

Mouse polyomavirus (MPyV) is small non-enveloped DNA virus. Although this virus has been studied for almost 60 years, it still remains unclear, how can virus transport its genetic information to the cell nucleus. Also, the mechanism of virion morphogenesis is not well understood. First part of this work is focused on endocytic pathway which is used by MPyV for trafficking toward the cell nucleus. Using dominant negative mutant of caveolin-1 we showed that caveolin-1-dependent endocytic pathway, described for SV40, is not used by MPyV for productive infection. MPyV is transported to early endosomes. Acidic milieu of endosomes is indispensable for productive infection. Preventing virus localisation into early endosomes (dominant negative mutant of Rab 5 GTPase) or endosomes alkalinisation (by ammonium chloride or bafilomycin A1) led to dramatic decrease of virus infectivity. Alkalinisation of endosomes entailed retention of MPyV in early endosomes. It indicates that virus is further transported to late endosomes. Finally, we confirmed by FRET that MPyV is in perinuclear space localized into recycling endosomes.

Another poor characterized process is virion morphogenesis. To characterize the participation of cellular proteins in virion precursor complexes, nuclear as well as whole-cell lysates of infected cells or cells transiently expressing VP1 were separated by blue native polyacrylamide gel electrophoresis (BN-PAGE). Several VP1 positive protein complexes were identified. Some of these complexes contained proteins from the heat shock protein 70 family. Although the interaction between VP1 and heat shock proteins has been described previously, this is the first time that BN-PAGE has been shown to detect several different forms of VP1-Hsp 70 complexes. None of the VP1-positive bands was abundant enough to be analysed by mass spectrometry. Therefore, we created plasmids which allow expression of VP1 fused with BioEase Tag (Invitrogen) at its N or C terminus. This tag ensures *in vivo* biotinylation and purification by affinity chromatography of fusion protein. Complexes of fusion proteins and cellular proteins were isolated by affinity chromatography and composition of the complexes were analysed by mass spectrometry. Cellular proteins Hsp 90 α , GAPDH and keratin type I were identified. Confirmation of interaction between VP1 and these cellular proteins as well as their roles in virion assembly and virus life cycle remains to be elucidated.

Key words: mouse polyomavirus, early endosome, VP1 protein, blue native polyacrylamide gel electrophoresis, Hsp 70, protein-protein interactions