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Viral Gene Expression in Cells Transformed by Simian Sarcoma Virus, an Infectious Primate Type C Retrovirus

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A. Introduction

Simian sarcoma virus (SSV) is a replication-defective transforming virus isolated in the presence of excess nontransforming associated virus (SSAV) from a spontaneous sarcoma of a woolly monkey. Previous studies have shown that the structural proteins of SSV(SSAV) are similar to the corresponding components of murine leukemia virus (MuLV). Studies reported here focus on viral gene expression in SSV-transformed virus producing marmoset monkey cells [HF/SSV(SSAV)] and SSV-transformed nonproducer marmoset cell lines (HF/SSV-NP).

I. Precursor Polypeptides in Virus Producing Cells

Antiserum specific for SSV(SSAV) p30 (kindly provided by Dr. H. J. Thiel) and another specific for SSAV envelope proteins were reacted with ^{35}S-methionine-labeled extracts of HF/SSV(SSAV) cells. Normal HF and HF/SSAV cells were tested in parallel. Analysis of radioimmunoprecipitates (RIP) by SDS-PAGE and fluorography demonstrated that anti-SSAV p30 precipitated predominantly polypeptides of approximately 30,000 and 60,000–62,000 daltons and, in addition, polypeptides of approximately 72,000 and 52,000 daltons (Fig. 1). Results of pulse-chase experiments indicated that the 60,000 dalton protein (pr60gag) is processed to yield p30, the major viral core protein (Fig. 1). Using antiserum specific for gp70 and p15E/p12E large amounts of an 82,000 dalton polypeptide were precipitated, and results of pulse-chase experiments indicated that it is an envelope precursor protein processed to yield p15E and p12E (Fig. 2). Further experiments are aimed at analyzing glycosylation of the *env* and *gag* gene products. A *gag* polyprotein or nonstructural polypeptide unique to transformed cells was not detectable by RIP SDS-PAGE with antisera for viral structural proteins or serum from tumor bearing marmosets.

II. Phosphorylated Proteins

Two virus-specific phosphoproteins, the pr60gag and p12, were revealed by SDS-PAGE of ^{32}P-labeled proteins. No phosphorylated proteins were precipitated by antiserum specific for SSAV envelope proteins, providing further evidence that SSAV has a phosphorylated core p12 and a nonphosphorylated 12,000 dalton envelope protein (p12E). It was further noted that IgG was phosphorylated in extracts of both HF/SSV(SSAV) and HF/SSAV cells, but not in extracts of HF/SSV-NP cells.

III. SSV Gene Expression

Establishment of HF/SSV-NP cells has been described previously (Bergholz et al. 1977). Presence of the SSV genome was demonstrated by rescue of transforming virus following superinfection with SSAV, GALV, or MuLV. Expression of the SSAV-related sequences by the SSV provirus was investigated by reacting an SSAV cDNA with increasing concentrations of RNA extracted from two HF/SSV-NP cell lines. The results (Fig. 3) indicated that SSAV-related sequences were transcribed but represented a smaller proportion of the total cell RNA relative to virus producing cells. Comparison of maximum levels of hybridization indicated that less of the SSAV genome is

represented in the SSV transcripts relative to RNA in SSAV-infected cells.

Virus-specific protein synthesis in five HF/SSV-NP cell lines was investigated by RIP and SDS PAGE. No viral polypeptides were precipitated by antiserum specific for SSAV envelope proteins. Anti-SSAV p30 and antiserum to Tween-ether disrupted virus detected one SSAV-related protein, pr60 gag, in only one cell line (HF/SSV-NP VE). The pr60 gag apparently is not processed to yield p30 or lower molecular weight structural proteins, suggesting that a helper virus gene function may be required for processing. The failure to detect virus-specific proteins in cell lines

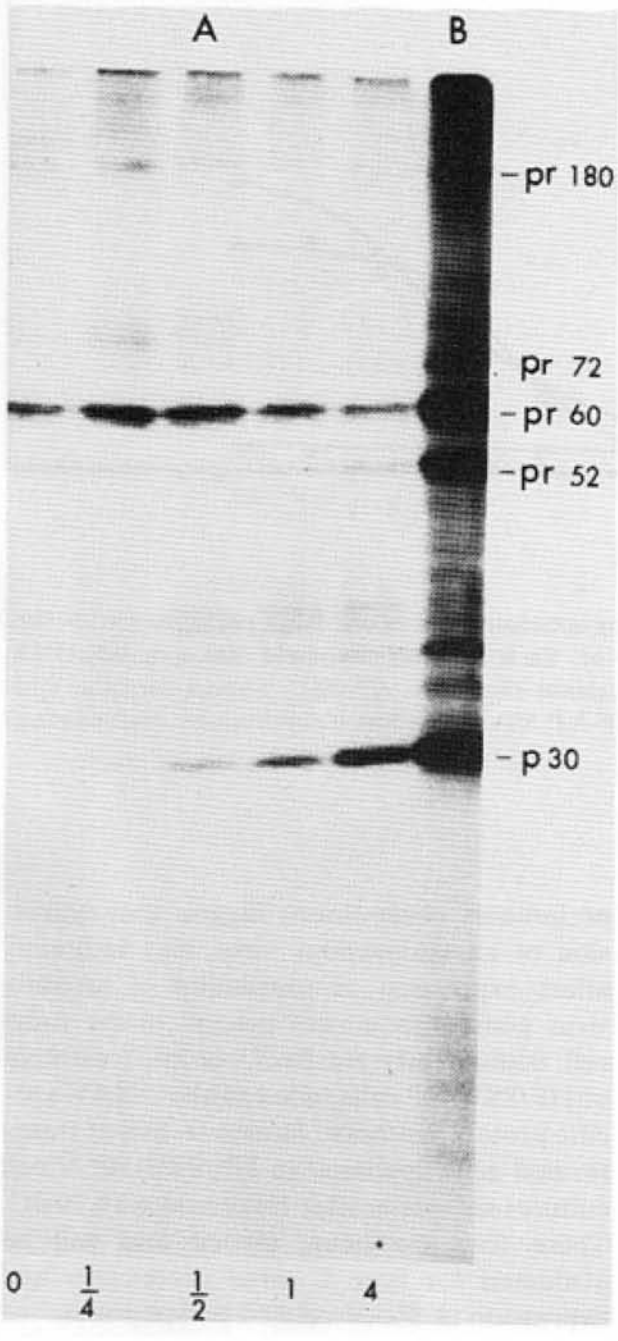

Fig. 1. Autoradiogram of 12% SDS-PAGE resolving ^{35}S-methionine-labeled proteins precipitated from HF/SSV(SSAV) cell extracts by anti-p30 serum. *A*, cells were pulse-labeled with 100 μci/ml ^{35}S-methionine for 15 min and were chased for 0, 1/4, 1/2, 1, and 4 hr before lysis. *B*, cells labeled for 4 h, then lysed

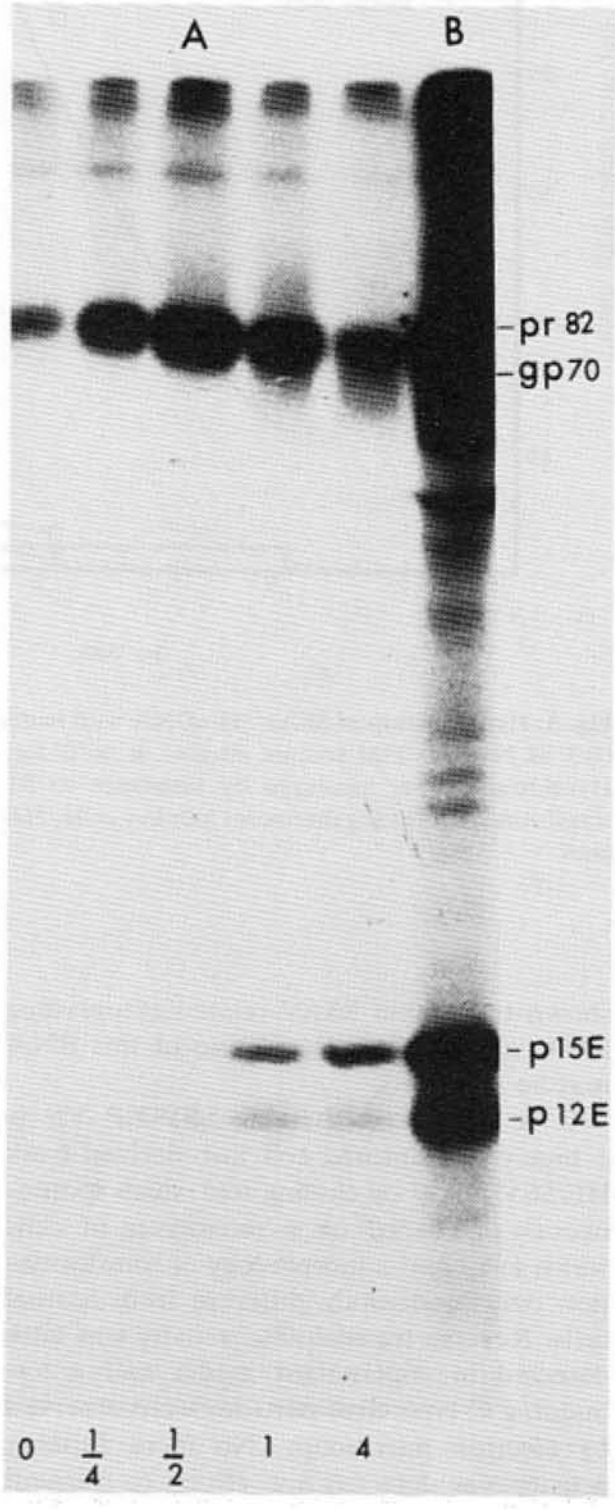

Fig. 2. Autoradiogram of 12% SDS-PAGE resolving ^{35}S-methionine-labeled proteins precipitated from HF/SSV(SSAV) cell extracts by antiserum for SSAV envelope proteins performed as described in Fig. 1

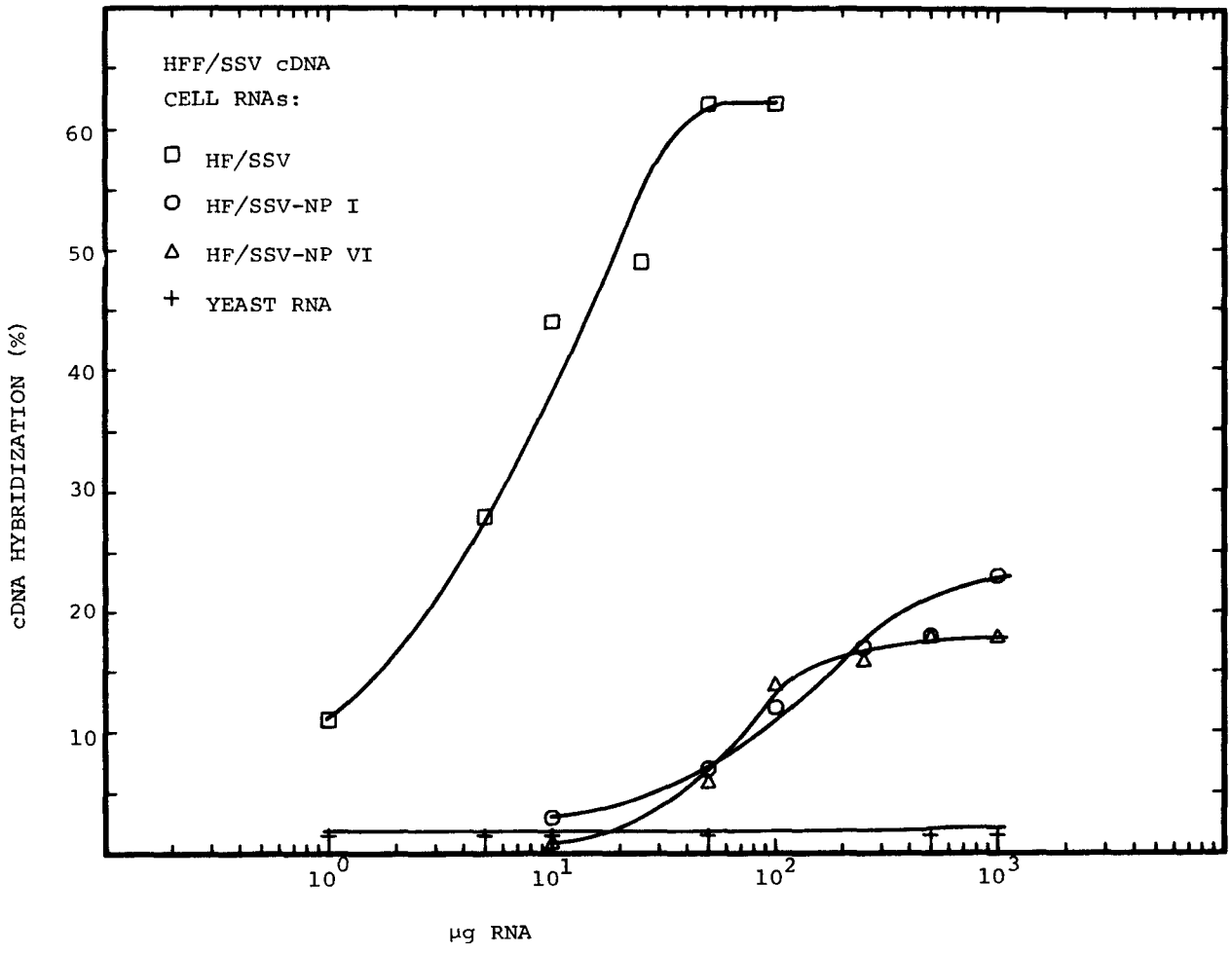

Fig. 3. Hybridization of SSAV ^{3}H-cDNA with increasing concentrations of cell RNA performed in 2X SSC (0.3 M NaCl, 0.03M sodium citrate) at 66°C for 20 hr. Each reaction contained 500 cpm ^{3}H-cDNA. Hybridization was measured by resistance to S1 nuclease digestion. *HF/SSV*, Simian sarcoma virus transformed producing marmoset monkey cells; *HF/SSF-NP*, SSV-transformed nonproducer marmoset cell lines

shown to contain SSAV-related cytoplasmic RNA suggests that translation of this RNA may be restricted.

It is of interest that HF/SSV-NP VE is a highly transformed cell line derived from HF/SSV-NP V by cloning foci which spontaneously appeared on a monolayer of cells which exhibited a morphology of transformation only moderately different from normal cells. Reverse transcriptase activity was later detected in supernatant media and a few budding C-type virus particles were observed by electron microscopy. No focus forming activity was detected and efforts to transmit virus to other cell lines were unsuccessful. The possibility that HF/SSV-NP VE cells are producing an endogenous marmoset virus is being investigated. As no viral structural proteins are detected in HF/SSV-NP V cells, these preliminary observations suggest that expression of an endogenous virus may indirectly affect expression or translation of sarcoma virus genetic sequences present in the same cell. Sodium butyrate, BrdU, or IdU treatment of HF/SSV-NP cells failed to alter SSAV-specific protein synthesis. However, foci of transformed cells appeared in HF/SSV-NP V cell cultures six weeks after treatment with BrdU. These foci are being cloned and will be evaluated for type C virus production and expression of SSAV-related polypeptides.

Reference

Bergholz CM, Wolfe LG, Deinhardt F (1977) Establishment of simian sarcoma, type 1 (SSV-1)-transformed nonproducer marmoset cell lines. Int J Cancer 58:104–111