Electron microscopy in analysis of mouse polyomavirus interactions with cellular structures.

ABSTRACT:

The mouse polyomavirus (PyV) is a small non-enveloped tumorogenic DNA virus. Its genome encodes six gene products, three early antigens (large, middle and small T antigens) and three structural proteins (VP1, VP2 and VP3). We used electronmicroscopic approaches to analyse the ultrastructural changes of mouse fibroblasts after PyV infection and to find out cooperation of the virus with cellular components during virion assembly and release of virus progeny from the cells. In late phases of infection, we observed increase of protein synthesis, indicated by the swollen rough endoplasmic reticulum. In addition, the compact nucleoli showed transformed character of infected cells. Localization of virus progeny suggested that cell nucleoli could play a role in virion assembly. We also found giant mitochondria in the cytoplasm of infected cells that confirm the induction of initial steps of apoptosis processes. However, in the late times of infection we observed necrosis in a majority of the cells probably caused by cytotoxic effect of the PyV structural proteins, whereas apoptosis observed already in early phases, could be induced by transformation ability of T antigens or, partly by defence of the cells against infection.

Immunoelectron microscopy revealed acumulation of H1 histon around the sites of virion assembly. It proves its elimination from the mature virions. We also localized the pleiotropic transcription regulator, YY1, in the proximity of virus progeny deposits. This finding support our hypotesis of a role of YY1 in morphogenesis of the polyomavirus.

We also prepared *in vitro* system, containing PyV genome, the major capsid protein VP1, and protein YY1, to test a role of YY1 in virion assembly. Interactions were analysed by spreading method in reduction conditions to prevent VP1 pentamer assembly. For elimenation of reduction agens from the reaction mixture, we constructed a recombinant plasmid for expression of VP1 gene with deletion of the last 31 C terminal amino acids, responsible for interpentameric contacts.

Besides, we made an ultrastructure analysis of mouse cells after their treatment with C35 synthetic peptide (prepared at UOCHAB). This peptide consisted of last 35 amino acids of the common C terminal part of VP2 and VP3. This sequence has similar

composition as protein transduction domains. Mammalian cells were incubated with the peptide and changes in their structure were analysed on ultrastructural level in dependence on incubation time. We have shown that peptide C35 causes damage of the cytoplasmatic membrane and induces apoptosis in short time (5-15 minutes) post adsorption. The peptide is internalised probably by both clathrin dependent and caveolae dependent endocytosis. After 30 minutes of incubation, some cells were regenerated, probably by elimination of the peptide.