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Understanding the molecular manipulation of DCAF1 by the lentiviral accessory proteins Vpr and Vpx

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ABSTRACT

Vpr and Vpx are primate lentivirus proteins that manipulate the cellular CRL4 ubiquitin ligase complex. While Vpr is common to all primate lentiviruses, Vpx is only encoded by HIV-2 and a limited range of SIVs. Although Vpr and Vpx share a high degree of homology they are known to induce markedly different effects in host cell biology through the recruitment of different substrates to CRL4. Here we explore the interaction of HIV-1 Vpr and SIVmac Vpx with the CRL4 substrate receptor DCAF1. Through mutational analysis of DCAF1 we demonstrate that although Vpr and Vpx share a highly similar DCAF1-binding motif, they interact with a different set of residues in DCAF1. In addition, we show that Vpx recruits SAMHD1 through a protein–protein interface that includes interactions of SAMHD1 with both Vpx and DCAF1, as was first suggested in crystallography data by (Schwefel, D., Groom, H.C.T., Boucherit, V.C., Christodoulou, E., Walker, P.A., Stoye, J.P., Bishop, K.N., Taylor, I.A., 2014. Structural basis of lentiviral subversion of a cellular protein degradation pathway., Nature, 505, 234–238).

Introduction

Many viruses encode proteins that manipulate the cellular ubiquitin proteasome system (UPS) to degrade cellular restriction factors. Human Immunodeficiency Virus type-1 (HIV-1) encodes three such proteins (Vif, Vpu, and Vpr). In addition, some primate lentiviruses (including HIV-2 and SIVmac) encode the Vpr paralog, Vpx. While the mechanism of UPS manipulation and the cellular targets of Vif and Vpu have been well characterized, the functions of Vpr and Vpx have been more difficult to elucidate (reviewed in Guenzel et al. (2014), Malim and Emerman (2008) and Romani and Cohen (2012)). In 2011, two groups independently identified SAMHD1 as the cellular protein targeted by Vpx (Hrecka et al., 2011; Laguette et al., 2011). More recently, Laguette et al. (2014) proposed that Vpr activates the SLX4 complex in a ubiquitin dependent manner.

Vpr is a short, 96-amino acid protein that is highly conserved among primate lentiviruses, which is expressed late during viral replication and is present in virions (Cohen et al., 1990; Müller et al., 2000). Vpr and its function appear to be crucial for HIV infection as no primary isolates have been described which lack Vpr (reviewed in Andersen et al. (2008)). While Vpr induces cell-cycle arrest at the G_2/M transition through the activation of the DNA damage sensor Ataxia Telangiectasia and Rad3-related protein (ATR) (Roshal et al., 2003), the significance of this cell cycle arrest in the virus life cycle remains unclear (reviewed in Andersen et al. (2008)). Recently, it was proposed that G_2 arrest is initiated by the Vpr-mediated activation of the SLX4, presumably generating aberrant damage to the host genome and activation of the cellular DNA damage response (Laguette et al., 2014).

In 1994, Vpr was shown to interact with a novel cellular protein (Zhao et al., 1994), subsequently named DDB1-Cullin 4-Associated Factor 1 (DCAF1). The significance of this interaction remained uncertain until DCAF1 was identified as a substrate receptor for the Cullin 4-RING E3 ligase (CRL4) (Angers et al., 2006; He et al., 2006; Higa et al., 2006; Jin et al., 2006). Subsequent studies demonstrated that induction of G_2 arrest by Vpr was dependent on the manipulation of $CRL4^{DCAF1}$ (Belzile et al., 2007; Dehart et al.,

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2007; Hrecka et al., 2007; Le Rouzic et al., 2007; Schröfelbauer et al., 2007; Tan et al., 2007; Wen et al., 2007).

In addition to Vpr, some primate lentiviral lineages encode the Vpr paralog, Vpx. Early studies identified a role for Vpx in the infection of myeloid lineage cells, dendritic cells and macrophages, by overcoming a block to viral reverse transcription (Yu et al., 1991; Goujon et al., 2008). Similar to Vpr, the Skowronski and Stevenson laboratories demonstrated that this Vpx-mediated effect requires the formation of a Vpx-CRL4^{DCAF1} complex (Sharova et al., 2008; Srivastava et al., 2008). In 2011, SAMHD1 was identified as the cellular protein targeted by Vpx, in the context of CRL4^{DCAF1} (Hrecka et al., 2011; Laguette et al., 2011).

Thus far, three mechanisms for viral directed ubiquitination of cellular proteins have been observed: 1) the encoding of a viral E3 ubiquitin ligase, as is the case of the ICP0 protein of Herpes Simplex 1 (Boutell et al., 2002; Everett, 2000); 2) the replacement of the substrate receptor of a cellular ubiquitin ligase by a virally encoded protein, as is the case of protein V from SV5 (Horvath, 2004) and Vif from primate lentiviruses (Mehle et al., 2004; Sheehy et al., 2003; Yu et al., 2003); and 3) mimicry of an endogenous substrate by the viral protein, which then ferries a cellular protein to be targeted for ubiquitination, as observed in the manipulation of CRL1$^{\beta TRCP}$ by HIV-1 Vpu to target CD4 (Bour et al., 2001; Margottin et al., 1998).

In this study we investigated the manner in which HIV-1 Vpr and SIVmac Vpx (hereafter referred to as "Vpr" and "Vpx", respectively) interact with DCAF1 resulting in the alteration of substrate specificity of CRL4^{DCAF1}. Using mutational analysis of the DCAF1 substrate-binding interface we found that although Vpr and Vpx share a highly homologous DCAF1-binding motif on their third alpha helix, Vpr and Vpx interact with CRL4^{DCAF1} using different residues on DCAF1. In addition, we identified the DCAF1 residues, D1092 which, when mutated, disrupted SAMHD1 degradation without impeding Vpx binding. Therefore, we surmise that the recruitment of SAMHD1 to DCAF1 by Vpx is mediated by a combination of residues in Vpx and DCAF1. Our results confirm and expand on a functional level the intermolecular interactions that were previously identified by Schwefel et al. (2014) via a co-crystal that included the DCAF1 WD40 domain, the C-terminal domain of SAMHD1 and Vpx.

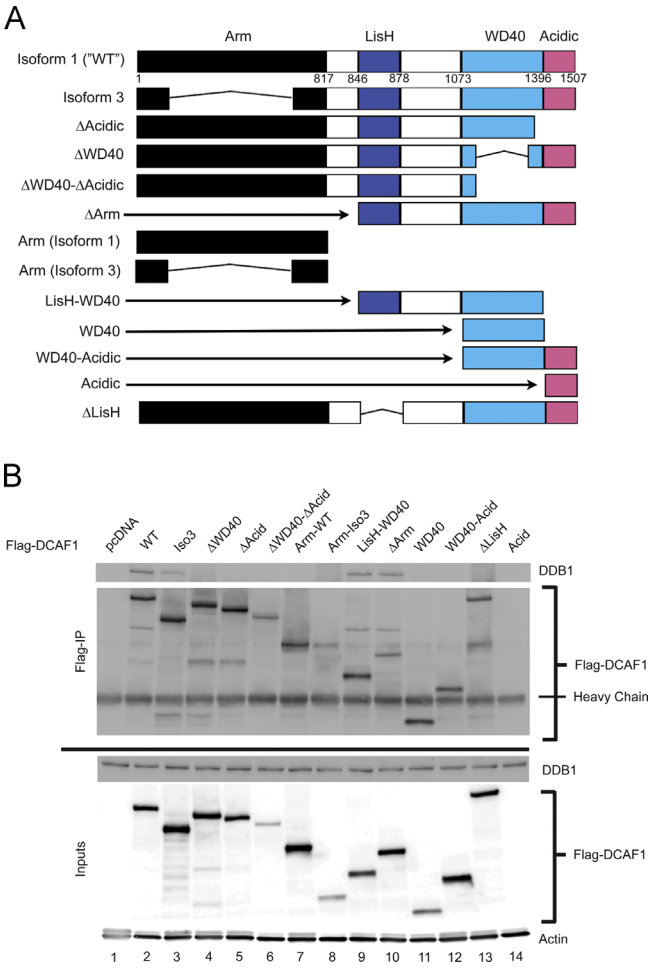

Fig. 1. The WD40 domain of DCAF1 is necessary for interaction with DDB1. (A) Domain architecture of WT DCAF1 (Isoform 1) and truncations. (B) 293FT cells were transfected with plasmids expressing FLAG-DCAF1 or the truncations described in (A). 36 h post-transfection cell lysates were subject to FLAG-immunoprecipitation (DCAF1) and analyzed for the presence of DDB1 by Western blot.

Materials and methods

Cell culture

Exponentially growing 293FT cells were cultured in Dulbecco minimal essential medium (DMEM; Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS) and 1% L-glutamine (Invitrogen). 293FT cells were transfected using the Calcium Phosphate method, as previously described (Zhu et al., 2001). Cells were harvested 36 h post-transfection, washed 2x with Phosphate Buffered Saline (PBS) and lysed as described below.

Plasmids

DCAF1 Iso1 cDNA (NCBI accession NM_014703) was amplified by PCR from a human cDNA library with the addition of an N-terminal 3x FLAG®-poly linker, then assembled into pCMV.Sport 6 (Invitrogen, Carlsbad, CA). DCAF1 truncations were made by generating unique restrictions sites which were indicated (Fig. 1A) using Quikchange Lightning site-directed mutagenesis (Agilent Technologies, Santa Clara, CA). DCAF1 WD40 point mutants were also generated via Quikchange. Myc-huSAMHD1 was purchased from OriGene (Rockville, MD). pcDNA3.1 was purchased from Invitrogen. HA-Vpr of HIV-1 and HA-Vpx of SIVmac were subcloned from pHR-HA-Vpr-IRES-GFP and pHR-HA-Vpx-IRES-GFP, respectively (Dehart et al., 2007) into pFIN-EF1-GFP-2a-mCherH-WPRE (a kind gift of Dr. Semple-Rowland) (Verrier et al., 2011) in substitution of the mCherry gene.

Immunoprecipitation and Western blots

For immunoprecipitation, cells were gently detached by incubation in PBS, pelleted and lysed with FLAG IP buffer (50 mM Tris HCl, pH 7.4, 15 0 mM NaCl, 1 mM EDTA and 1% TRITON X-100) in the presence of protease inhibitors (Complete EDTA free tablets; Roche, Indianapolis, IN). Lysate protein concentrations were determined by Pierce™ BCA (Thermo Scientific, Rockford, IL) and brought to equal protein concentration. Lysates were subjected to immunoprecipitation using Anti-FLAG® M2 Magnetic Beads (SIGMA-ALDRICH, St. Louis, MO). Briefly, lysates were incubated with beads for 2 h (at RT) to overnight (at 4 °C). Beads were washed 5x with lysis buffer and proteins eluted with 3x-FLAG® Peptide, 100 μg/ml, for 1 h at RT. Cells used in degradation assays were lysed in SET Buffer (1% SDS, 50 mM Tris HCL, pH 7.4, 1 mM EDTA); lysates were thoroughly denatured by boiling for 5 min. Lysates and immunoprecipitation samples were resolved by SDS-PAGE on 4–10% Criteron™ TGX™ gels (Bio Rad, Hercules, CA) as per manufacturer's recommendations and transferred to PVDF membrane (EMD Millipore, Billerica, MA). The following antibodies were used: