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Identification and functional characterization of Epstein-Barr virus encoded deconjugases

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av

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ABSTRACT

The post-translational conjugation or deconjugation of proteins by ubiquitin (Ub) or ubiquitin-like molecules (UbLs: e.g. SUMO, NEDD8, ISG15) has emerged as a major regulatory mechanism of various cellular activities. The controlled processes include protein transcription, translation, trafficking and degradation, signal transduction, replication and apoptosis. All modifier get covalently linked to their substrates via the cascade activity of three enzymes (E1, E2, E3). After execution of the modification mediated signaling, deconjuagases, belonging to the class of deubiquitinating enzymes (DUBs) or Ubl-specific proteases (ULPs), cleave the modifier of its substrate for possible recycling. Given the central role of the conjugation and deconjugation pathway in all aspects of cellular physiology, it is understandable that viruses have developed mechanisms to exploit those pathways for their own benefit. RNA and DNA viruses express their own E3 ligases or manipulate cellular E3 ligases to facilitate the modification of mostly cellular proteins, which, in the case of ubiquitination, often leads to their degradation. Also several viral encoded DUBs or ULPs have been described, e.g. facilitating the suppression of ubiquitination and ISGylation mediated antiviral effects.

Epstein-Barr virus (EBV) is a large double-stranded DNA tumor virus encoding ~100 open reading frames (ORFs). EBV belongs to the human herpes virus (HHV) family. The virus establishes latent infections in 90% of the human population worldwide and like other herpesviruses has a latent and lytic life cycle. EBV is associated with a variety of malignancies of lymphoid cells, like Burkitt´s lymphoma and Hodgkin´s lymphoma as also of epithelial cells, like nasopharyngeal carcinoma and gastric carcinoma.

The overall aim of this study was to identify and functional characterize EBV encoded deconjugases. We developed a bacterial screening assay based on the usage of Ub/Ubl-GFP reporter constructs. We screened an EBV-ORFeome library for their activity against Ub-, NEDD8-, SUMO-1,-2,-3 and ISG15-GFP reporter. As a result we discovered that the BSLF1- and BXLF1-ORF comprised deubiquitinating activity. We were further able to confirm the earlier described deubiquitinating function of the BPLF1-ORF. BPLF1 is a large tegumental protein, comprising DUB activity in its N-terminus, which was described for all of its HVV-family homologues. In our screening assay we could also detect that BPLF1-N cleaves the NEDD8-GFP reporter with similar efficiency as the Ub-GFP reporter. Following this observation we could show that BPLF1-N was able to process Ub- and NEDD8-linked functional probes with similar efficiency suggesting equal affinities towards ubiquitinated and neddylated substrates. We could show that BPLF1-N binds to and deneddylates cullins, which are assembled in cullin-RING ligases (CRLs). This CRL deneddylation facilitated the stabilization of their substrates involved in cell cycle regulation. Those accumulated BPLF1-N controlled CRL substrates were essential for an S-phase like cellular environment and endoreduplication in BPLF1-N expressing cells. We further demonstrated that the impact of BPLF1-N expression on viral genome replication was dependent on stabilization of the DNA licensing factor CDT1.