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Involvement of a Spliced and Defective Human Foamy Virus in the Establishment of Chronic Infection

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Human foamy retrovirus (HFV) is found as two proviruses (HFV and ΔHFV) which differ by a splice-induced deletion within the *bel1* transactivator gene. The defective ΔHFV (which lacks a functional Bel1 but harbors an intronless *bet* gene) is predominantly found in nonlytic infections in vitro as well as in vivo. Here, we show that infection of cell lines stably transduced by ΔHFV DNA with the highly lytic HFV leads to chronic infections characterized by an absence of lysis, a balanced ratio of HFV to ΔHFV, and a persistent Bet expression accompanied by a shutoff of structural genes. While this system only partially reflects the natural situation, in which target cells are infected by HFV and ΔHFV simultaneously, it strongly suggests that ΔHFV is a defective interfering retrovirus. Accordingly, previous or concomitant exposure to ΔHFV viruses greatly enhances the formation of lysis-resistant clones in culture after HFV infection. The inability of ΔHFV proviruses encoding a mutated *bet* gene to induce chronic infection suggests a role for Bet in this process. Through a specific, splice-induced, genomic deletion, resulting in a switch from Bel1 to Bet expression, the lytic properties of HFV are progressively lost. Such programmed inactivation of a key gene represents a new regulatory mechanism of gene expression in retroviruses.

Foamy viruses are a large family of complex animal retroviruses which induce characteristic foam-like cytopathic effects in tissue culture. Some chronically infected cell lines have also been described (39). Natural infection is well documented in simians, and sporadic cases in humans have been described (8, 35). In vivo, no clear cytopathic effect could be demonstrated, although chronic persistent infection is well established in animals. Consequently, this group of viruses was considered innocuous (37, 40). However, expression of a human foamy virus (HFV) genome or the early viral genes in transgenic mice leads to progressive myopathy and encephalopathy (4), raising questions about this idea. Several important aspects of HFV biology remain to be clarified, particularly the molecular basis for induction of a chronic state of infection.

HFV has a complex structure in that, in addition to *gag*, *pol*, and *env* structural genes, it harbors three regulatory genes in the 3′ end of its genome (Fig. 1). An internal promoter within the *env* gene controls the transcription of these regulatory genes independently from the long terminal repeat (LTR) (21). The *bel1* gene encodes a nuclear 36-kDa phosphoprotein which transactivates the HFV LTR as well as the internal promoter (21, 34). Several Bel1 responsive elements which exhibit no consensus sequence have been identified (20, 21). This, as well as the absence of an identifiable DNA-binding motif, suggests that Bel1 acts indirectly through association with cellular proteins, as is the case with several other viral transactivators (25). Bet is an abundant protein consisting of the first 88 amino acids of Bel1 spliced to the entire Bel2 coding sequence (Fig. 1). The functions of Bet, as well as those of the related Bel2 protein, are yet unknown. Finally, the *bel3* gene product was proposed to be a viral superantigen (41, 42).

We have previously shown that in cell cultures as well as in experimentally infected animals and in an HFV-infected myasthenic patient, HFV proviruses exist in two distinct molecular forms (35, 36); the shorter one (ΔHFV) contains a 301-bp deletion in the *bel1* gene, generated by a single splicing event in the genomic RNA, and is consequently defective in the absence of exogenous Bel1. This splice leads to the formation of an intronless *bet* gene (Fig. 1). ΔHFV is distinctly more abundant in chronic forms of infection (36), suggesting that it might interfere with the expression and/or replication of wild-type HFV. This situation evokes that of defective interfering (DI) viruses, natural mutants found in a variety of animal and plant viruses which provide negative feedback towards their cognate viruses. DI viruses may be involved in induction of chronic infections or in vivo modulations of virulence (15, 17). The genetic defects which induce the formation of these DI viruses are generally poorly characterized.

In this report, we demonstrate that ΔHFV is a DI virus. Cell lines which harbor ΔHFV exhibit a rapid or immediate resistance to HFV-induced lysis, leading to a chronic state characterized by a shutdown of structural protein expression and a conserved level of Bet. Exposure to ΔHFV viruses interferes with HFV-induced cell lysis. The Bet protein seems to be required for induction of lysis resistance by ΔHFV, although the exact molecular mechanisms underlying chronicity are not fully understood. In this ex vivo model, the balance between HFV and ΔHFV, and hence between the Bel1 transactivator and the Bet protein, controlled by a single splicing event, appears to regulate the biological behavior of HFV.

MATERIALS AND METHODS

Cells and virus. AV3, a human amniotic cell line, was maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. Mycoplasma-free HFV stocks were grown on U373MG cells, a human neural cell line. ΔHFV cell lines were obtained by cotransfection of ΔHFV plasmid with the plasmid DSP hygro followed by antibiotic selection. Cells were transfected with Lipofectin reagent (Gibco, BRL) according to the manufacturer's instructions. Selection of hygromycin B-resistant clones was done in Dulbecco's modified Eagle's medium containing 110 U of hygromycin B per ml (Boehringer Mannheim) added 48 h posttransfection. Different chemical agents, such as dimethyl sulfoxide (20% [1 min]), 5′-azacytidine (20 μM [7 days]), tetradecanoyl phorbol

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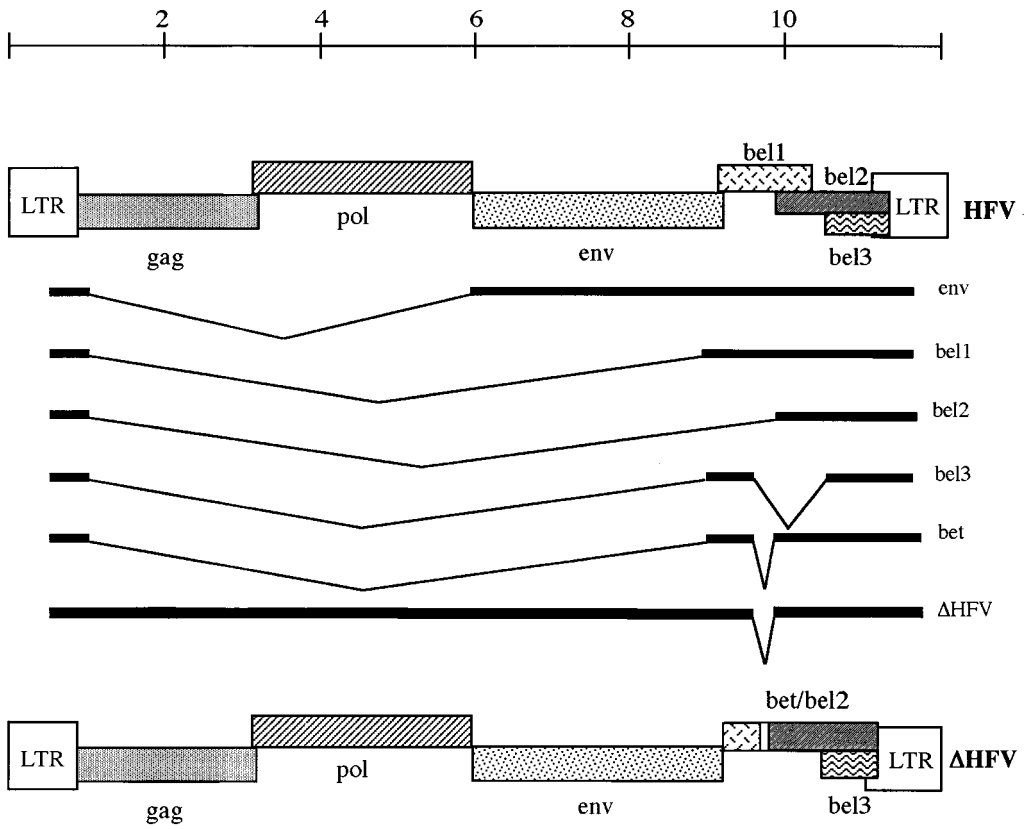


FIG. 1. Schematic representation of HFV and ΔHFV genomes. The principal singly and multiply spliced RNAs are indicated. The spliced genome is reverse transcribed to generate the ΔHFV provirus. Note that the splicing in ΔHFV disrupts the Bel1 open reading frame and creates an intronless *bet* gene.

acetate (100 ng/ml [1 day]) and bromodeoxyuridine (10 μg/ml [7 days]) (39), were used in an attempt to activate ΔHFV in AV3 stably transfected cell lines. The Bet expression vector was pbcBet, a kind gift of R. Flügel.

DNA and RNA analysis. DNA from stably transfected cells was analyzed according to standard procedures with *Eco*RI and *Nco*I as restriction enzymes (38). The 475-bp *Pst*I-*Nco*I DNA fragment (from nucleotides 4329 to 4805 in the *bel1* gene) was used as a probe.

For Northern blot analysis, total cellular RNAs were extracted with a Bioprobe Systems RNA extraction kit. About 10 μg of RNA was loaded on formaldehyde-agarose gels, blotted to nitrocellulose filters according to the method of Fourney et al. (9), and hybridized as previously described. The glyceraldehyde 3-phosphate dehydrogenase (GAPDH) probe (kindly provided by F. J. Benham [2]) and p13HSRV (22) were used as probes.

Generation of the HFV mutant. The *Cla*I-*Cla*I fragment, containing the *bel* genes, was isolated from the ΔHFV vector (36) and subcloned in pBluescript KS+, resulting in pBlu-p13. A frameshift mutant of the *bet* gene was generated via *Bgl*II digestion of pBlu-p13, which was filled in with Klenow enzyme (38). This resulted in a stop codon 18 amino acids 3′ of the *Bgl*II site, which was controlled by sequence analysis. The mutated *Cla*I-*Cla*I fragment was reinserted into ΔHFV, giving rise to the ΔHFVΔBet plasmid.

Protein analysis. For immunoprecipitation assays, 10^7 HFV acutely or chronically infected cells were labeled with [^{35}S]methionine (50 μCi/ml; specific activity, 1,245 Ci/mmol [Amersham]) for 7 h in minimal essential medium lacking methionine and supplemented with 5% fetal calf serum. Cells were lysed in 50 mM Tris-HCl (pH 7.4)–100 mM NaCl–5 mM $MgCl_2$–1% Triton X-100–0.5% sodium dodecyl sulfate (SDS) for 30 min at 4°C. After centrifugation, the supernatant was collected and immunoprecipitated with a rabbit anti-whole virus antiserum as described previously (12). For Western blotting (immunoblotting), cells were directly lysed in Laemmli sample buffer and proteins were loaded on an SDS-polyacrylamide gel (18). The proteins were transferred onto a polyvinylidene difluoride membrane (Immobilon-P; Millipore), which was incubated with D11, an anti-Bet monoclonal antibody, and subsequently revealed with DAB substrate (Sigma).

Database searches with single sequences. Motif searches were done with the computer program developed by Brenner (5). Sequence alignment was performed with the BOXSHADE program (7). Statistical significance was tested with the MACAW program of the National Center for Biotechnology Information, Bethesda, Md.

RESULTS

Generation of ΔHFV stable transfectants. The abundance of ΔHFV in lysis-resistant HFV-infected cells suggested that the defective virus might be involved in the induction of interference (36). To investigate the role of ΔHFV, we generated stable transformants in HFV-permissive AV3 cells (see Materials and Methods). Five clones were obtained, and Southern blot analysis revealed that the defective integrated genomes appeared intact. Southern blotting with *Eco*RI, which cuts twice in the HFV or ΔHFV genome, showed that these clones harbored, respectively, 0, 2, 15, 35, and 40 proviruses, and are subsequently designated by the number of ΔHFV copies per genome. Northern blot analysis demonstrated that very low levels of the 2.2-kb Bet mRNA could be detected in cell lines harboring more than 15 ΔHFV genomes, while no Bet (or any other viral) protein could be found by either immunofluorescence, Western blotting, or immunoprecipitation. Interestingly, the presence of ΔHFV induced morphological changes in the cells (particularly in those that harbor multiple copies), most notably a cytoplasmic shrinkage (smaller cellular size) which was associated with a slower growth rate. These morphological alterations might be the consequence of low levels of Bet expression, because attempts to stably express Bet led to a grossly altered cell morphology and cell death, at least in these AV3 cells. Because a fraction of Bet appears to be excreted, such an inability to overexpress Bet might be caused by the presence of an RGD motif (amino acids 394 to 396), which might interfere with both cell adhesion and the cytoskeleton.

In an attempt to activate ΔHFV expression, these cell lines

were challenged with a number of viral transactivators. While transfection with HFV Bel1- or SFV-6 Taf (the simian homolog of Bel1, kindly provided by J. Tobaly and J. De Celis)-encoding vectors induced ΔHFV protein expression (Bet as well as all structural viral proteins was observed by Western blotting) and cell lysis (consistent with our previous findings [36]), neither Tat from human immunodeficiency virus type 1 (HIV-1) nor Tax from human T-cell leukemia virus type 1 (HTLV-1) induced ΔHFV protein expression. Infection with herpes simplex virus type 1 or adenovirus type 1 also failed to induce ΔHFV expression, in contrast to HIV-1, which can be reactivated from its latent stage by herpes simplex virus type 1 (30). Different chemical agents, such as dimethyl sulfoxide, 5′-azacytidine, tetradecanoyl phorbol acetate, or bromodeoxyuridine, known to activate latent cellular or viral genes (39) also failed to induce ΔHFV expression. Finally, although *ets*-like and AP1 sites were found in the LTR (positions 321, 330, and 335 in reference 27), transient expression of neither *jun*, *fos*, nor *ets* enhanced ΔHFV expression.

HFV infection of ΔHFV cell lines. To test the hypothesis that ΔHFV might interfere with HFV replication, we infected these ΔHFV cell-lines with HFV. Strikingly, the three cell lines which harbor more than 15 copies of ΔHFV were resistant to lysis upon exposure to HFV but progressively established a chronic form of infection. The cell line harboring two defective genomes exhibited the opposite behavior, lysing twice as quickly as parental AV3 cells; however, lysis-resistant clones emerged rapidly and in large numbers, in sharp contrast to control cells in which they only occasionally occurred 3 weeks postlysis (Fig. 2). After 3 weeks of culture, all of these HFV-infected ΔHFV cell lines behaved similarly in terms of growth, cell morphology, and molecular results (see below). ΔHFV did not induce the synthesis of interferons, because 2′,5′-oligoadenylate synthethase activity remained unchanged (data not shown). These results establish that depending upon the abundance of the defective genome, ΔHFV can exert both negative and positive effects on the early phases of infection, but in all cases, ΔHFV makes a major contribution to the establishment of chronic forms of infection.

To extend these results, we transfected clone 40 with a Bel1 expression vector and transferred the supernatant (containing ΔHFV in a viral form) onto permissive AV3 cells. Southern blot analysis 3 days later revealed the exclusive presence of ΔHFV DNA in the recipient cells (data not shown). Similarly to clone 2, when challenged with HFV, these cells established lysis-resistant clones at a much higher level of efficiency than did controls. Supernatant from HFV-infected ΔHFV clones at the lysis-resistant stage directly induced a typical chronic infection with little, if any, transient lysis. Therefore, previous or concomitant exposure to ΔHFV viruses interferes with the lytic properties of HFV. The same results were obtained with the human glioblastoma cell line U373MG as the recipient. Taken together, these results strongly suggest that ΔHFV is a DI virus.

To clarify the basis of interference, the molecular status of HFV in these ΔHFV cell lines was investigated at the DNA, RNA, and protein levels during the onset of HFV infection. The kinetics of HFV infection of three representative clones (0, 2, 40) were first analyzed by Southern blotting (Fig. 3A). Control cells (clone 0) show a progressive accumulation of both deleted and wild-type viruses up to total lysis. At this point, they harbor about 200 HFV proviruses per genome (determined by densitometry), with a fivefold excess of HFV to ΔHFV. Early postinfection (days 2 and 3), cells initially harboring two copies of ΔHFV (clone 2) replicate and accumulate both HFV and ΔHFV sequences more rapidly than controls.

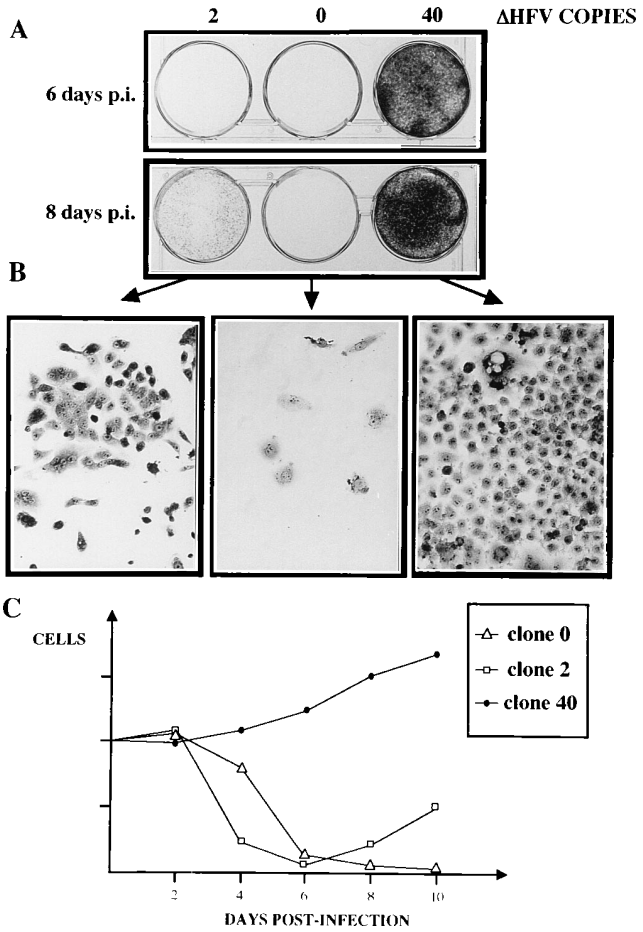


FIG. 2. ΔHFV modulates HFV-induced lysis. (A) View of clones harboring 0, 2, or 40 ΔHFV proviruses 6 days postinfection (p.i.) and 8 days postinfection with 1 PFU of wild-type virus per cell stained with phenol blue. (B) High-power-field view of cells 8 days postinfection. (C) Schematic diagram of cell survival after infection. Note that two copies of ΔHFV enhance HFV-triggered lysis but induce rapid resistance, while clone 40 is lysis resistant from the beginning. Cell survival was estimated by repeated measurements of phenol blue-stained cells and is expressed in arbitrary units.

However, at the time of lysis (day 8), clone 2 contains twofold less HFV but as much ΔHFV as the control (Fig. 3A and data not shown). Finally, in clone 40, multiplication of HFV provirus is dramatically delayed. These observations are consistent with the kinetics of cell lysis and suggest that accumulation of wild-type virus parallels lysis. Note that the behavior of clone 2 is biphasic, with an initial boost in HFV replication (days 2 and 3) followed by a decrease in viral replication (day 8). Later in infection, at the chronic lysis-resistant state, all clones contain approximately equal numbers of deleted and intact proviruses. This situation is analogous to the one found in two spontaneously lysis-resistant clones (Dami H9 chronically infected cells [12b, 43]).

When viral RNA expression was studied in these cell lines, related findings were noted (Fig. 3B). At early stages of infection, the main RNA species are those of the regulatory genes; later, at the time of lysis, large amounts of genomic RNAs (some of which are incomplete and/or partially degraded, leading to smears) are found (28, 31). In clone 40, little viral expression is observed until day 8, when low levels of Bet and Bel2 transcripts became apparent. In clone 2, the expression of Bet mRNAs (relative to the internal GAPDH control) was

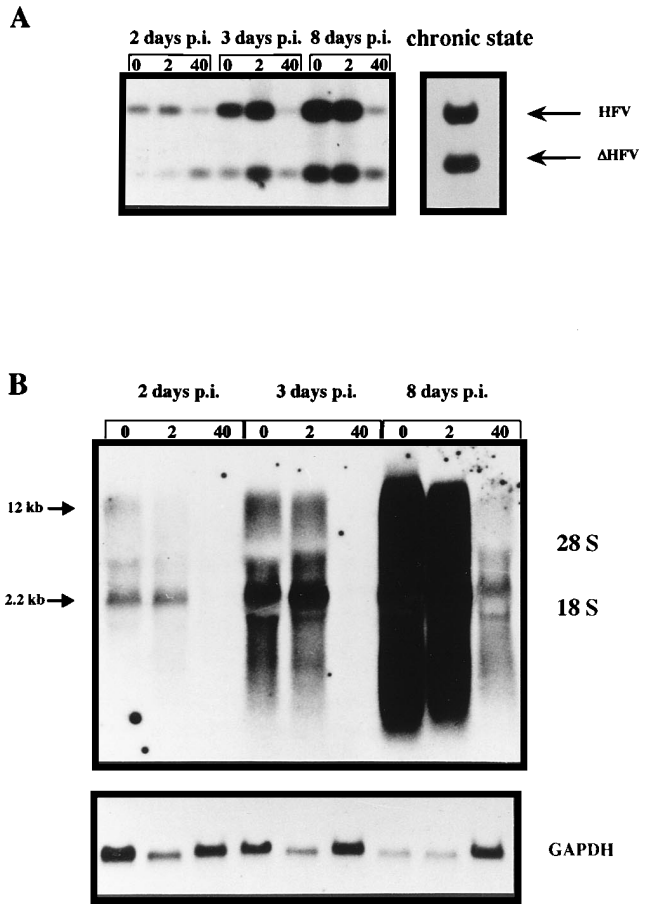


FIG. 3. Kinetics of viral DNA and RNA accumulation during HFV infection of ΔHFV clones 0, 2, and 40. (A) Southern blot analysis of total cell DNA (5 μg hydrolyzed with *Eco*RI and *Nco*I) hybridized with an HFV probe revealing the deletion (see Materials and Methods). ΔHFV proviruses yield a 371-bp fragment, while HFV genomes display a 672-bp fragment. The time course is indicated, and the last point corresponds to complete lysis in the control cells. p.i., postinfection. (B) Northern blot analysis of the RNAs extracted from the same samples hybridized with an HFV or GAPDH probe (see Materials and Methods). Note that early postinfection, only the 1.8- and 2.1- to 2.8-kb mRNAs from regulatory genes are expressed (31). As infection proceeds towards lysis, the 12-kb genomic mRNA is expressed at increasing levels. The 5.4-kb band corresponds to the Env mRNA (some samples contain less RNA, as seen with the GAPDH hybridization).

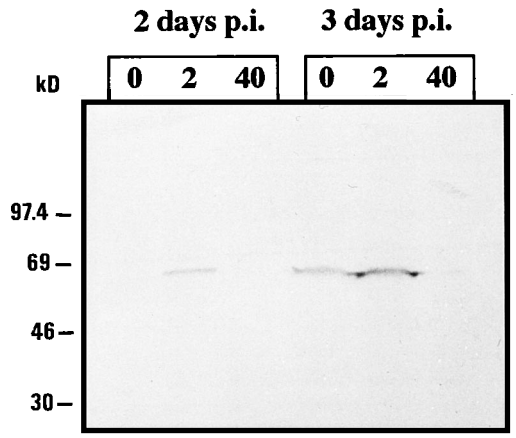


FIG. 4. Western blot analysis with an anti-Bet monoclonal antibody of clones 0, 2, and 40 at 2 and 3 days postinfection (p.i.). The p62 Bet protein is transiently overexpressed in clone 2.

three- to fivefold higher at days 2 and 3 than in control clone 0. However at day 8, two- to threefold lower levels of viral RNA were present compared with those in the control (Fig. 3B and data not shown). In line with these observations, Western blot analysis showed a clearly higher level of expression of Bet protein at days 2 and 3 in clone 2 compared with that in the control (Fig. 4). These results are consistent with HFV-induced expression of ΔHFV (in particular of the Bet protein).

At the chronic, lysis-resistant stage, all cell lines exhibited the same RNA profile, characterized by a drastic decrease in genomic RNA expression and an even greater reduction in the Env mRNA (Fig. 5A; note that a 10-fold excess of RNA from chronically infected cells compared with acutely infected cells was used to obtain similar amounts of genomic RNA in the right panel). Protein analysis showed that the p62 Bet is the most abundant viral protein detected by immunoprecipitation with an anti-HFV polyclonal serum from chronically infected rabbits which recognizes all HFV proteins (12). The identity of the p62 band was confirmed with a monoclonal anti-Bet antibody (D11 [12a]). Other regulatory or structural products can only be revealed by a longer exposure, particularly the Env proteins (gp130, gp70 to gp80, and gp48 present in the positive control), whose expression is decreased 50-fold relative to that during an acute infection (Fig. 5B). Western blot analysis provided similar results (data not shown). The viral status in these engineered chronic cells is identical to that of spontaneously resistant DAMI cells used in the original molecular cloning of the ΔHFV provirus (36). These results demonstrate that, relative to acute infections, in chronic infections, conserved Bet expression is accompanied by a shutoff of the structural genes.

A mutation in Bet inhibits interference by ΔHFV. To directly test the hypothesis that Bet could be a key factor in the initiation of chronic infection observed in the ΔHFV clones, we generated two AV3 clones harboring approximately 15 intact ΔHFV genomes with a frameshift mutation in the Bet open reading frame (but no effects on the Bel1 or Bel3 reading frames [see Materials and Methods]), leading to a C-terminally truncated Bet protein (molecular mass, 40 kDa). Infection with the wild-type HFV leads to cell lysis, which appeared more rapidly than in controls (as observed for clone 2). However, in contrast to the situation encountered in clone 2, the ΔHFVΔBet cell lines behave as the AV3 control, because no lysis-resistant clones were detected up to 3 weeks postinfection (Fig. 6 and data not shown). This observation (together with the overexpression of Bet during establishment of ΔHFV-induced chronicity) strongly suggests that Bet plays a direct role in the establishment of chronicity in HFV-infected cells.

A possible clue to the role of Bet in viral biology stems from protein sequence analysis, which revealed that two regions of Bet are similar to conserved motifs present in Rev-like proteins. Several regions similar to the HIV-Rev RNA binding domain are present in Bet (24); one of them (positions 395 to 412) was relatively similar to HIV-1 Rev (positions 35 to 53) (Fig. 7A). These alignments were significant as assessed by the MACAW program. A leucine-rich effector domain, thought in the case of lentiviral Rev to interact with cellular proteins (16, 23), is also found in Bet (positions 9 to 56). For this domain, the similarities expand to the Rex proteins of HTLV (16) (Fig. 7B). In that case, the similarities between both HFV and HTLVs or HFV and HIVs are significant, while that between HTLVs and HIVs is not.

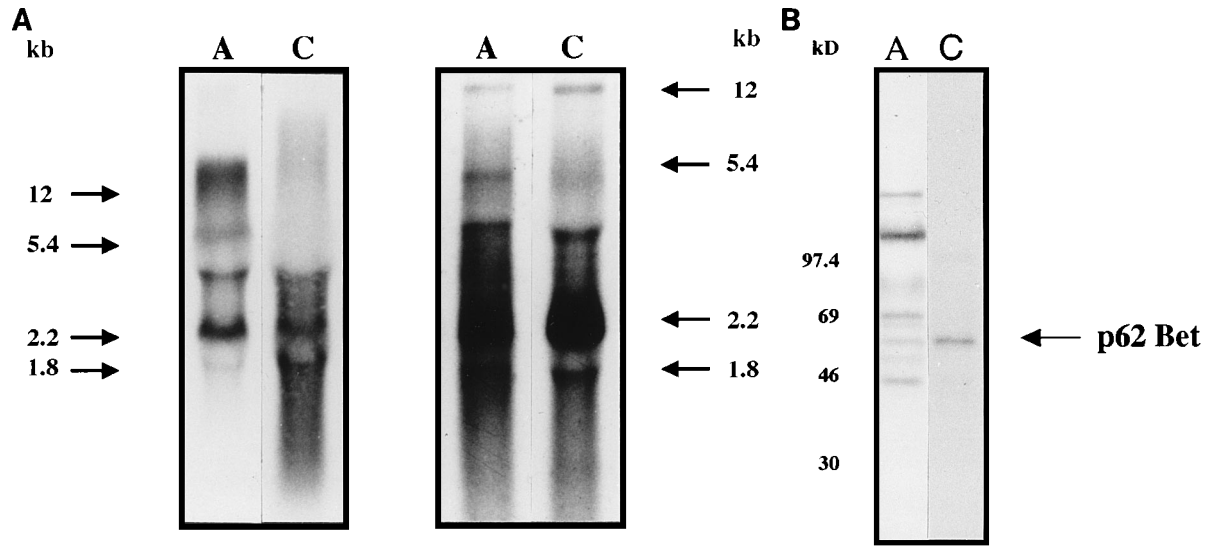


FIG. 5. (A) Northern blot analysis of HFV acutely infected (5 days postinfection) (lanes A) or HFV chronically infected (lanes C) ΔHFV cells. On the left, identical amounts of viral RNAs were loaded. In the experiment shown on the right, a 10-fold excess of RNA of chronic cells was used to demonstrate the diminished Env/genomic RNA ratio in chronic infections. (B) HFV protein expression in chronically infected cells as revealed by immunoprecipitation analysis with a rabbit anti-HFV polyclonal serum (see Materials and Methods). The Bet protein is the major viral protein expressed. (The same result was obtained with any of the chronically infected clones 0, 2, or 40.) In acutely infected cells, we observed a classical profile of HFV polypeptides (12).

DISCUSSION

This report demonstrates that ΔHFV is a DI virus. We propose that through the splicing out of an essential part of its genome, HFV changes the balance between the Bel1 transactivator and the Bet protein, switching its biology from acute to chronic ex vivo.

We show that the presence of ΔHFV prior to HFV infection is a key factor directing cells towards chronic states of infection. The initial behaviors of clones 2 and 40 after infection are different. We propose that the accelerated lysis observed in clone 2 is due to HFV-induced expression of the structural genes of ΔHFV. The slow onset of chronic infection in all three clones with more than 15 ΔHFV genomes excludes clonal variation as the cause of resistance. The initial resistance of clone 40 to HFV infection may reflect a titration of the Bel1 transactivator by the defective virus, the inability of the virus to enter the cell because of homologous interference through Env protein binding to cell surface receptor, or a negative effect of ΔHFV-derived Bet on HFV gene expression. However, our failure to detect Env mRNA and protein in these cells does not

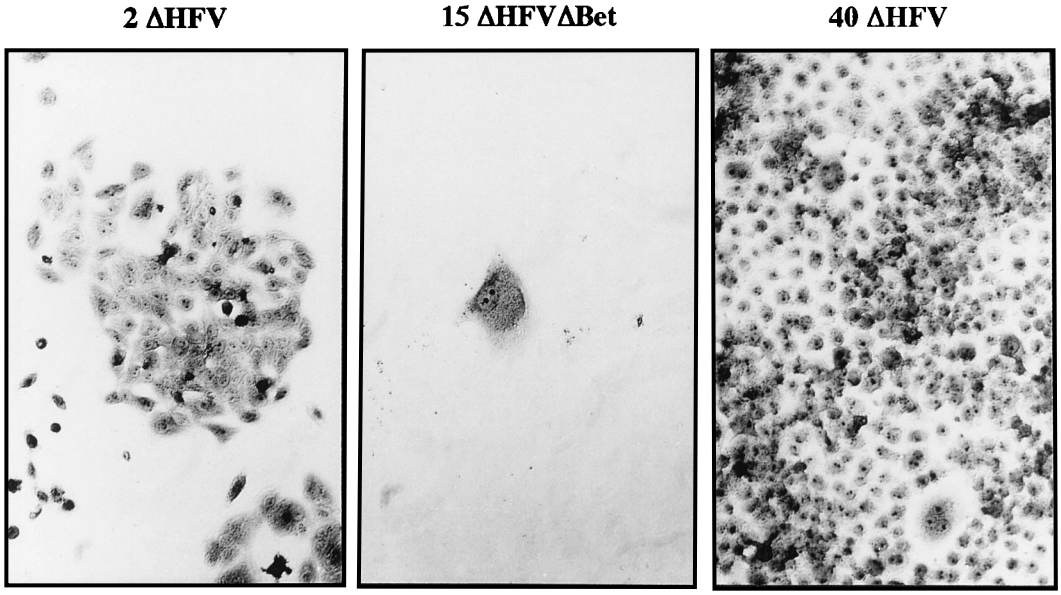


FIG. 6. Infection of clone 2, clone 40, and a ΔHFVΔBet AV3 clone by HFV. While clone 40 is lysis resistant, many resistant clones appeared in clone 2 after total lysis, 8 days postinfection. The lysis resistance or emergence of resistant cells was not observed in the ΔHFVΔBet clone, which harbors 15 copies of mutated ΔHFV genome, demonstrating that Bet plays a key role in the establishment of lysis resistance clones. A representative field is shown. Cell survival in clone ΔHFVΔBet was similar to that of clone 0 in Fig. 2.

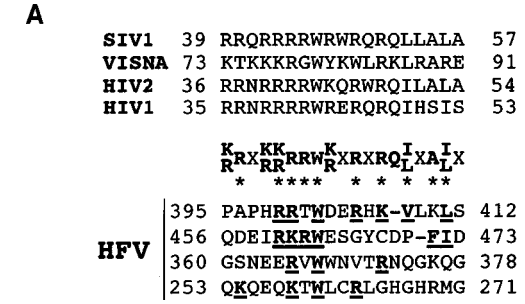
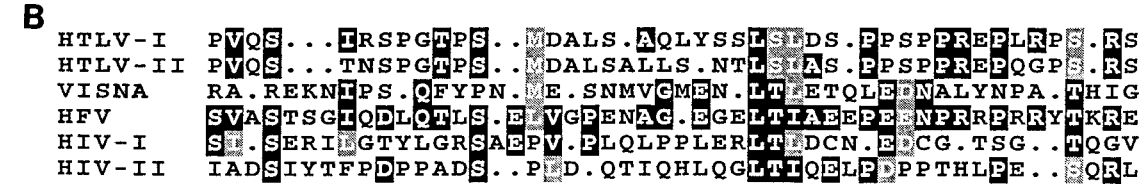


FIG. 7. Sequence similarities between Bet and Rev proteins. (A) RNA binding, oligomerization, and nuclear localization domains of Rev proteins from lentiviruses. The consensus sequence is shown below, with conserved residues in boldface. Numbers refer to sequence positions. Several amino acid stretches in HFV Bet show similarities to this consensus sequence (boldface and underlined). The 395-to-412 sequence is similar to HIV-1 Rev. (B) Effector domain. Conserved residues are boxed (black boxes, identity; gray boxes, similarity). In this case, the similarity expands to the Rex proteins of HTLVs.



support the hypothesis of Env interference. Moreover, the fact that 15 copies of ΔHFVΔBet proviruses are unable to induce a chronic state favors a Bet-dependent process rather than Bel1 titration (see below).

Clearly, the occurrence of 40 copies of ΔHFV genomes prior to HFV infection is an unlikely natural event ex vivo and, furthermore, in vivo. However, our previous data reporting the abundance of ΔHFV in all forms of chronic infections either ex vivo or in vivo and the drift of clone 2 towards lysis-resistant states suggest that formation of ΔHFV may be commonly used to induce chronic states of infection (35, 36). Nonrandom deletions in the *bel* region have also been reported in animal spumaretroviruses, suggesting that this splice of the genome may be a general mechanism by which this group of viruses modulate their lytic properties (14, 33). The demonstration that either a mix of ΔHFV and HFV viruses or supernatants from chronically infected cells (presumably containing similar amounts of both forms) directly induce a chronic state is important proof that in a more physiological system, ΔHFV is an interfering virus.

The main features of the chronic state of HFV infections are very low levels of structural protein expression together with a relatively high level of Bet RNA and protein expression, evoking the latent state of several other retroviruses (6, 32). At the molecular level, a drastic decrease in both genomic and Env mRNAs, together with relatively conserved Bet mRNA expression, could indicate either a promoter switch from the LTR to the internal promoter or a change in the ratio between spliced and unspliced LTR-driven transcripts. The sharp decrease in the ratio of *env* to genomic RNA during chronic infections (Fig. 5A) would favor the existence of this last phenomenon. The molecular events underlying these changes in viral gene expression are not yet mechanistically understood. However, such a drastic decrease in *env* gene expression (also observed at the protein level [Fig. 5B]) likely plays a role in the suppression of virus-induced cell fusion and, consequently, the absence of cell lysis (1, 10).

This viral interference between ΔHFV and HFV most likely involves the Bet protein, because (i) ΔHFV bearing a mutation in Bet is no longer able to induce chronic infection, (ii) Bet is the major protein expressed in chronically infected cells, and (iii) Bet is transiently overexpressed in clone 2 during the establishment of chronicity. Unfortunately, our inability to stably overexpress Bet precludes a direct assessment of the role of Bet in inducing chronic states. However, when we transfected a Bet-expressing vector into AV3 cells (approximately 40% of the cells were positive for Bet by immunofluorescence) and further infected them 48 h posttransfection with HFV, a sharp delay in the appearance of lysis as well as in viral gene expression (assessed by Western blot analysis) was observed compared with the mock-transfected control (data not shown). This experiment favors a direct role of Bet in the establishment of chronicity, although a general toxic effect cannot be excluded. We are currently constructing inducible Bet-expressing vectors and testing different cell systems to further address this point.

The functions performed by Bet are yet unidentified. The partial sequence similarity between Bet and lentiviral Rev may indicate that these proteins share some functions, but at the present time, there is no molecular indication for a Rev function of Bet, and HFV does not seem to need a posttranscriptional regulatory protein (19). The high level of Bet expression in chronic states, in which structural genes are shut off, does not favor the hypothesis that Bet has a typical Rev activity to enhance expression of unspliced mRNAs. Further experiments are needed to precisely determine the contribution of this protein to viral biology.

In our experimental model, the state of infection seems to depend on the ratio between HFV and ΔHFV and, consequently, the balance between Bel1 and Bet. As the wild-type virus replicates, a fraction of the genomic mRNA will be spliced, yielding ΔHFV viruses. As infection progresses, increasing amounts of defective viruses will be formed. Such accumulation of ΔHFV will progressively increase Bet levels in infected cells. We propose that beyond a certain Bet/Bel1 threshold, infection will drift towards chronic states. Because Bel1 shares its first 88 amino acids with Bet, they may compete for the binding of essential cofactors; note however, that in transient transfections, Bet did not appear to alter the function of Bel1 (13).

In this model, the principal role of ΔHFV is to induce and perpetuate a disequilibrium between Bel1 and Bet (Fig. 8). That transfection of Bel1 into these ΔHFV chronically infected cells led to a burst of lysis, followed by a progressive return to the chronic state (data not shown), fulfills an important prediction of this model. Conversely, high-efficiency transient transfection of Bet prior to infection sharply delayed the occurrence of cell lysis. Naturally, we cannot exclude the possi-

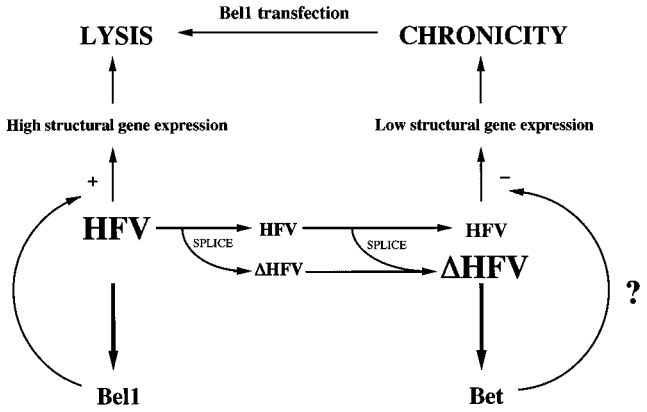


FIG. 8. Proposed model for the molecular basis of chronicity in HFV. HFV replicates and gives rise to ΔHFV through genome splicing. This defective virus can also replicate in the presence of HFV Bel1. In the presence of Bel1 (acute infection), structural genes are highly expressed, leading to cell lysis. With a high ΔHFV load, Bet is overexpressed, which may account for the drastic decrease in structural gene expression; chronicity ensues. Transfection of Bel1 into chronically infected cells leads to cell lysis.

bility that other mechanisms (e.g., incomplete reverse transcription, persistent unintegrated DNA, integrated silent provirus, and incompletely transcribed provirus [6, 11]) could contribute to the establishment of viral resistance for HFV. It is interesting that in HIV infection, several reports have described a high prevalence of *tat*-defective genomes in the peripheral blood mononuclear cells of infected individuals (26, 29), suggesting that inactivation of the transactivator gene may be a feature shared with other retroviruses. Therefore, the concepts outlined by the present study may extend to other retroviruses. In this sense, it has recently been shown that the presence of HIV-1 genomes, defective within the *pol* gene, leads to a drastic inhibition of wild-type HIV replication (3).

Our data show that apart from the highly lytic infection, HFV can establish chronic ones, likely arising through a virus-dependent mechanism conserved in this family of viruses. We propose that such behavior favors the constitution of cellular reservoirs of HFV and ΔHFV proviruses, which might later be reactivated by virus- or host-derived Bel1-like activities. These observations linking defective retroviruses to chronicity may be related to the multitude of endogenous defective retroviral sequences found in mammalian genomes, which may have played a role in protection against lytic retroviral infections during evolution. Such a selective, splice-derived loss of key viral sequences appears to be a new regulatory mechanism of gene expression used by retroviruses to modulate their behavior.

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