A Comparative interactomics study reveals the involvement of the Ubiquitin Proteasome System in influenza A viruses infection

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Introduction

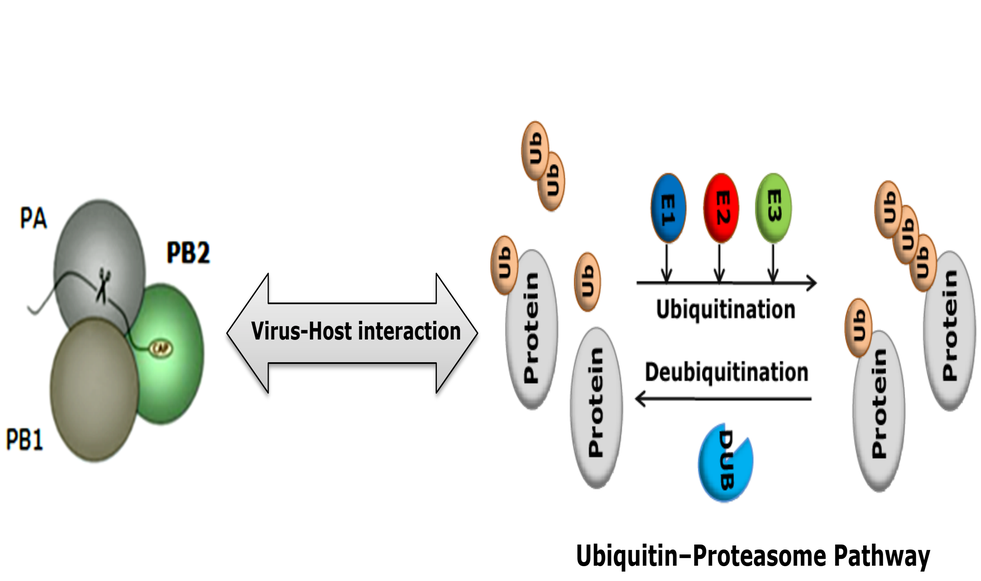

Figure 1: Influenza A viruses (IAV) polymerase - Host Ubiquitin Proteasome System (UPS) Interplay. The PB2 subunit of the trimeric RNA dependent RNA polymerase of IAV is known to be a major interface between the viral polymerase and the host proteome. The host UPS is critical in regulating diverse cellular functions, therefore is a prime target of several viruses.

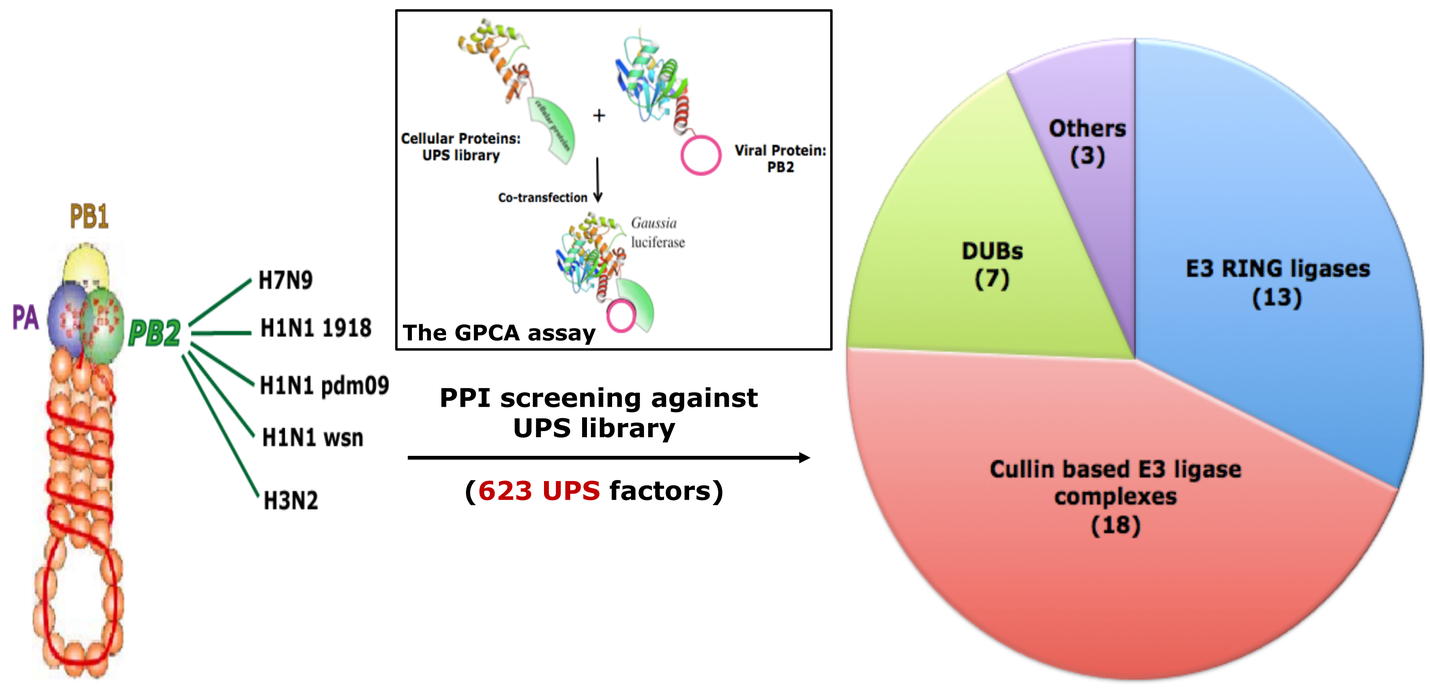

Figure 2: Interaction mapping of the PB2 sub-unit of IAV polymerase with UPS library. 41 UPS factors are found to interact with PB2 from five different strains of IAV when screened against an UPS library using Gaussia princeps luciferase Protein Complementation Assay (GPCA). Among these PB2-interacting UPS factors, Cullin-based E3 ligase complexes are the most prevalent targets of PB2.

Results

siRNA mediated depletion of PB2-UPS targets resulted in significant reduction of virion production

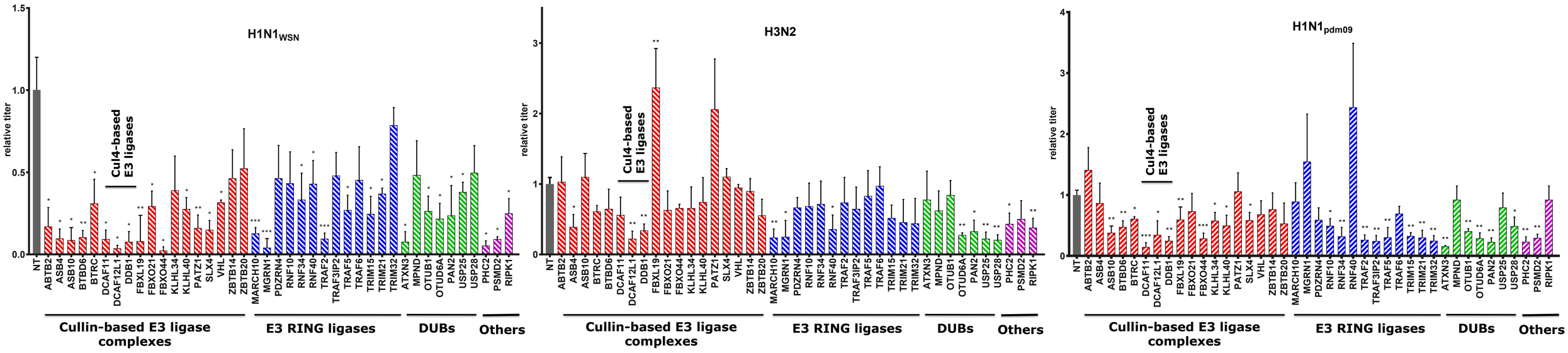

Figure 3: Functional evaluation of the involvement of UPS-PB2 partners in $H1N1_{WSN}$, H3N2 and H1N1pdm09 infection. A549 cells were transfected with Non-Target (NT) or UPS targeting siRNA for 48 hours, then infected by 0,0001 moi of $H1N1_{WSN}$, or 0,001 moi of H3N2 or $H1N1_{pdm09}$ for 24 hours. Viral titers were determined by plaque forming assay and the ratio to NT are given. Factors belonging to Cullin-based E3 ligases (18), E3 RING ligases (13), DUBs (7) and others (3) are grouped as indicated. Data represent means ±s.e.m of three independent experiments, p values were calculated with a two-tailed non-parametric Student's *t*-test, with * indicating 0.05>p>0.033, **0.033>p>0.002, ***0.002>p.

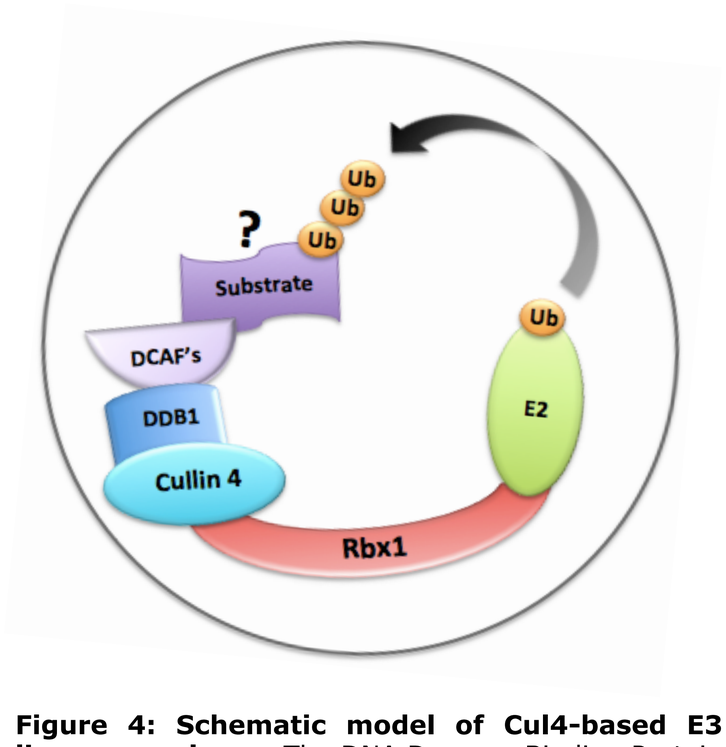

Figure 4: Schematic model of Cul4-based E3 ligase complexes. The DNA Damage Binding Protein 1 (DDB1) interacts with the N-terminal of the Cul4 scaffold, and acts as an adaptor between Cul4 and the DCAFs. DCAFs are substrate recognition factors, which specifically recognize the substrate of ubiquitination. The RBX protein brings the ubiquitin molecule close to the substrate through binding to the Cul4 and E2 conjugating enzyme.

CUL4-based E3 ligases interact with different domains of PB2

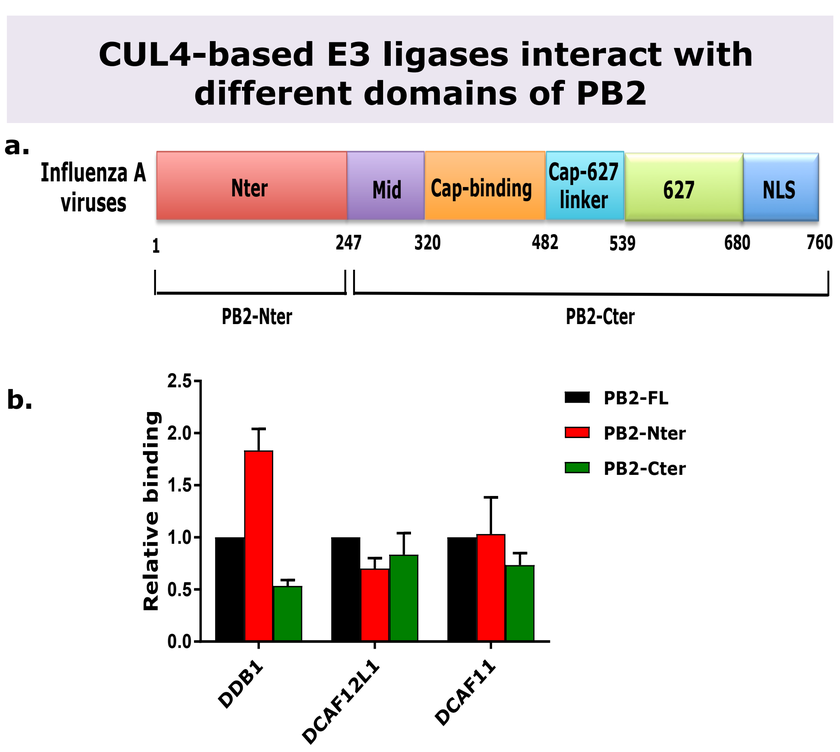

Figure 5: Interaction of Cul4-based E3 ligases with different domains of PB2 protein. Schematic diagram of PB2 domains in different Influenza A viruses. N-ter & C-ter represents 1/3 and 2/3 region of the PB2 respectively (a). Identification of DDB1, DCAF12L1 and DCAF11 binding site with full-length (FL), N-ter and C-ter of PB2 using GPCA. The ratio to FL are given and data represents the mean ±s.e.m of triplicates (b).

DCAF12L1 & DDB1 bind to polymerase subunits PB2 and PA

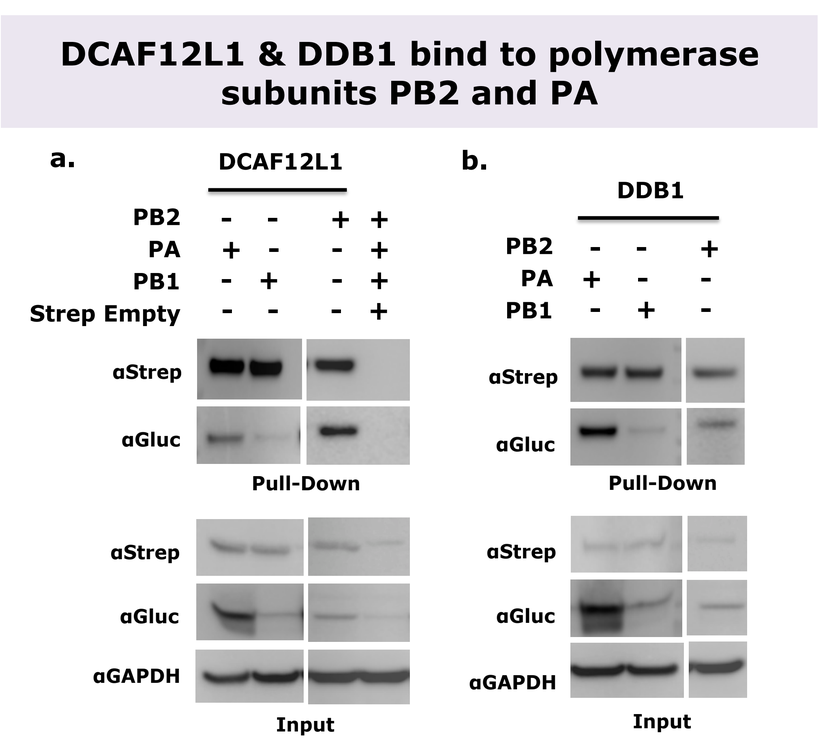

Figure 6: Interaction of DCAF12L1 & DDB1 with influenza polymerase proteins. The binding of DCAF12L1 (a) or DDB1 (b) with polymerase proteins were detected in Co-Immunoprecipitation. Cells were transfected with Gluc-tagged polymerase proteins and Strep-tagged DCAF12L1 or DDB1 proteins which were precipitated with streptavidin beads and immunoblotted as shown. Equivalent amount of input were probed as indicated.

Conclusions

- Knockdown of identified PB2 interacting UPS factors significantly affects viral production in a strain-independent or strain-specific manner.
- The PB2 protein interacts with several factors belonging to the Cul4-based E3 ligase complex machinery such as DDB1 and DCAF's.
- DDB1 binds to the N-ter domain of PB2 whereas the DCAF's might dock into the interface bewteen N-ter and C-ter domain of PB2.
- The interaction of DDB1 and DCAF12L1 with the polymerase subunits (PB2, PA) indicates their potential role on viral polymerase activity.

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