The interactome of the Epstein-Barr virus nuclear antigen 5 suggests novel roles in RNA and protein metabolism

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Avhandlingen baseras på följande arbeten:

I. **Epstein-Barr virus nuclear antigen 5 inhibits pre-mRNA cleavage and polyadenylation.**
Dufva, M., **Flodin, J.**, Nerstedt, A., Rüetschi, U., and Rymo, L.
Nucleic Acids Res. (2002) May;30(10):2131-2143

II. **Identification of intracellular proteins associated with the EBV-encoded nuclear antigen 5 using an efficient TAP procedure and FT-ICR mass spectrometry.**
Forsman, A., Rüetschi, U., **Ekholm, J.**, and Rymo, L.
Journal of Proteome Research (2008) Jun;7(6):2309-2319

III. **Epstein-Barr virus nuclear antigen 5 is a multi-functional protein with a possible role in the chaperone-mediated protein folding and ubiquitin-proteasome degradation systems.**
Ekholm, J., Forsman, A., Kashuba, E., Andersson, M. K., Rüetschi, U., and Rymo, L.
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The interactome of the Epstein-Barr virus nuclear antigen 5 suggests novel roles in RNA and protein metabolism

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Epstein-Barr virus (EBV) is a human herpes virus that infects B and epithelial cells of the oropharynx. EBV is transmitted by saliva and establishes a lifelong latency in over 90% of the world's population. During latency, the virus exists predominantly as multicopy episomes in the nuclei of memory B cells. If these memory B cells are activated they can produce EBV virions and the person may shed the virus. Most people are exposed to the virus as children, when the disease produces no noticeable symptoms or only flu-like symptoms. Latent infection is associated with several malignancies, including Burkitt's lymphoma, Hodgkin's disease, nasopharyngeal carcinoma, and lymphoproliferative disorders in the immunosuppressed patients. A compromised immune system and an aberrant EBV latent gene expression are thought to play roles in the aetiology of EBV malignancies. EBV immortalizes human B cells through expression of at least 12 viral genes, which include the EBNA5 protein. Elucidation of EBNA5 functions has been guided by identification of interacting cellular and viral proteins. The functions of these cofactors implicate EBNA5 as a potential modulator of apoptosis, cell cycle processes, and transcriptional pathways. This thesis will add to the knowledge about how EBNA5 contributes to EBV biology. By using luciferase and CAT as model proteins to study EBNA5-regulated gene expression in lymphoid cells, we found that EBNA5, at high but still biologically relevant levels, acted as a promiscuous repressor of gene expression from transfected plasmids. However, EBNA5 was more selective in the regulation of chromosomal genes; only two out of 588 human genes, thereof the pro-apoptotic BNIP3 gene, were down-regulated. The decrease in expression from transfected plasmids was partly explained by the ability of EBNA5 to inhibit luciferase pre-mRNA 3´-end cleavage and polyadenylation. To gain further insight into molecular pathways in which EBNA5 may have a regulatory role, we searched for cellular targets for EBNA5. By using an improved tandem affinity purification procedure, we identified 147 novel interaction partners of which 37 were validated with independent methods. The validated proteins could be grouped into three main classes depending on their biological function: I) protein folding and degradation, II) pre-mRNA processing, and III) ribosome biogenesis. We further showed that EBNA5 is part of high molecular complexes. Of particular interest, we verified interaction between EBNA5, Hsp70, and BAG2, a co-chaperone with the ability to inhibit ubiquitinylation, and as a consequence, protein degradation of Hsp70 clients. Further studies on the effect of EBNA5 on luciferase activity demonstrated a correlation between luciferase activity and level of soluble luciferase protein. In addition, substantial amounts of insoluble luciferase had accumulated in the nuclei. Insolubilisation was accompanied by translocation of luciferase, EBNA5, Hsp70, and BAG2 to the nucleoli and to a small number of nuclear foci. An EBNA5 mutant (ΔCR3) was neither able to induce a similar translocation nor able to associate with BAG2. Therefore, we identified BAG2 as a major target of EBNA5. Moreover, over-expression of Hsp70 blocked EBNA5-induced insolubilisation of luciferase. Our results indicate that EBNA5 is likely to play a regulatory role in the decision between degradation and folding.

Keywords: *Epstein-Barr virus, EBNA5, chaperone, proteasome, nucleolus*
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