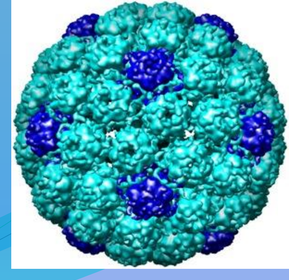
Study of mouse polyomavirus minor capsid proteins

Faculty of Science, Charles University in Prague, Vinicna 5, Prague, Czech Republic

S. Huerfano, A.Kozmanová, O. Vít, V.Žíla and J.Forstová



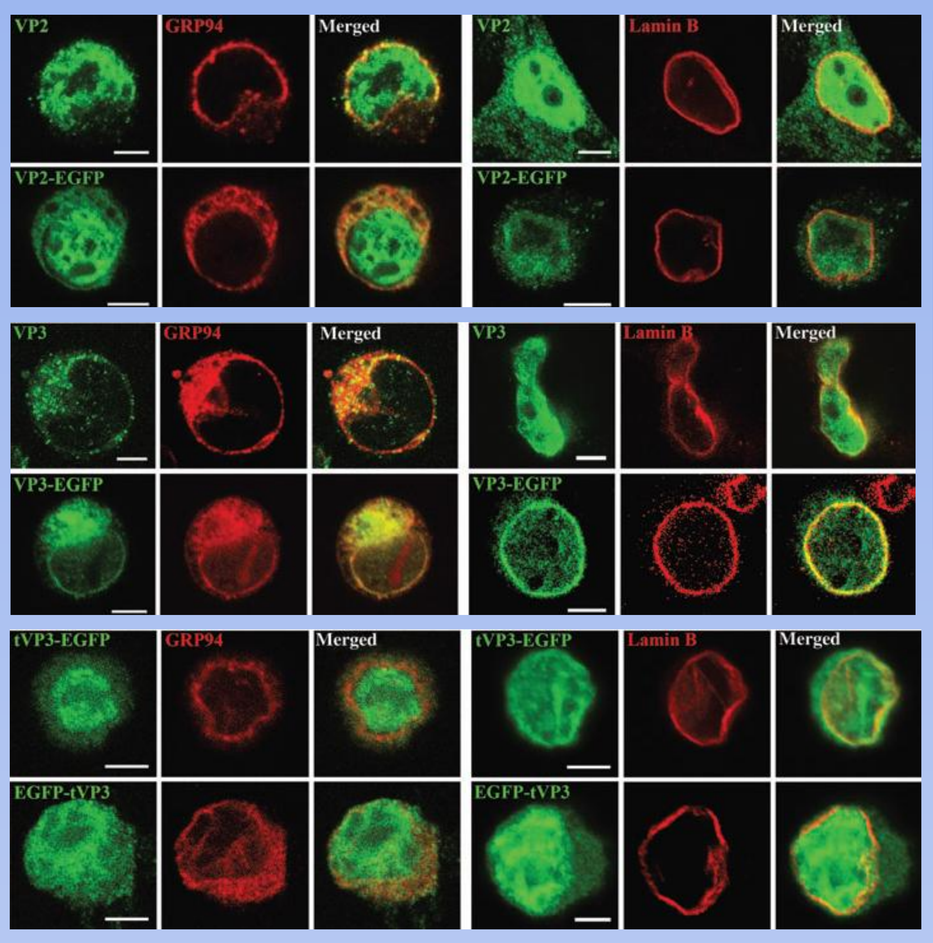
Introduction

Capsids of Polyomaviruses, small tumorigenic DNA viruses, are composed of 72 pentamers of the major capsid protein, VP1. Each VP1 pentamer is associated with one molecule of the minor capsid protein VP2 or its shorter variant - VP3. Mutated virions lacking either VP2 or VP3 lose infectivity but the function of the minor structural protein is not clear. Three hydrophobic domains (1–3) for VP2 and two domains (2 and 3) for VP3 were predicted in VP2/VP3 sequence, which may play important role in protein functions.

Aim

To contribute to understanding functions of the minor structural proteins of mouse polyomavirus, VP2 and VP3 in the virus life cycle.

1. To reveal cell localization and properties of MPyV minor capsid proteins during their transient expression in mouse fibroblasts and the extent and character of cytotoxicity induced by them.
2. To create plasmids for expression of VP2 and VP3 with deleted individual putative hydrophobic domains for studies of their involvement in interactions of the proteins with membranes.
3. To study possible oligomerisation of VP3 protein by bimolecular fluorescence complementation and by immunoprecipitation.



Localization of VP2, VP3 and their fusion variants in transfected 3T3 cells. Selected confocal microscopy sections of 3T3 cells, 4 h post-transfection, are presented. Cells were stained with antibody against the GRP 94 ER marker, or with lamin B (red). Minor structural proteins were stained with anti-VP2 / 3 IgG (green), and EGFP fused variants were enhanced with anti-VP2 / 3 IgG (green). (A) VP2 and its EGFP variants. (B) VP3 and its EGFP variants. (C) EGFP variants of tVP3. Bars, 5 lm.

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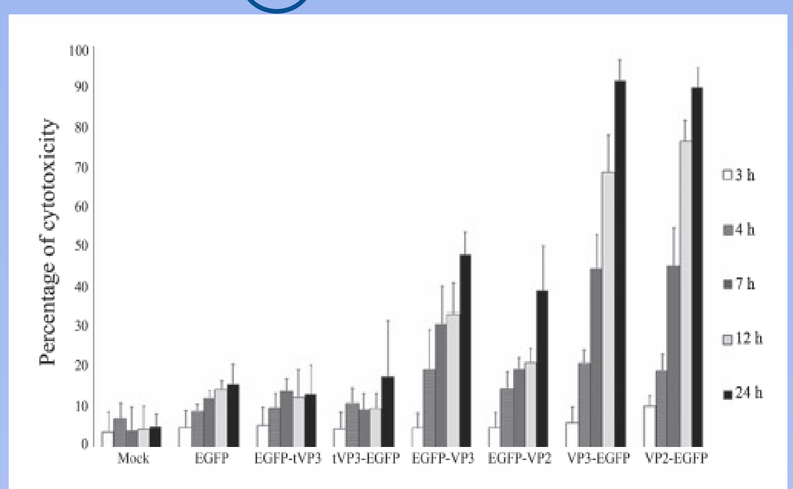
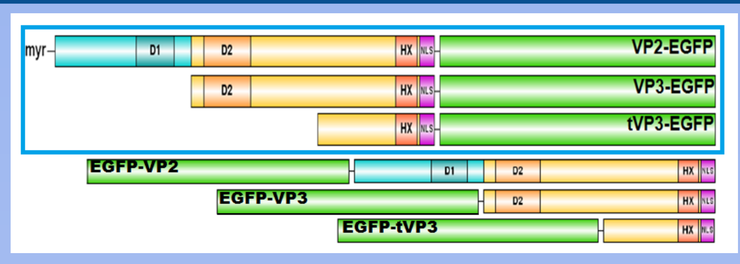
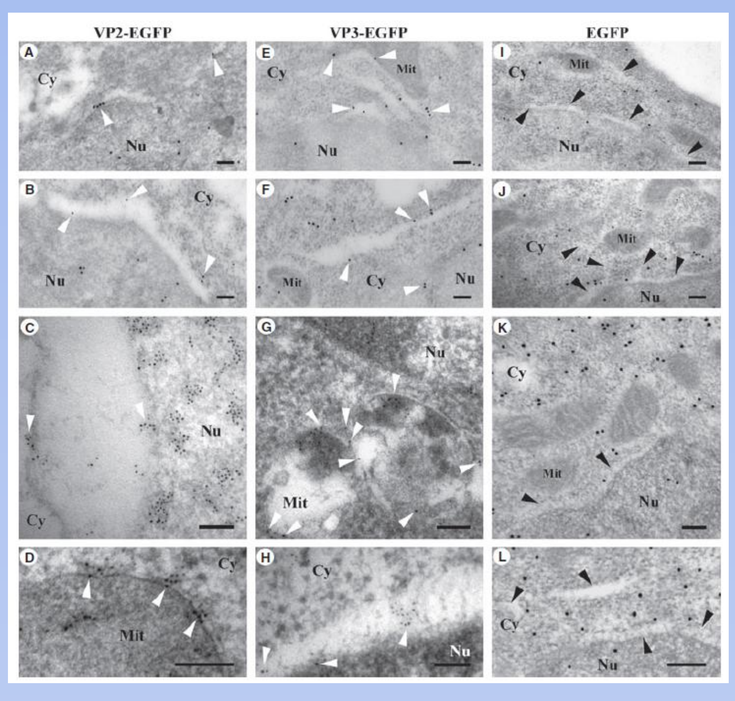


Cytotoxicity of VP2 or VP3 fusion proteins. Cytotoxicity of individual protein variants transiently expressed in 3T3 cells was followed by measuring LDH leakage from transfected cells into the medium at the indicated time-points post-transfection. Values are presented relative to that of LDH release obtained by treatment of cells with 9% Triton X-100 (=100%). Data represent mean values measuring duplicates of three independent experiments. Mock-transfected cells were used as a negative control.

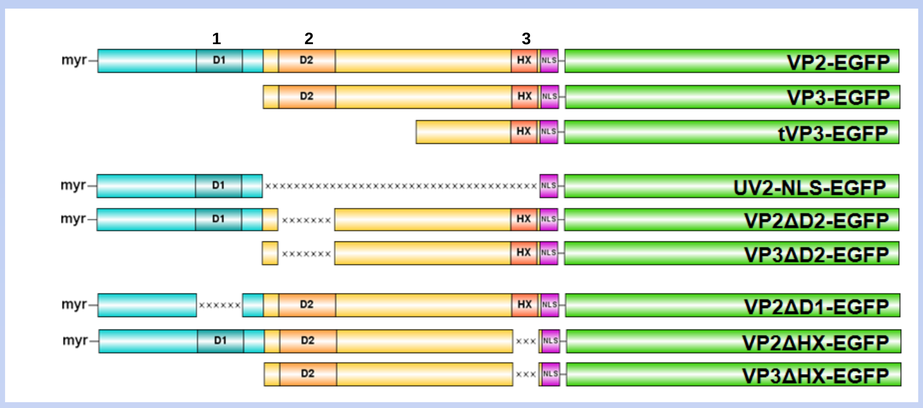
Immuno-electron microscopy on ultrathin sections of 3T3 cells expressing VP2–EGFP, VP3–EGFP or EGFP only. Cells were fixed 5 h post-transfection. Fused minor capsid proteins were detected by incubation of cell sections with anti-GFP IgG followed by incubation with the secondary antibody conjugated with 10-nm gold particles (A, B, E, F, I–L) or 5-nm gold particles (C, D, G, H). Selected gold particles are indicated by white arrowheads. Black arrowheads indicate ER cisternae on sections of cells expressing EGFP only. Bars 100 nm. Cy, cyto- plasm; Mit, mitochondria; Nu, nucleus.

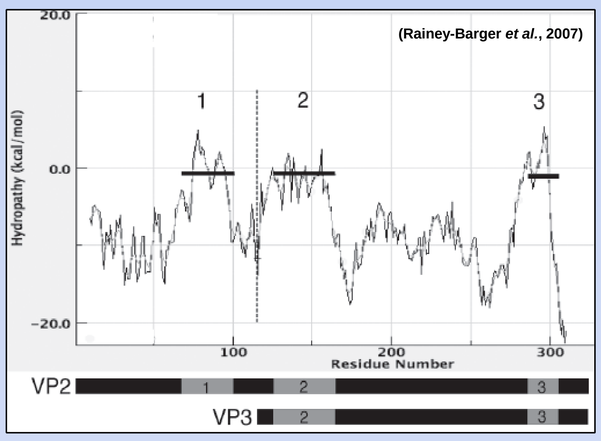


Plasmids for expression of EGFP fusion variants of VP2 or VP3 or VP3 truncated from its N-terminus were prepared and their cytotoxicity, cellular localisation and affinity to intracellular membranes examined using confocal microscopy and FACS analysis.



Result 1: Wildtype VP2 and VP3 appeared to be highly cytotoxic and exhibited, besides nuclear localization, evident affinity for the nuclear envelope and the ER. Similar findings were observed with the minor capsid proteins fused with EGFP at their C-terminus (VP3–EGFP and VP2–EGFP). By contrast, the minor structural proteins fused with EGFP at their N-terminus (EGFP–VP2 and EGFP–VP3) as well as both fusion variants of tVP3 (the hydrophobic domain 2 cut out) had substantially lower affinity or no affinity to membranes and were much less cytotoxic . EM pictures of cells at early timepoints post-transfection (5h) were obtained, showing the presence of VP2–EGFP and VP3–EGFP on the membranes of a swollen ER and also on damaged mitochondria. VP3–EGFP was seen to be associated with the nuclear membrane, often located between the inner and outer layers.



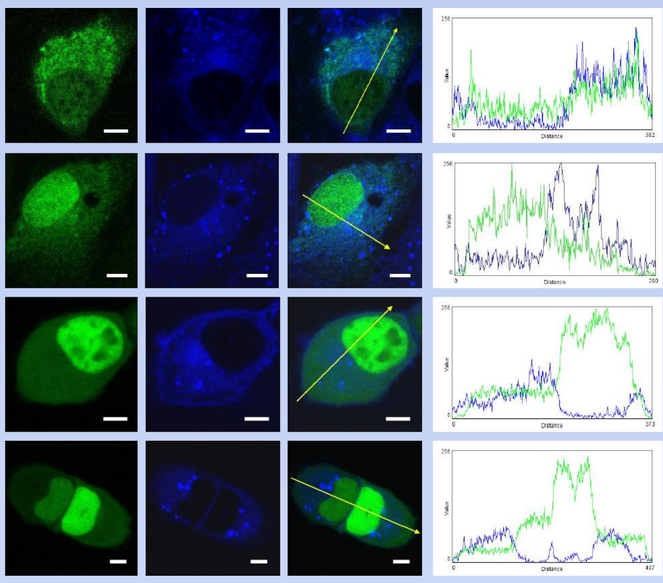


2.

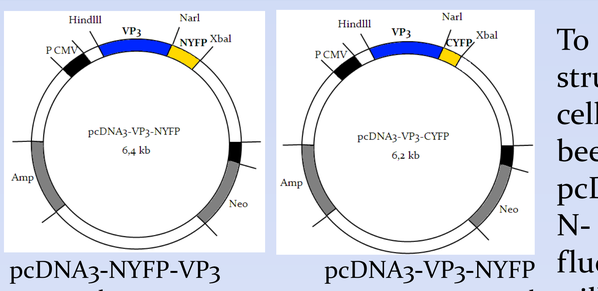
✓	membrane affinity observed
×	no membrane affinity observed
☠	cytotoxicity observed
↓	low cytotoxicity observed
↓↓	very low cytotoxicity observed
?	data not available

	Membrane affinity	toxicity
VP2-EGFP	✓	☠
VP3-EGFP	✓	☠
tVP3-EGFP	×	↓↓
EGFP-VP2	×	↓
EGFP-VP3	×	↓
EGFP-tVP3	×	↓↓
UV2-NLS-EGFP	×	↓
VP2ΔD1-EGFP	?	?
VP2ΔD2-EGFP	×	?
VP3ΔD2-EGFP	×	?
VP2ΔHX-EGFP	?	?
VP3ΔHX-EGFP	✓	☠

Result: Localization of UV2-NLS-EGFP and VP2ΔD2-EGFP protein was mainly nuclear, only low level of EGFP fluorescence was seen in the cytoplasm. Confocal microscopy analysis revealed that neither UV2-NLS-EGFP nor VP2ΔD2-EGFP had affinity to intracellular membranes. Deletion of hydrophobic domain 2 lead to loss of affinity to intracellular membranes. Localization of VP3 ΔD2-EGFP protein was similar to that of both VP2 mutants and also, VP3 protein with deleted membrane domain 2 did not possess affinity to cellular membranes.

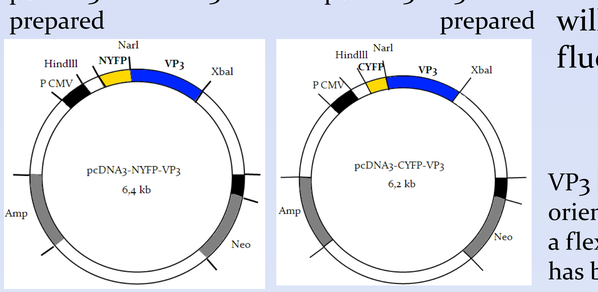


Localization of mutated proteins fused with EGFP in living mouse fibroblasts 3T3. Individual cross section by confocal microscope. Green fluorescence of proteins fused with EGFP , blue are membranes stained with 1,6-difeny-1,3,5-hexatrien. In the third column is overlap of both signals. Graphs in right column illustrate intensity of blue and green signals, which was measured by yellow arrow direction, illustrating overlap of both signals at pictures.



pcDNA3-NYFP-VP3 prepared

pcDNA3-VP3-NYFP prepared



pcDNA3-VP3-CYFP

pcDNA3-CYFP-VP3

To examine wheather MPyV minor structural proteins form oligomers on cellular membranes, VP2 or VP3 have been inserted into pcDNA-NYFP, or pcDNA-CYFP plasmids to be fused with N- or C-terminal part of the yellow fluorescent protein (YFP). Constructs will be exploited for bimolecular fluorescence complementation .

VP3 has been introduced to the plasmids in both orientation in respect to YFP.. Into each plasmid, a flexible linker between VP3 and YFP sequences has been introduced.

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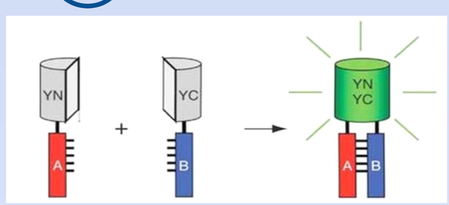

BiFC- bimolecular fluorescent complementation – an investigation method for precise visualization of protein-protein interactions based on association of two yellow fluorescent protein fragments.

Conclusion

The minor proteins linked to EGFP at their C-terminus (VP2–EGFP, VP3–EGFP) were found to display properties similar to their non-fused, wild-type version. They induce apoptosis of mouse 3T3 cells. Despite of nuclear localization signal, a substantial subpopulation of VP2–EGFP and VP3–EGFP was detected in the cytoplasm, co-localizing with intracellular membranes. Toxicity results suggest that VP2–EGFP and VP3–EGFP possesses properties similar to those of natural VP2 or VP3 and truncation of the N-terminal part of VP3 decreases its toxicity as well as its affinity to membranes. Examining of deletion mutants of VP2 and VP3 revealed the importance of the hydrophobic domain 2 for their affinity to cellular membranes.

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