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Generation of a life-expanded rhesus monkey fibroblast cell line for the growth of rhesus rhadinovirus (RRV)

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Summary. RRV, the rhesus macaque equivalent to HHV-8 or kaposi's sarcoma-associated herpesvirus (KSHV) was recently isolated from a simian immunodeficiency virus (SIV) infected macaque with a lymphoproliferative disorder. The growth of RRV in tissue culture requires propagation of primary rhesus monkey fibroblasts (RFs). In an effort to extend the life of these primary cells in tissue culture, the catalytic subunit of telomerase (hTERT) was introduced into RF cells using a recombinant retrovirus. This new cell line, Telo-RFs, have currently been passed in tissue culture over 80 times compared to a maximum passage number of 38 for wild type RFs, remain fully permissive for RRV DNA replication and production of infectious virus. Viral gene expression of immediate-early and early RNA transcripts was virtually identical to that observed in wild-type (wt) RFs. In addition, transfection experiments show that telo-RFs are easily and more efficiently transfected than wtRFs.

Introduction

The study of many viruses requires the growth and maintenance of primary cells. Some viruses, such as some of those in the herpesvirus family, can only be grown in normal diploid fibroblasts. In order to carry out experiments involving viral attachment, permissive gene expression and viral DNA replication, the steady supply of tissue to generate primary cell lines is needed. In many cases, acquiring a supply of human or non-human primate tissue can be difficult since viable tissue can be limited. In addition, even when tissue is acquired and cell lines are made, primary cell lines have as a major disadvantage a limited life in tissue culture. Our goal was to increase the life span and availability of Rhesus Macaque fibroblasts to enhance the study of Rhesus Macaque Rhadinovirus (RRV) lytic DNA replication.

In an effort to extend the life of primary cell lines, several investigators have successfully immortalized cells by transforming them using various viral encoded oncogenes. One method used to transform primary cell lines is to introduce the oncogenes E6 and E7 encoded by human papillomavirus (HPV) [3]. These genes are usually delivered by transfection of a plasmid encoding E6/E7 followed by selection for cells having both resistance to a drug selection marker and an extended life span. This method has been used to immortalize a human fibroblast cell line that maintains HCMV permissiveness [3]. Although these cells are permissive for HCMV, the effects of E6 and E7 on cellular processes may confound some experiments by affecting levels of p53 and retinoblastoma tumor suppressor protein (Rb) [15]. Studies involving the effects of viral gene expression on cell cycle, cell signaling or, downstream regulation can be difficult to interpret in cell systems that have been immortalized by addition of E6/E7 because of this alteration of p53 and/or Rb. In addition, these cells can exhibit phenotypic changes and aberrations in growth characteristics bringing into question the usefulness of these transformed lines when studying viral replication and host cell effects.

In addition to the method of immortalization mentioned above, other methods have been explored to increase the lifespan of primary cells in culture. One recent method involves the introduction of the gene encoding telomerase into primary cells [2]. Life-span extension by telomerase not only reduces senescence in cells, but life-expanded cell lines appear to have biological function and cellular characteristics of early passage cells avoiding growth arrest and senescence associated with shortened telomeres [5, 7, 10]. Telomerase is a ribonuclear protein complex that has a reverse trancriptase (hTERT) catalytic domain and an RNA template domain [1]. The recent subcloning of hTERT allows for the delivery of this component of the enzyme into normal cells [9]. Several reports have successfully "life-expaned" primary cells by transfection of hTERT. The result has been an increase in normal life span in tissue culture consistent with the expression of telomerase and elongation of telomeres [2]. In addition, life-expanded cells due to the introduction of hTERT appear to retain wt function of p53 and Rb [8].

In this report we evaluate the ability of life-expanded rhesus fibroblasts to support replication of the recently isolated Rhesus Rhadinovirus (RRV) [4, 13].

Materials and methods

Cells and virus

ØNX cells, a retrovirus packaging cell line, was supplied by Gary Noland (Stanford University). Primary Rhesus fibroblasts were isolated as described previously [13]. Both cell types were cultured in Dulbecco's modified Eagle's medium (Cellgro) supplemented with 10% fetal bovine serum (FBS) in humidified incubators at 37 °C and 7% CO_2. RRV isolate 17577 was grown in primary rhesus fibroblasts [13].

Retroviral production and transduction

The ORF encoding the catalytic subunit of telomerase protein (hTERT) was subcloned into the retrovirus vector pLXIN (Stratagene) by cleaving pGRN145 (Geron, Inc.) with EcoRI, which releases the hTERT coding sequence, and ligating it into pLXIN in the proper orientation for

gene expression. The telomerase construct, pLXIN-hTERT, was transfected into ØNX cells using Lipofectamine 2000 (Lifetechnologies) according to the manufacturers' specifications. Forty-eight hours after transfection, supernatants containing retrovirus were harvested and filtered using a 0.22 μM syringe filter. Primary Rhesus fibroblasts (RFs) were trypsinized and 1×10^5 cells were pelleted at 3000 × **g** using a table top centrifuge, the resulting pellet was re-suspended in 2 mls of media containing pLXIN-hTERT retrovirus, and plated 3 cm Retronectin dishes (TaKara). After a 2 h incubation, two mls of media was removed from the dish and refreshed with 2 ml of new virus containing media. Cells were split into selection media (DMEM plus 150 μg per ml G418 Sulfate Solution) after a 24–48 h incubation. Individual colonies were visible after 2–3 weeks and expanded for further studies. Telo-RFs were maintained on DMEM supplemented with 100 μg per ml G418.

RT-PCR

Total cellular RNA was extracted from 5×10^6 life-expanded and wt RFs using RNAEasy Mini Kit (QIAGEN). Two micrograms of total RNA was used to make cDNA using M-MLV reverse transcriptase (Panvera) according to manufacturers assay conditions. Five microliters (∼ 100 ng) of cDNA was combined with 45 μl Platinum Taq (Lifetechnologies) and 1 μl of each specific primer. Telomerase message was identified using primers specific for the catalytic subunit of telomerase, LT5 (CGGAAGAGTGTCTGGAGCAA) and LT6 (GGATGAAGCGGAGTCTGG) generating a 146 base pair fragment. Beta-actin specific primers (TGGCTACAGCTTCACCACC and GTGGCTACAGCTTCACCAC) were used as a control generating a 498 base pair product. PCR conditions were as follows: 94 degrees for 2 min, followed by 31 cycles of 94 °C for 45 sec, 60 °C for 45 sec, and 72 °C for 90 sec. PCR products were resolved on a 1% agarose gel.

TRAP assay

Extracts from life-expanded Rhesus fibroblasts (passage 65) and ØNX cells were prepared by the Chaps-lysis method [6]. R8 quantification standard (Pharmingin) and ØNX cell extracts were used as positive controls. For the heat-killed control, 20 μl of cell extract was incubated at 95 °C for 5 minutes. Twenty microliters of cell extract was incubated with 1 μl of RNase A (0.1 mg/ml, Qiagen) for 15 min at room temperature for RNase treated controls. Telomerase activity of cell extract was determined by a modified TRAP assay (TP-TRAP) as described previously [14] with the following exceptions. Instead of using 1 μCi (Me-^{3}H) dTTP, 50 μM dNTPs were used, and gel was stained with ethidium bromide. Two micrograms of each cell extract was added to 46 μl of reaction mixture. The main steps of the assay include extension of MTS primer by telomerase, amplification of the telomerase products using two reverse primers RP and RPC3. MTS acts as a template for telomerase as well as a forward primer for PCR amplification. PCR products were resolved using a 12% nondenaturing polyacrylamide gel. The gel was stained for 30 minutes with ethidium bromide and visualized by UV transillumination.

Cell proliferation and RRV infection

Wt and Telo-RFs were plated at 1000 cells per well in 96 well tissue culture plates and incubated a 7% CO_2 at 37 °C. RRV was added at an approximate MOI of 0.05 to one plate of each cell type. After 24 h, 20 μl of Cell Titer 96 Aqueous-one solution was added to 12 of the 96 samples. Cells were incubated at 37 °C for 2 h and absorbance at 490 nm was read in a CERES 900 plate reader. This process was repeated every 24 h for 5 days in uninfected cells and for 8 days in RRV infected cells. To determine the viral DNA replication in the

two cell types, WT-RF's and telo-RF's were plated at 1–2 million cells per well in 6 well tissue culture plates and infected with Rhesus Rhadinovirus at an approximate MOI of one. Cells were lysed using PUREGENE cell lysis solution. Proteins were precipitated, and DNA was harvested from the supernatant by precipitating in isopropyl alcohol. The sample was centrifuged for 5 min at 16,000 × **g** at 25 °C. The pellet was washed with 70% ethanol and re-suspended in DEPC treated water. Cells were harvested every 24 h from 48 h to 168 h. Two mg of DNA was cleaved with EcoRI, resolved on a 1% agarose gel and transferred to Zeta-Probe nylon membrane (Bio-Rad). Blots were probed with a ^{32}P-labeled EcoRI fragment of cosmid 28a, RRV nucleotide sequence 56645–60744. Blot was exposed to X-ray film overnight and relative DNA accumulation was determined by using Scan-analysis software (Biosoft). Error bar values were determined from three separate experiments. Both cell types show a linear accumulation of viral DNA from day 2 to day 7.

Northern blot

Telo and wt RFs were plated in 6 cm dishes at a cell density of 1×10^6 cells per dish. Cells were infected with RRV using an approximate MOI of 5. Total cellular RNA was harvested using TRIsol solution 2–4 days post infection. RNA was separated on a 1.2% formaldehyde agarose gel, transferred to a nylon membrane and hybridized with either a single-stranded ^{32}P-labeled riboprobe (for 40–41) or random-primer ^{32}P-labeled ORF50 probe. For riboprobes, probes were hybridized in 1.5 × SSPE, 50% formamide, 1% SDS, 0.5% Blotto, 100 μg per ml sonicated salmon sperm DNA and 50 μg per ml yeast tRNA at 65 °C for 15 h. Blots were washed twice with 2 × SSC, 0.1% SDS at room temperature and then twice with 0.1 × SSC, 0.1% SDS at 65 °C. Blots were exposed to X-ray film for 5–15 h at −80 °C.

For random-primer probes, hybridizations were done in 1.5 × SSPE, 7% SDS, 10% 65 °C. Washes were performed the same as with riboprobes.

Transfection of EGFP expression plasmid

Telo and wt RFs (passage 6) were plated in 6 cm dishes (1×10^6) 24 h prior to infection. wtRFs were from the same batch as those used to generate the life-expanded cell line. Cell were transfected with pEGFP-N1 (Clontech) using TRANSIT (Mirrus). Three micrograms of plasmid DNA and 7 μl of TRANSIT were incubated with cells for 15 h. Cells were photographed at 4 × magnification 48 h after transfection.

Results

To efficiently deliver the telomerase gene to primary fibroblasts, we chose to subclone the hTERT component into a retroviral vector. The use of retroviruses to transduce cells has several advantages over transfection. Retroviruses allow efficient gene transfer to a large number of cells grown in culture, efficiently integrating into the genomic DNA of recipient cells, whereas transfection efficiencies vary with the method used [11]. Also, primary fibroblasts are traditionally more difficult to transfect, and we have successfully used retroviral transduction of to deliver foreign genes to primary fibroblasts [12]. Figure 1 is a schematic showing the construction of the retroviral plasmid (pLXIN-hTERT) encoding the catalytic subunit of telomerase, hTERT.

To determine if cells with G418 resistant colonies were life-expaned, selected colonies were analyzed for the expression of the hTERT component of telomerase.

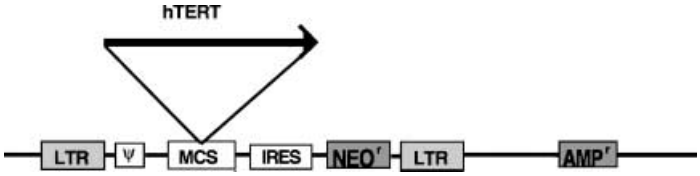


Fig. 1. Construction of Retrovirus Vector encoding the catalytic subunit of telomerase (hTERT). hTERT was subcloned from pGRN145 (Geron), a plasmid encoding the catalytic subunit of telomerase. The hTERT plasmid was cleaved with EcoRI and the resulting 3453 bp insert was ligated into pLXIN (Clonetech). pLXIN is a bicistronic retroviral vector that allows coordinated translation of the gene of interest along with a neomycin resistance gene. This retroviral vector has a virus-packaging signal Ψ that allows FNX cells to package the retrovirus and release it into culture media. The resulting retrovirus (pLXIN-hTERT) was harvested and used to infect RF cells using neomycin resistance for selection

Since the retrovirus used to transduce RFs encoded hTERT, we performed RT-PCR to test for the presence of hTERT specific transcripts. RT-PCR was performed using primers specific for the catalytic subunit of telomerase [9]. Total cellular RNA was extracted from G418 resistant cells and the resulting PCR product of 146 bp corresponded to the predicted size of the hTERT-amplified product. This specific amplification product was only observed in cells transduced with pLXIN-hTERT retrovirus (top panel, Fig. 2A). Although the result of only one selected colony is shown in figure 2, in all, five out of five colonies selected were shown to express hTERT mRNA. Cells which express hTERT mRNA were expanded and analyzed for telomerase activity. As a control wtRFs were also transduced with a retrovirus containing empty vector. Although initially some G418 resistant colonies were selected from these cells, we were unable to expand these cells due to senescence.

A modified telomeric repeat amplification protocol (TP-TRAP) assay was employed to determine if telomerase activity was present in cell colonies transduced with pLXIN-hTERT [14]. In this assay, telomerase adds 6 bp repeats to the MTS primer and telomerase activity is indicated by the appearance of a 6 bp telomeric ladder. Figure 2B shows the appearance of this ladder in a representative sample from cells infected with pLIXN-hTERT (Fig. 2B, top panel, see brackets). Telomerase activity was shown in RF cells transduced with pLXIN-hTERT (top panel, Fig. 2B, lane 2) and also shown in to ØNX cell extracts, a cell line known to contain telomerase activity (top panel, Fig. 2B, lane 1). This activity was eliminated by heat treatment of lysates (top panel, Fig. 2B, lane 3) or by treatment of lysates with RNase A (top panel, Fig. 2B, lane 5). No telomerase activity was observed in wtRFs (data not shown). These results confirm the RT-PCR data indicating that the hTERT gene is present and functional within RF cells. Also, consistent with the introduction of telomerase, we have currently passed telo-RFs over 80 times compared to a life span of only 38 passages for wt RFs (wt-RFs). Consequently, the expanded life span observed in telo-RFs is consistent with the expression of hTERT and is similar to the life-span expansion observed in other cell types [2].

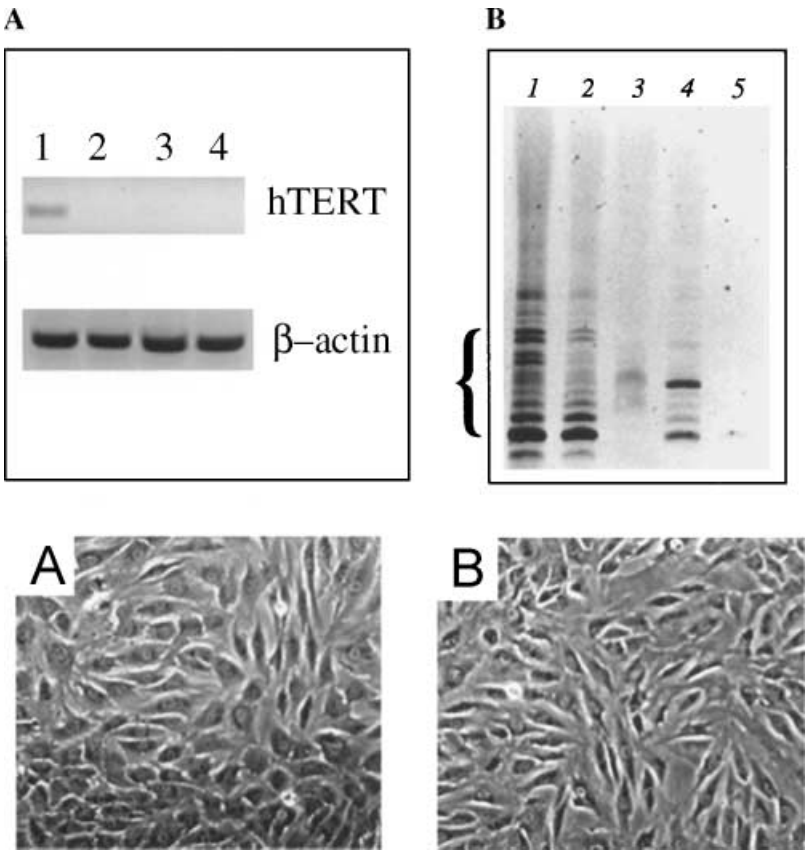


Fig. 2. *Top Panel*: **A** Detection of telomerase mRNA in RFs infected the pLXIN-hTERT. Total cellular RNA was isolated from 1–2 million cells using RNAeasy Mini Kit (Qiagen). RNA was treated with DNase, phenol/chloroform extracted and ethanol precipitated. RNA was re-suspended in nuclease free water. Two micrograms of RNA was used for each RT-RCR experiment. Telomerase message was identified using primers specific for the catalytic subunit of telomerase: LT5htrt (CGGAAGAGTGTCTGGAGCAA) and LT6htrt (GGATGAAGCGGAGTCTGG). RT-PCR amplification detected a 146 bp fragment corresponding to the mRNA of hTERT. In addition, primers were also used to amplify β-actin as a control, showing comparable RNA in each sample. *1* Telo-RF RNA; *2* normal RF RNA; *3* human foreskin fibroblast RNA; *4* human embryonic lung cells. **B** Detection of telomerase activity in Telo-RFs. Telomerase activity in cell extracts was analyzed using a modified telomeric repeat amplification protocol (TRAP). Cell extracts were isolated using the CHAPS lysis method and two micrograms of extract was used for each experiment. The main steps of the assay include extension of MTS primer by telomerase, amplification of the telomerase products using two reverse primers RP and RPC3. MTS acts as a template for telomerase as well as a forward primer for PCR amplification. PCR products were resolved using a 12% nondenaturing gel polyacrylamide gel. The gel was stained or with ethidium bromide and the PCR products were visualized by UV transillumination. *1* ØNX Cell extract; *2* Telo-RF Cell extract; *3* Telo-RF extract inactivated by heating at 90 °C for 5 min; *4* R8 quantification standard; *5* Telo-RF extract inactivated by treatment with RNase A. Bracket denotes telomeric ladder indicative of telomerase activity in the sample. *Bottom Panel*: Microscopic evaluation of normal and telo-RFs. Cells were seeded in 6 well culture plate at 5–6 million cells per well. Cells were photographed 48 h after plating. Pictures were taken using 20 × magnification on a Nikon Diaphote microscope. Both cell types exhibit contact inhibition and appear phenotypically identical. **A** Telo-RFs. **B** Normal RFs

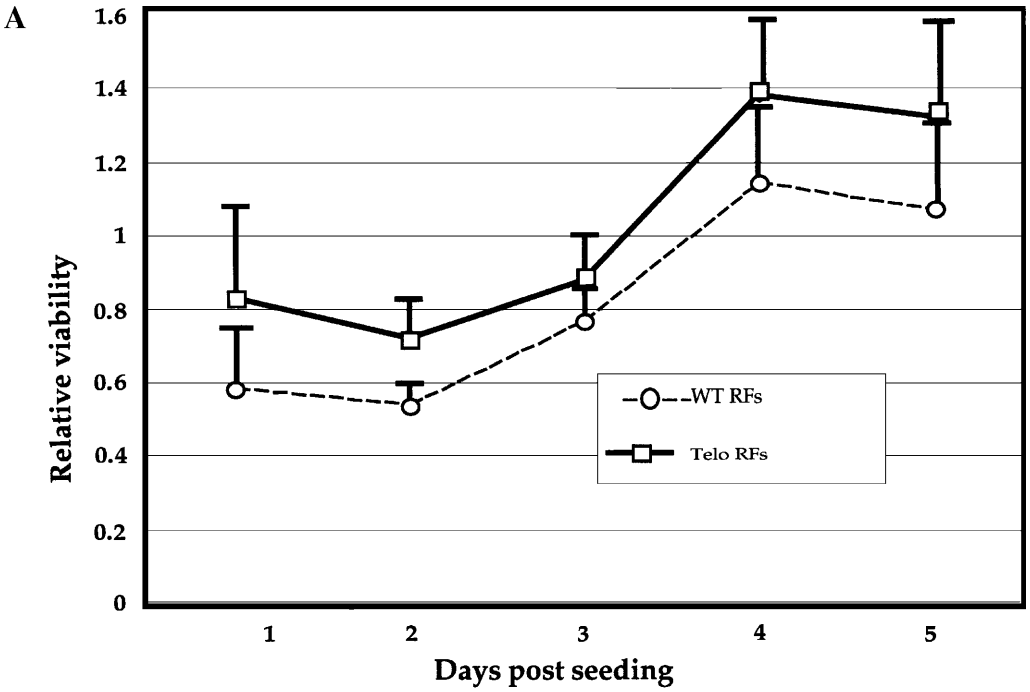
Now that it was established that telo-RFs were expressing the functional telomerase gene, we compared phenotype and cell growth characteristics of wt-RFs and telo-RFs. Figure 2, bottom panel, is a photomicrograph of wt-RFs and telo-RFs showing an identical cellular phenotype between the two cell types. Both cell types exhibit contact inhibition and show no significant phenotype differences.

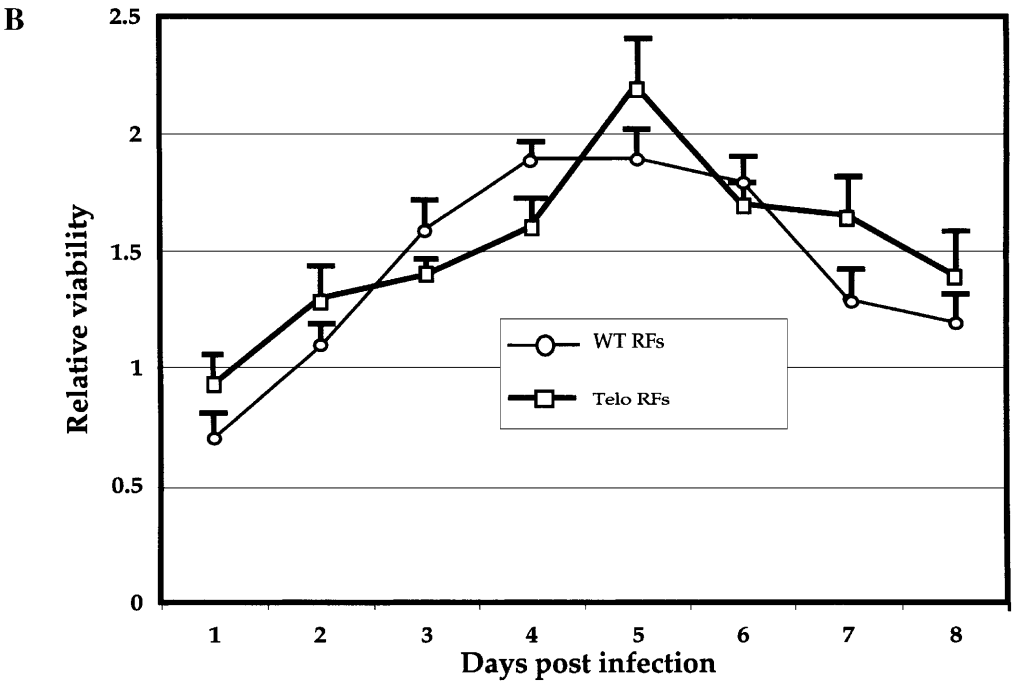
We next examined the growth characteristics of wt and life-expaned RFs. Equal numbers of cells (1000) were plated into each well of a 96 well plate and cell growth assays were performed. In parallel experiments, cellular proliferation was measured at 24 h increments. Figure 3A is a graph comparing normal and telo-RF cell doubling times. Cell growth assays showed almost identical growth patterns and cell numbers at all time points, indicating that no abnormal changes in doubling times resulted from the expression of hTERT.

After evaluating cell growth under normal conditions, it was necessary to evaluate cell growth for RRV infected cells. As before, cell growth assays were performed in 96 well microtiter plates with equal number of cells in each type of plate. RRV was added to the growth medium at the time of plating using an MOI of 0.05. Growth was monitored at 24 h intervals. Figure 3B is a graph comparing cellular growth of wt and telo-RFs infected with RRV. Cellular growth patterns appear to be almost identical in both cell types suggesting that viral infection does not alter growth patterns in telo-RFs and that the presence of telomerase has no observed effect on cell growth in the presence of virus. Virus cytopathic effect (CPE) was clearly visible at 7 days post infection. This was also indicated as a decrease in cell viability initially observed at day 6 post infection (Fig. 3B).

In addition to the effects of viral infection on cell growth, viral DNA replication was also evaluated in normal and telo-RFs. Wt and telo-RFs were plated in 6 well tissue culture plates and infected using a MOI of 3. Total cellular DNA was harvested starting at 48 h post infection, and continued thereafter in 24 h intervals. DNA was cleaved and resolved on a 1% agarose gel. Southern blots were performed using a probe specific for the RRV genomic DNA to measure the accumulation of viral DNA in both infected cell types at various times post infection. Figure 4 is a bar graph analysis of Southern blots showing the relative amounts of viral DNA accumulation in infected normal and telo-RFs. Accumulation of viral DNA occurred to the same degree in both wt and telo-RFs. Both cell types show a linear accumulation of viral DNA from day 2 to day 7 (Fig. 4). This data suggests that viral infection occurred normally in telo-RFs and viral DNA accumulation is unaffected by the presence of telomerase. Infectious virus production was also shown to be similar when RRV was used to infect wt or telo-RFs. Viral titers obtained from virus propagated in either wt RFs or telo-RFs were determined by standard plaque assay. Supernatant virus was harvested from infected cells grown in 6 cm dishes at 5 days post infection. Titers were determined to be 2×10^4 plaque forming units per ml (pfu/ml) (± 0.47) for virus originating from wt-RFs and 3×10^4 pfu/ml (± 0.56) for virus originating from telo-RFs. Standard deviations were calculated from 3 separate plaque assay experiments.

Since future experiments with RRV will involve the study of gene expression, we decided to examine the steady state mRNA levels of viral immediate early and early transcripts. For the study of immediate-early gene expression we selected ORF50, the proposed homolog of EBV Rta [13]. ORF50 is present in infected





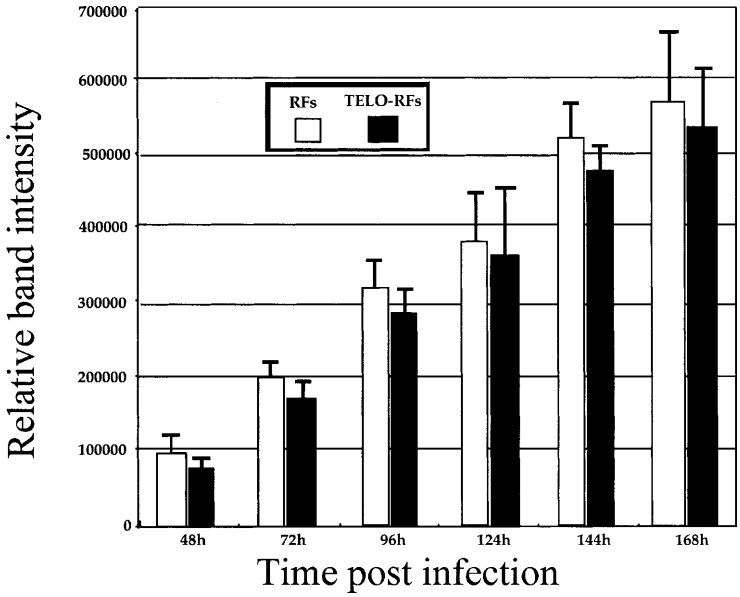


Fig. 4. Accumulation of RRV DNA from infected wt and telo-RFs. Wt and Telo-RFs were plated at a density of 1×10^5 cells per well in 6 well tissue culture plates and infected with RRV at an approximate MOI of one. Total cellular DNA was harvested every 24 h from 48 h to 168 h. Two micrograms of DNA was cleaved with EcoRI, resolved on a 1% agarose gel and transferred to Zeta-Probe nylon membrane (Bio-Rad). The blot was probed with a single-stranded riboprobe generated from a labeled EcoRI fragment sub-cloned from cosmid 28a, corresponding to RRV nucleotide sequences 56645–60744. Blot was exposed to x-ray film and relative DNA accumulation was determined by using Scan-analysis software (Biosoft). Error bar values are the standard deviations from three separate experiments

cells shortly after induction of the HHV-8 viral lytic cycle. Accumulation of a putative early transcript was examined by hybridizing RNA blots with a probe specific for ORF 40–41. These ORFs encode the putative primase-associated factor [13]. Figure 5 is an autoradiogram of a Northern blot showing that similar steady state levels of the viral transcript encoding ORF50 was observed in RNA samples harvested from wt-RFs and telo-RFs at 24 h post infection (Fig. 5, lanes

Fig. 3. Cellular growth assays for wt and telo-RFs under normal and RRV infected conditions. **A** Wt RFs and telo-RFs were seeded in 96 well tissue culture plates at 2000 cells per well and incubated at 37 °C in a 5% CO_2 incubator. To measure cellular proliferation, every 24 h, 20 μl of Cell Titer 96 Aqueous-one solution (Promega) was added to 12 wells and plates were incubated for 2 h at 37 °C. Absorbance at 490 nm was read using a CERES 900 plate reader to determine relative cellular viability. Error bars are the standard deviations from the average of 36 different samples at each time point. **B** Cellular growth assays for infected wt and telo-RFs. Cells were seeded in 96 well tissue culture plates at 2000 cells per well and infected at seeding at an approximate MOI of 0.5. Cell proliferation was determined as described A. Error bars are the standard deviations from the average of 12 different samples at each time point

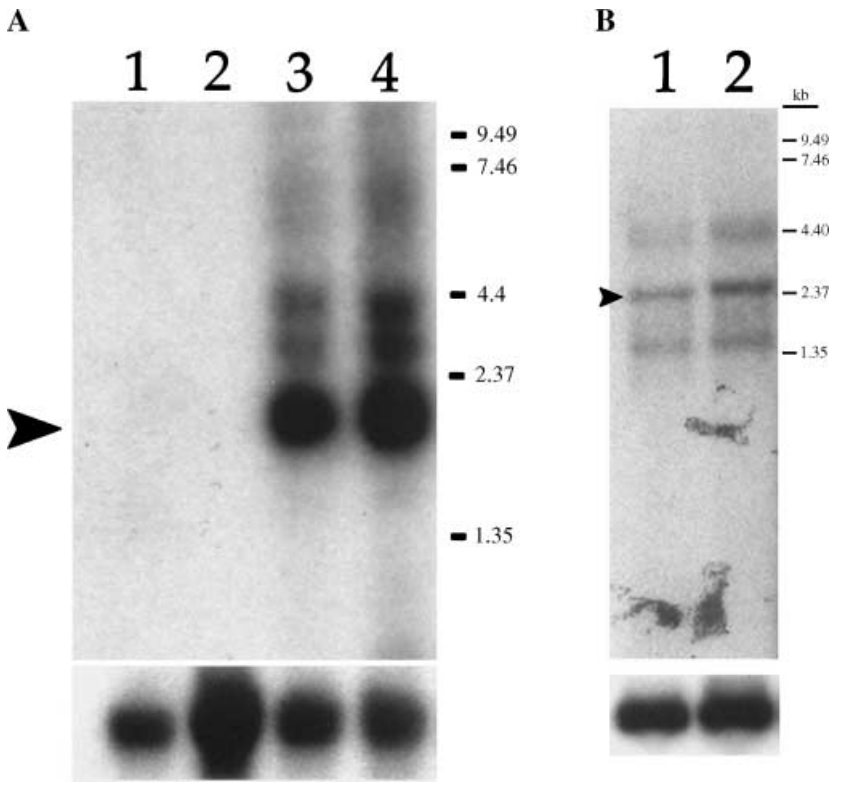


Fig. 5. Northern analysis of RRV transcripts in RFs and telo-RFs. RFs and telo-RFs (1×10^5 cells) were plated in 6 cm dishes and infected with RRV. For **A**, RNA was hybridized with a ^{32}P-labeled random primer generated DNA probe corresponding to nts 67661–69205 corresponding to RRV ORF 50. *1* Uninfected wt-RFs; *2* uninfected telo-RFs; *3* 24 h post infection of wt-RFs; *4* 24 h post infection of telo-RFs. **B** RNA was probed with a single-stranded riboprobe generated from a DNA fragment corresponding to RRV genomic nts 55,964–57,224 corresponding to ORFs 40/41 of RRV. The arrow shows the 40–41 spliced transcript (2.3 kb). The lower panels are autoradiograms of the same filters hybridized with a probe for 18S RNA indicating that approximately the same amount of total RNA was loaded on the agarose gel

3 and 4, see arrow). Transcripts for the early gene, PAF, also displayed a similar pattern in Northern analysis (Fig. 4B, lanes 1 and 2). Although several bands are present on the blot, the arrow indicates the spliced transcript arising from the ORFs 40–41 locus. The lower band is the result of a separate smaller transcript initiating from the ORF40 locus with a molecular weight of approximately 1.4 kb. Splicing was confirmed by RT-PCR (data not shown). The band present a 4.4 kb is the result of cross hybridization with 28S RNA.

Although an obvious application of telo-RFs is to perform infection and gene expression studies, many experiments involve the transfection of plasmids expression exogenous or viral genes. We evaluated the transfectablity of telo-RFs by transfecting an EGFP expressing plasmid. RFs and telo-RFs were plated and transfected with pEGFP-N1 (Clontech). Cells were visualized using epiflorescence

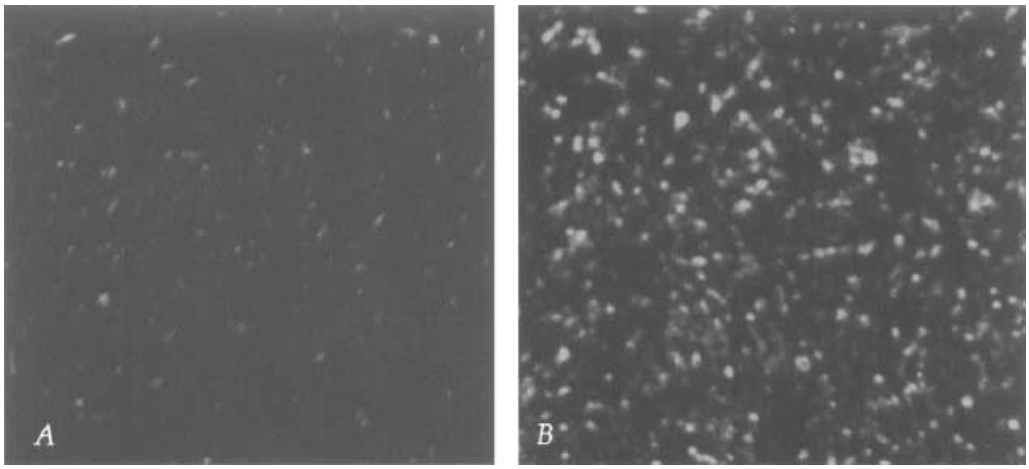


Fig. 6. Evaluation of transfection efficiencies of pEGFP in RFs and Telo-RFs. Cells were transfected with 3 μg of pEGFP-N1 using Transit (Mirrus) transfection reagent. **A** Transfection of RF cells; **B** transfection of telo-RFs

microscopy, photomicrographs are shown in Fig. 6. Telo-RFs cells displayed not only a higher intensity of green but also more cells appeared to be transfected when compared to wt RFs (compare panel A to panel B).

Discussion

We have shown that the telomerization of RF cells allows for expanded live span in cell culture while retaining permissiveness for DNA replication and production of infectious RRV. From the data presented here, telo-RFs can be used for the study of viral gene expression, transcript mapping and lytic replication experiments. Although the technique for the telomerization of primary cells has been described previously, we have now demonstrated that the propagation of a herpesvirus, RRV, in life-expanded cells, is virtually identical to results observed in wt cells. Telo-RFs have similar growth patterns as normal RFs and exhibit viral replication and proliferation that is unaffected by telomerase activity. We observed the same growth patterns between the two cell lines in infected cells along with the same time for the appearance of viral CPE. This suggests that viral processes leading to the production of infectious virus is unimpaired in telo-RFs. In addition, virus yields were virtually identical in both cell lines. This makes the telo-RF cell line attractive for the propagation of virus and to generate viral stocks. Telo-RFs have also been used successfully to propagate Rhesus Monkey Cytomegalovirus (P. Barry, UC Davis, pers. comm.).

One distinction between the two cell lines is that telo-RFs appear to transfect with greater efficiency than wt RFs. Telo-RFs will be ideal for studies requiring efficient transfection, for example transient assays for promoter evaluation and transient replication assays. Although we have shown that RNA levels of immediate early and early transcripts are identical to those observed in wt RFs upon infection, the higher transfection efficiency of telo-RFs allows for greater

expression levels of proteins assayed in a transient transfection and can be readily used for replication assays and the production of mutant viruses (unpublished results, D. AuCoin, K. Colletti, V. Kirchoff, S. Wong and G. S. Pari).

Unlike the human homolog, which is primarily latent in culture, infection of primary fibroblasts with RRV in culture results in a lytic infection. This feature makes RRV an attractive model for the study of the human counterpart in that the production of viral mutants and the study of lytic replication may be less complicated. Now with the generation of a life-expanded cell line permissive for RRV, these studies will be made even easier. The maintenance of a cell line with an extended life span will alleviate the burden for the continual supply of fresh primary cells to study the lytic replication of RRV.

Acknowledgement

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