  $\text{Cot}_{1/2}$  was determined for the entire heterogeneous reaction.

At a Cot of 40,000 mol sec  $^{-1}$ ,  $^3\text{H}$  30S HL23V-BAB RNA hybridized 54% to DNA from AS/HOS/A7573 cells (Fig. 1). The  $\text{Cot}_{1/2}$  of hybridization was approximately  $4 \times 10^3$  mol sec  $^{-1}$ .

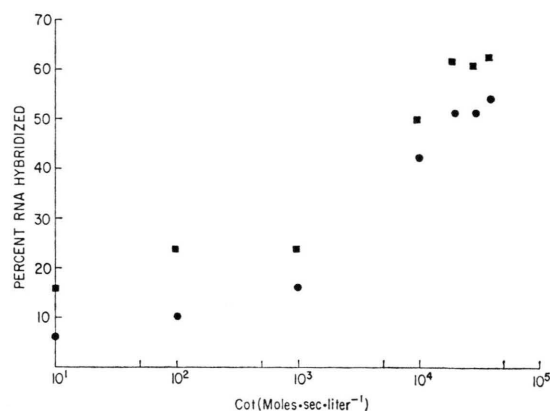


Fig. 1. Kinetics of hybridization of  $^3\text{H}$  30S HL23V-BAB RNA. Hybridization mixture consisted of 1 mg of sonicated DNA, 1000 cpm of  $^3\text{H}$ -30 S viral RNA (specific activity approximately  $1 \times 10^6$  cpm/ $\mu\text{g}$ ) 0.001 M EDTA, 0.05% SDS in 125  $\mu\text{l}$  of 0.4 M phosphate buffer, pH 6.8. For Cots of  $10^0$  and  $10^1$  mol sec  $^{-1}$ , 0.05 M phosphate was used. For Cot of  $10^2$ , 0.1 M phosphate and for Cot of  $10^3$  and above, 0.4 M phosphate was used. An appropriate correction to 0.12 M phosphate was made. The hybridization was carried out in tightly stoppered tubes. After boiling for 5 min at 100 °C, the hybridization mixture was quickly transferred to a 65 °C water bath. Hybridization tubes containing 0.125 ml of hybridization mixture were removed at different time intervals and diluted with  $2 \times \text{SSC}$ . One-half of each sample was then treated with pancreatic ribonuclease A and  $T_1$ , and the other half served as control. The data are plotted as percentage of viral RNA hybridized as a function of Cot. ■, Baboon Liver DNA *P. hamadrys*; ●, AS/HOS/A7573 DNA.

#### Kinetics of hybridization of $I^{125}$ 60S HL23V-BAB RNA to DNA from *P. papio* or AS/HOS/A7573 cells

At a Cot of 40,000 mol sec  $^{-1}$ ,  $I^{125}$  60S HL23V-BAB RNA hybridized 53% to DNA from the spleen of a normal *P. papio* (Fig. 2). The  $\text{Cot}_{1/2}$  of hybridization was  $2 \times 10^3$  mol sec  $^{-1}$ . At a Cot of 40,000 mol sec  $^{-1}$ ,  $I^{125}$  60S HL23V-BAB RNA hybridized 55% to DNA from AS/HOS/A7573 cells (Fig. 2). The  $\text{Cot}_{1/2}$  of hybridization was approximately  $4 \times 10^3$  mol sec  $^{-1}$ .

#### Competition hybridization between $^3\text{H}$ 30S HL23V-BAB RNA and homologous unlabeled 60S RNA with DNA from *P. hamadrys*

At a Cot of 40,000 mol sec  $^{-1}$  in presence of 1–4  $\mu\text{g}$  of yeast RNA,  $^3\text{H}$  30S HL23V-BAB RNA hybridized an average of 63% to DNA from a *P. hamadrys* liver and approximately 4% to normal murine DNA. Unlabeled 60S HL23V-BAB RNA at a concentration of 2–4  $\mu\text{g}$  per 250  $\mu\text{l}$  inhibited

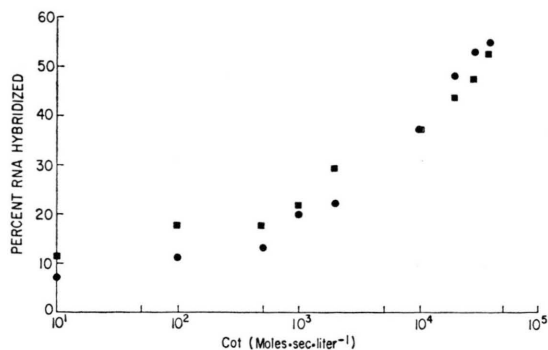


Fig. 2. Kinetics of hybridization of  $I^{125}$  60S HL23V-BAB RNA. The conditions of hybridizations were exactly the same as in Fig. 1, except that 1200 cpm of  $I^{125}$  60S BAV RNA (specific activity approximately  $10^8$  cpm/ $\mu$ g) were used. ■, Baboon Spleen DNA from *P. papio*; ●, AS/HOS/A7573 DNA.

the hybridization by 90%. The 50% inhibition level by homologous unlabeled 60S RNA was obtained at a concentration of  $5 \times 10^{-2}$   $\mu$ g per 250  $\mu$ l. The normalized results are presented in Fig. 3.

*Competition hybridization between  $^3H$  or  $I^{125}$  HL23V-BAB RNA and homologous unlabeled 60S RNA to DNA from West African Baboon (*P. papio*)*

At a Cot of 40,000 molsec $^{-1}$ ,  $I^{125}$  60S HL23V-BAB RNA hybridized an average of 42% to DNA from the spleen of a normal *P. papio* in presence of 1–4  $\mu$ g/250  $\mu$ l of yeast RNA. Unlabeled 60S HL23V-BAB RNA at a concentration of  $5 \times 10^{-2}$   $\mu$ g/250  $\mu$ l caused 50% inhibition. The normalized results are presented in Fig. 4. A competition hy-

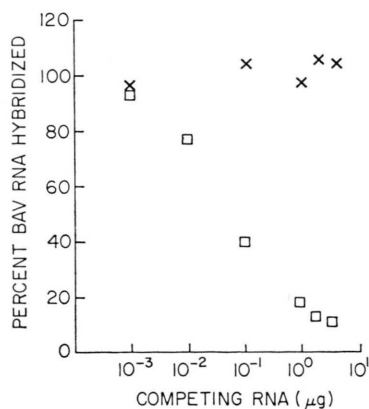


Fig. 3. Hybridization of  $^3H$  30S HL23V-BAB RNA with an excess of liver DNA from *P. hamadrys* in presence of increasing concentrations of unlabeled homologous RNA. The procedure is described in Materials and Methods. X, yeast RNA; □, HL23V-BAB 60S RNA.

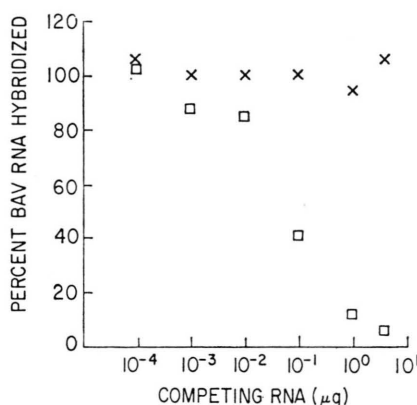


Fig. 4. Hybridization of  $I^{125}$  60S HL23V-BAB RNA with an excess of spleen DNA from *P. papio* in presence of increasing concentrations of unlabeled homologous RNA. The procedure is described in Materials and Methods except that DNA concentration was 8 mg/ml. X, yeast RNA; □, HL23V-BAB 60S RNA.

dization experiment with brain DNA from *P. papio* and  $^3H$  30S HL23V-BAB RNA gave identical results (data not shown).

*Competition hybridization between  $^3H$  30S HL23V-BAB and homologous unlabeled RNA with DNA from a cell line producing HL23V-BAB*

At a Cot of 40,000 molsec $^{-1}$ ,  $^3H$  30S HL23V-BAB RNA hybridized an average of 54% to DNA from AS/HOS/A7573 cells in presence of 1–4  $\mu$ g/250  $\mu$ l of yeast RNA. Unlabeled 60S BAV RNA at a concentration of 2–4  $\mu$ g/250  $\mu$ l inhibited the hybridization by 98% and 50% competition was obtained at a concentration of  $1.5 \times 10^{-2}$   $\mu$ g/250  $\mu$ l. The normalized results are presented in Fig. 5.

*Competition hybridization between  $^3H$  30S HL23V-BAB RNA and homologous unlabeled 60S RNA to DNA from dog thymus cells producing M-7 BAV*

At a Cot of 40,000 molsec $^{-1}$ ,  $^3H$  30S HL23V-BAB RNA hybridized an average of 51% to DNA from M-7 (8155) cells in presence of 1–2  $\mu$ g of yeast RNA. Unlabeled 60S BAV RNA from HL23V-BAB at a concentration of 1–2  $\mu$ g/250  $\mu$ l inhibited the hybridization by 96%. Unlabeled 60S HL23V-BAB RNA at a concentration of  $1.6 \times 10^{-2}$   $\mu$ g/250  $\mu$ l inhibited the hybridization by 50%. The normalized results are presented in Fig. 6.

## Discussion

Competition hybridization between labeled and unlabeled RNA to template DNA in large excess is



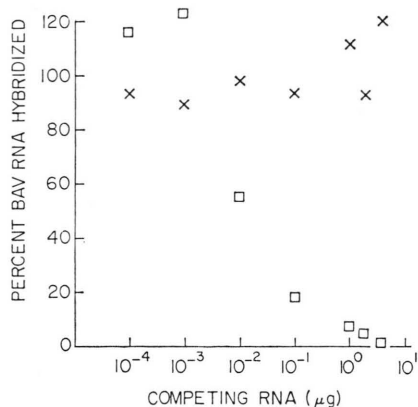


Fig. 5. Hybridization of  $^3\text{H}$  30S HL23V-BAB RNA with an excess of AS/HOS/A7573 DNA in presence of increasing concentrations of unlabeled homologous RNA. The procedure is described in Materials and Methods. X, yeast RNA; □, HL23V-BAB 60S RNA.

used to determine the sequence homology between two species of RNA. In this study we have used DNA of different Baboon species to assess the sequence homology between HL23V-BAB and different Baboon proviruses. We have used 30S genomic RNA and large excess of cellular template DNA to determine the sequence homology of the entire viral genome. Competition hybridization results presented in this study have shown that HL23V-BAB is highly related to viruses of *P. hamadryas*, *P. papio* and M-7 (8155). Thus the baboon virus component of HL23 virus is an endogenous baboon virus widely distributed in different baboon species. Radio-immunoassay competition has shown that P-30 protein is highly conserved in *P. cynocephalus*, *P. anubis*, *P. hamadryas* [4]. The kinetics of hybridization of cDNA and mRNA to cellular DNA in excess can be used to determine the copy number of a gene from the  $\text{Cot}_{1/2}$  of the hybridization reaction [20, 21]. From the  $\text{Cot}_{1/2}$  of hybridization, it appears that DNA from normal baboon tissues contains approximately twice as much viral DNA as dog cells infected *in vitro* which produce baboon virus.

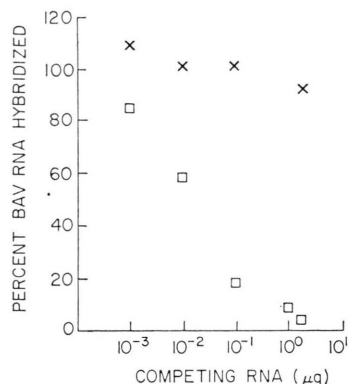


Fig. 6. Hybridization of  $^3\text{H}$  30S HL23V-BAB RNA with an excess of M7(8155) DNA in presence of increasing concentrations of unlabeled homologous RNA. The procedure is described in Materials and Methods. X, yeast RNA; □, HL23V-BAB 60S RNA.

The amount of unlabeled 60S BAV RNA required for fifty percent inhibition of hybridization of labeled viral RNA to different baboon DNAs reflects the concentration of BAV proviruses in the baboon tissues or infected cell lines. The concentration of unlabeled 60S BAV required for fifty percent competition of  $^3\text{H}$  HL23V-BAB 30S RNA was 3–4 times greater for baboon tissue DNA than for BAV infected dog thymus cell DNA (*i.e.*  $5 \times 10^{-2} \mu\text{g}/250 \mu\text{l}$  for baboon tissues DNA and  $1.5 \times 10^{-2} \mu\text{g}/250 \mu\text{l}$  for BAV infected cell line DNA). Therefore, the normal baboon tissues appear to contain 3–4 times more BAV proviral DNA sequences than the infected dog thymus cells, AS/HOS/A7573 or M-7 (8155) [24]. Our data are in agreement with the results of Benveniste and Todaro [24] who have detected 5–15 copies of BAV proviral DNA per haploid cell genome in DNA from baboon tissues and 1 copy per haploid cell genome in DNA from BAV infected cell lines.

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