Review of the doctoral thesis by Sandra Huérfano-Meneses, M.S., entitled "Studies of the mouse polyoma virus: Properties of the minor structural proteins, requirements for virion productive trafficking to the nucleus and observed side effects of DNA transfection.

The doctoral thesis of Sandra Huérfano-Meneses explores mainly the role of minor structural proteins of the mouse polyoma virus (MPyV), VP2 and VP3, in the interactions with the host cell and in the viral life cycle, virus trafficking to the nucleus and induction of interferon (IFN) beta by nucleofection. Accordingly, the thesis is divided in three distinct parts and each part was summarized in a paper, with two of them being published and one of them being in the process of preparation/submission. Sandra is the first author of the two published papers and a co-author of the third one. In addition to the papers related to the thesis topic, Sandra is the first author or a co-author of 7 papers published previously.

As mentioned above, the thesis is divided in three distinct parts that are preceded by a General introduction. Each part then contains a specific Theoretical background, Aims, Materials and Methods, Results, Discussion and Conclusions. The References are presented together for all three parts. In the end, the two published papers and one manuscript are presented in three Appendices. In total, the thesis counts 116 pages without appendices. It is well organized and carefully prepared, nevertheless misspelled words or confused formulations are occasionally found in the text.

I would have several questions and comments:
Ad I
Regarding the first part of the thesis which describes the effects of expression of MPyV minor proteins using different expression vectors with different tags and differences in the resulting cell death, I find very hard to reconcile different results obtained by different methods in various times post infection. Specifically, it is not clear to me in which instances Sandra refers to the cell lysis or necrosis and in which to apoptosis. Apparently, when she refers to a cell death, she mostly means cell lysis or necrosis; but this is not correct as necrosis and apoptosis are different types of cell death. On the other hand, Sandra is positively correlating cell toxicity characterized by LDH release with characteristics of apoptosis like activation and/or cleavage of caspase-9, cleavage or activity of caspase 3 or cleavage of a death substrate PARP (eg. in Figs. 13 and 14 or in Fig.17). Another problem is comparing methods based on cell lysates (like western blot analysis or enzyme activities) with methods based on analysis of single cells (like flow cytometry and Annexin V positivity or microscopy of individual cells).
- In summary, I would like Sandra to clearly summarize what is the predominant type of cell death at which time post infection by MPyV and how it is affected or modified by MPyV minor proteins. Further, I would like her to distinguish between the toxicity and the primary and secondary necroses.
- I would like Sandra to discuss possible explanations for different intracellular localization and different types and kinetics of cell death caused by N- and C- terminal tagged VP2 and VP3. Could there be also of importance the availability or the accessibility of a putative nuclear localization signal? Further, as both proteins localize to the ER, did you explore or speculate about activation of capase-4 or 12 in addition to caspase 9, cytochrome c release or changes in intracellular levels of calcium?
- Since the minor proteins of MPyV seem to reveal their own effects on the host cell and possibly in the early phases of infection, I think it would be appropriate to ask if they should be classified as structural proteins or if they might be considered as functional proteins.

Ad III

Regarding the third part of the thesis which analyses the induction of interferon beta by transfection of DNA,
- I would like Sandra to summarize the time course and proves of causality of events following the nucleofection – production of ROS, DNA damage, IFN beta expression, and NF-kappa B translocation. NF-kappa B is a redox sensitive transcription factor, while both nucleofection and IFN beta might induce production of ROS.
- NF-kappaB is a transcription factor involved in expression of many proteins that could be themselves involved in additional signaling cascades. Consequently, I would like Sandra to comment on the possibilities how to prove if activation of NF-kappaB is really involved in the induction of IFN beta in this system.
- I have to disagree with the conclusion that the vectors with mutated ATG would be the best negative control for transfection, as protein expression, folding and other processing could induce additional effects like consumption of ATP or ER stress by missfolded, overexpressed proteins. It might be difficult to find the appropriate control sequence, but I believe, protein expression should occur also.
- I would like to add two additional comments.

In the early 90's of the last century, Dr. John Lewis from SUNY, HSCB made the observation that transfection of DNA is inducing interferon. In that time, he probably used the classical calcium phosphate method of transfection, possibly also dextran.

During a lytic infection of epithelial cells with vaccinia virus, we have observed processing of caspase-3 and formation of various dimmers from its cleaved products that was dependent on the type of insertion vector used to make a recombinant vaccinia virus (we made a conclusion that a presence of Lac operon and/or expression of Lac I, not the recombinant protein, was critical; we did not analyze IFN beta expression, but vaccinia virus is in vitro resistant to type I IFN). Therefore, I would like to ask how much is the effect of any plasmid sequence likely to be affected by a concomitant expression of other genes.

Minor comments:
Ad. I
Method section:
- p. 26 Cytotoxicity determined based on 1 well lysate = 100% LDH release and 1 well LDH released in culture supernatant
- p. 27 Poor description of Flow cytometry analysis and of Quantification of caspase 3 activity and caspase inhibition assay.

Ad II
- p. 68 Fig. 5B Are there any statistically important differences?
- p. 72 Lines 11-12 …cell cytotoxicity by quantifying spontaneous LDH release (LDH is released only during cell death).

Ad III
- p. 102-103, Fig. 12 Translocation of NF-kappaB into the nucleus is suggestive, but not a direct prove of its involvement in IFN beta expression

In conclusion of my review, I recommend this thesis to be accepted as fulfilling the requirements for the title of Philosophiae Doctor.

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