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XPB mediated retroviral cDNA degradation coincides with entry to the nucleus

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ABSTRACT

Retroviruses must integrate their cDNA to a host chromosome, but a significant fraction of retroviral cDNA is degraded before integration. XPB and XPD are part of the TFIIH complex which mediates basal transcription and DNA nucleotide excision repair. Retroviral infection increases when XPB or XPD are mutant. Here we show that inhibition of mRNA or protein synthesis does not affect HIV cDNA accumulation suggesting that TFIIH transcription activity is not required for degradation. Other host factors implicated in the stability of cDNA are not components of the XPB and XPD degradation pathway. Although an increase of retroviral cDNA in XPB or XPD mutant cells correlates with an increase of integrated provirus, the integration efficiency of pre-integration complexes is unaffected. Finally, HIV and MMLV cDNA degradation appears to coincide with nuclear import. These results suggest that TFIIH mediated cDNA degradation is a nuclear host defense against retroviral infection.

Introduction

All retroviruses reverse transcribe a genomic RNA to a linear cDNA molecule (Coffin et al., 1997). The cDNA is part of a pre-integration complex (PIC) which includes at least the viral proteins reverse transcriptase, matrix, and integrase (Miller et al., 1997). The retroviral PIC integrates the cDNA into a host chromosome to continue the viral life cycle. Retroviruses, including Molony murine leukemia virus (MMLV), require cellular division and breakdown of the nuclear envelope for the PIC to enter the nucleus. Lentiviruses, such as HIV, do not require cellular division and can traverse an intact nuclear envelope. Previous studies have shown that most of the cDNA that completes reverse transcription will be degraded before integration (Barbosa et al., 1994; Brussel and Sonigo, 2003; Butler et al., 2002; Van Maele et al., 2003; Vandegraaff et al., 2001).

Several host proteins have been shown to inhibit viral replication before integration (Goff, 2004). Most notably, APOBEC3G deaminates cytosines during reverse transcription (Harris et al., 2003; Mangeat et al., 2003). APOBEC3G has also been shown to reduce the accumulation of cDNA, but this finding is controversial (Bishop et al., 2006). The proteasome has been shown to play a role in cDNA stability (Butler et al., 2002; Schwartz et al., 1998). In the presence of proteasome inhibitors more retroviral cDNA accumulates. Similarly, mutations of the host XPB (ERCC3) and XPD (ERCC2) proteins correlate with an increase of retroviral cDNA (Yoder et al., 2006). The kinetics of cDNA accumulation is affected by the rate of reverse transcriptase synthesis and the rate of degradation. Degradation of cDNA may be analyzed by abrogating synthesis with the non-nucleoside reverse transcriptase inhibitor efavirenz. Treatment of XPB or XPD mutant cells with efavirenz following infection resulted in a significantly slower decrease of HIV cDNA compared to wild type cells, suggesting that these proteins participate in a cDNA degradation pathway (Yoder et al., 2006).

XPB and XPD are part of TFIIH, a ten-subunit complex that participates in both basal transcription and DNA nucleotide excision repair (NER; Giglia-Mari et al., 2004; Ranish et al., 2004). During transcription TFIIH unwinds promoter DNA allowing RNA polymerase access to the DNA template. TFIIH participates in NER by unwinding the DNA helix at the site of DNA damage. XPB and XPD are ATPase helicases with opposing polarity. XPB 3′→5′ helicase activity is required for transcription while XPD 5′→3′ helicase activity is required for NER (Coin et al., 2007). The XPB ATPase activity is also required for NER, possibly acting as a wrench to initialize separation of the DNA helix (Fan et al., 2006). Three diseases are associated with mutations of XPB or XPD: xeroderma pigmentosum (XP), XP with Cockayne syndrome (XP/CS), and trichothiodystrophy (TTD) (Lehmann, 2001). XP patients are prone to skin cancer due to an inability to complete NER. The XP associated mutation XPB(F99S)

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reduces interaction of XPB with TFIIH subunit p52, which stimulates XPB ATPase activity at sites of DNA damage (Coin et al., 2007). The XP associated mutation XPD(R683W) affects interaction of XPD with p44 and helicase activity (Dubaele et al., 2003). Hence it is possible to identify and isolate cell lines from XP patients with mutations of XPB or XPD that affect NER. However, deletion of any TFIIH complex gene is lethal due to its essential role in transcription (Friedberg and Meira, 2006).

Previous studies have shown that XPB and XPD mediated degradation of retroviral cDNA is associated with NER activity, but did not directly address the transcription activity of these proteins during cDNA degradation (Yoder et al., 2006). Here we show that XPB and XPD mediated cDNA degradation is not due to altered transcription or translation of other host factors. This degradation pathway is distinct from APOBEC3G or proteasome host defense pathways. While degradation of cDNA leads to less integrated provirus *in vivo*, the integration efficiency *in vitro* of PICs isolated from XPB or XPD mutant cells is similar to PICs from wild type cells. Finally, studies of cells arrested by aphidicolin indicate that TFIIH mediated cDNA degradation coincides with entry to the nucleus.

Results

XPB and XPD do not affect cDNA accumulation by transcription

Cell lines were derived from XP patients expressing the XPB(F99S) mutation or the XPD(R683W) mutation (Gozukara et al., 1994; Riou et al., 1999). These cell lines were complemented with the respective wild type gene to generate isogenic cell lines XPB-wt and XPD-wt (Gozukara et al., 1994; Riou et al., 1999). XPB(F99S) and XPD(R683W) cell lines are more sensitive to UV irradiation compared to the complemented cell lines, indicating defective NER (Yoder et al., 2006). Previous studies have shown that retroviral infection, both HIV and MMLV, is more than 100% greater in XPB(F99S) cell lines compared to cells expressing the wild type gene; XPD(R683W) cells have 15% greater retroviral infection efficiency compared to cells expressing wild type XPD (Yoder et al., 2006). The XP cell lines were infected with HIV or MMLV vector particles expressing GFP following successful integration. The cells were analyzed for GFP expression by flow cytometry (Fig. 1A). XPB(F99S) cells showed greater than 100% increase in HIV or MMLV transduction efficiency compared to XPB-wt cells (HIV $P=0.0063$, MMLV $P=0.022$). XPD(R683W) cells also display an increase in HIV or MMLV transduction efficiency (HIV $P=0.0003$, MMLV $P=0.0005$).

The role of XPB and XPD in basal transcription suggests the possibility that shortly following viral entry to the cell, new host factors are transcribed that could be responsible for the observed host defense against retroviral cDNA. To determine whether the TFIIH proteins are transcribing nascent host factors that mediate cDNA degradation, XPB-wt and XPB(F99S) cells were treated with the transcription inhibitor α-amanitin for 2 h (Fig. 1B). In the continued presence of α-amanitin, cells were infected with an HIV based retroviral vector encoding GFP (Follenzi et al., 2000). These vector particles have previously been shown to faithfully recapitulate the HIV life cycle from reverse transcription through integration (Butler et al., 2002). DNA was collected at 6 h post infection (hpi), a time point previously shown to display significant differences in cDNA accumulation between mutant and wild type cells (Yoder et al., 2006). The DNA samples were analyzed for HIV late reverse transcripts (LRT) by quantitative PCR (qPCR). The LRT qPCR primer set spans the primer binding site and amplifies all forms of full length HIV cDNA including linear unintegrated cDNA, 1LTR circles, 2LTR circles, and integrated provirus (Butler et al., 2001). In both the presence and absence of α-amanitin, the NER mutant cell line XPB(F99S) showed greater accumulation of cDNA than XPB-wt cells ($P<0.0001$ with or without α-amanitin, data not shown). If XPB mediates cDNA degradation through transcription of new host factors, then treatment with α-amanitin will prevent the appearance of nascent defense factors and cDNA accumulation will increase. However, there was no significant difference (XPB-wt $P=0.54$, XPB(F99S) $P=0.19$) in cDNA accumulation between untreated cells and cells treated with α-amanitin, suggesting that the transcription activity of TFIIH does not affect retroviral cDNA stability (Fig. 1B). This data is similar to

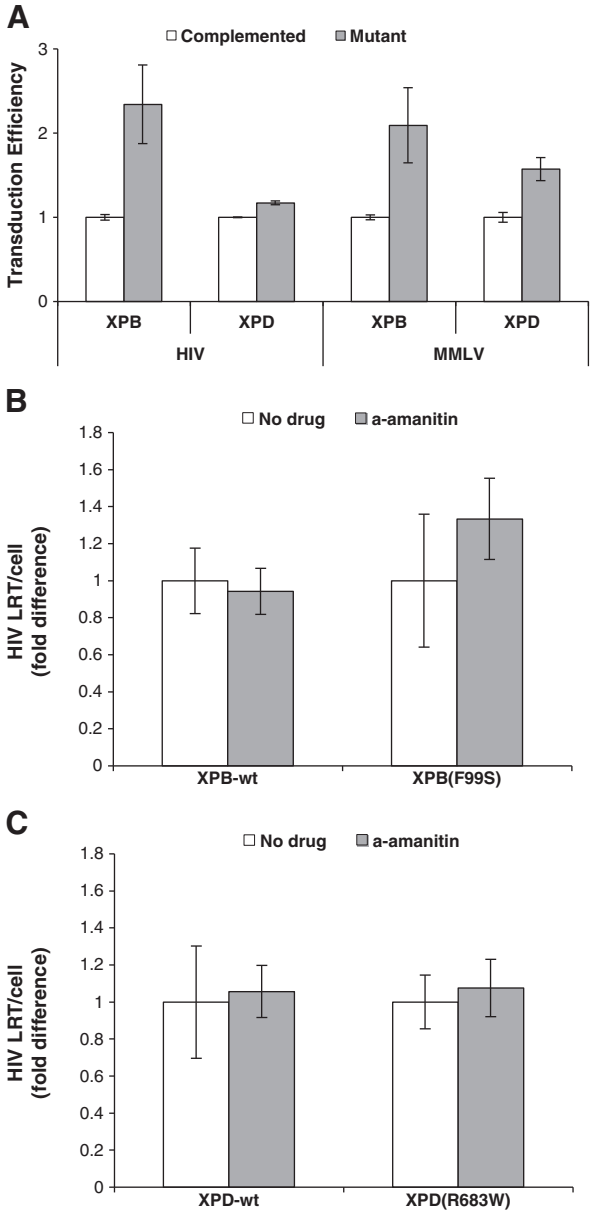

Fig. 1. Retroviral infection of wild type and NER mutant cell lines. (A) An equal number of NER mutant (XPB(F99S) and XPD(R683W)) and complemented cells (XPB-wt and XPD-wt) were infected with HIV or MMLV retroviral vectors expressing GFP following integration to the host genome. At 72 hpi the cells were analyzed by flow cytometry for GFP expression. Transduction efficiency is expressed relative to the complemented cells expressing the wild type gene. (B and C) HIV late reverse transcripts in wild type and NER mutant cells treated with the transcription inhibitor α-amanitin. An equal number of wild type and NER defective cells were treated with the RNA polymerase II inhibitor α-amanitin for 2 h and infected with an HIV based retroviral vector in the continued presence of α-amanitin (a-amanitin). DNA was collected at 6 h after the addition of HIV. HIV late reverse transcripts (LRT) and the cellular 18S gene were measured by qPCR. The quantity of HIV LRT was divided by the number of cellular genomes to obtain the number of HIV LRT per cell (HIV LRT/cell). (B) XPB-wt and NER defective XPB(F99S) cell lines. (C) XPD-wt and NER defective XPD(R683W) cell lines. Data from α-amanitin treated cells is expressed relative to untreated cells (No drug). Error bars indicate the standard deviation between triplicates at two MOI in three independent experiments.