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Epstein-Barr Virus encoded deconjugases
and
AmotL2 in control of cell topology

AKADEMISK AVHANDLING
Som för avläggande av medicine doktorsexamen vid Karolinska Institutet
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av Sebastian Hildebrand

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**ABSTRACT - EPSTEIN-BARR VIRUS ENCODED DECONJUGASES**

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. Epstein-Barr virus (EBV) is a large double-stranded DNA tumor virus encoding ~100 open reading frames (ORFs). The overall aim of this study was to identify and functional characterize EBV encoded Ub- or UbL-deconjugases. 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 could also detect that the large tegumental protein 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.

**ABSTRACT - AMOTL2 IN CONTROL OF CELL TOPOLOGY**

During developmental morphogenesis, cells migrate, differentiate and organize into multicellular structures. As a distinct step in organ formation, epithelial cells join together via cell-cell junctions to form sheets of cells that separate cellular compartments from each other. Endogenous forces, generated by contractile actin, are transmitted over cell-layers in part by the connection to adhesion junctions and E-cadherin. These forces affect cellular geometry (cell size and shape) and topology (connectivity among cells in a tissue). Exactly how E-cadherin connects to the actomyosin network has been less clear.

In our work, we show that the scaffold protein AmotL2 p100 binds to the adherens junction components E-cadherin and MAGI1 and associates to contractile actin fibers which connect cells over multiple layers. Silencing of AmotL2 in epithelial cells in vitro and in zebrafish keratinocytes in vivo resulted in loss of actin filaments perpendicular to cellular junctions and dramatic changes in cellular geometry. As a consequence, the packing of epithelial cells in the typical hexagonal patterns was severely perturbed and the ability to form 3-D structures was lost. Cells depleted of AmotL2 also showed increased fluidity and elasticity when subjected to mechanical force. We propose that AmotL2 is a critical component in the adhesion junctions that controls intracellular contractility as well as relaying forces between cells.