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M-2. The mechanisms of YB-1 nucleocytoplasmic translocation

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The Y-box binding protein 1 (YB-1) is a DNA and RNA binding protein that performs numerous functions both in the cytoplasm and in the nucleus. Its nuclear localization has been observed at the G1/S cell cycle boundary [1], in stress conditions of various types [2-5], and under adenovirus infection [6]. The sequence of YB-1 contains a nuclear localization signal (NLS) [7] which is responsible for its nuclear translocation. The YB-1 NLS is recognized by transportin 1 [8] and classified with signals of the PY-NLS type characterized by the presence of an N-terminal positively charged or hydrophobic cluster, the residue R, and the C-terminal dipeptide PY. For better understanding of NLS functioning, we generated a few forms of YB-1 with mutations in its NLS region, where major structural elements of NLS were modified. We compared subcellular localization of the WT YB-1 with that of its mutant forms both in normal growth conditions and with stimulation of its translocation to the nucleus. Methods: The mutations were performed by site-directed mutagenesis. Transfection of eukaryotic cells was made according to the manufacturer's recommendations. Results: The comparison of subcellular localization of WT YB-1 with that of its mutant forms showed that only the removal of the entire NLS resulted in the loss of the nuclear translocation ability of YB-1. The removal or replacement of separate structural elements of the NLS did not prevent YB-1 translocation to the nucleus either in normal growth conditions or under stimulated translocation. Conclusions: There is increasing evidence in the literature that some PY-NLS found in various proteins interact with transportin 1. Besides, transportin 1 can recognize PY-NLS lacking the dipeptide PY [9-12]. The YB-1 NLS has a highly charged N-terminal cluster which presumably makes the greatest contribution to the interaction with transportin 1.

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M-3. HIV 1 Tat induces cell type specific expression of host genes in B cells

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In European countries and North America, high rates of Burkitt lymphoma are found preferentially among patients infected with HIV. Interestingly, HIV is mostly associated with B cell lymphomas although it does not infect B cells. Oncogenesis in HIV infected patients may be connected with the action of a small viral protein Tat, which is able to exit infected T cells and enter other cells via its cellpenetrating domain. As Tat affects expression of host cell genes, we hypothesized that Tat protein could affect expression of B cell genes; this could trigger lymphomagenesis in HIV infected patients. To analyze the effect of HIV 1 Tat, we developed B cell lines ectopically expressing Tat protein. To discover genes that are regulated by Tat, total RNA was collected and RNA seq-based differential expression analysis was performed. We identified six KEGG pathways affected by Tat including virus response, cytokine cytokine receptor interaction pathway, ubiquitin mediated proteolysis pathway. Unexpectedly, when comparing genes modulated by Tat in B cells with those deregulated by Tat in T cells, we observed just a small overlap between the two sets. In conclusion, Tat protein appears to behave differently in B cells and T cells, exploiting distinct mechanisms to generate a specificenvironment in different tissues.

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N-1. Large-scale distinct H2A and H2B redistribution detected in live Jurkat cells after Doxorubicin

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We used confocal microscopy to detect prefixed immunfluorescently labeled histones and laser scanning cytometer to detect histone aggregation. Results: Using Confocal Microscopy (CLSM), we observed marked differences between the effect of doxorubicin (Dox), applied in a concentration range between 0.6-36 μM, on the intracellular distribution of H2A vs. H2B in Jurkat cells. Aggregation was assessed by Laser Scanning Cytometry (LSC), via the retention and consequential accumulation of histones in permeabilized nuclei of cells showing no signs of apoptosis. Aggregation was observed only in the case of H2A, while the dominant effect of the anthracyclin on H2B was the massive accumulation of the histone in the cytoplasm concommitant with its disappearance from the nuclei. The latter phenomenon was not affected by inhibitors of protein and RNA synthesis, by Tanespimycin, an hsp70 inhibitor, and by leptomycin B, a nuclear export inhibitor. On the other hand, cytoplasmic accumulation was completely diminished by PYR41, an inhibitor of ubiquitylation,