Specific RNA- and protein-binding characteristics of the nucleoprotein of a South African rabies virus isolate

by

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Co-supervisor: Dr. J. Theron

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I declare that the thesis, which I hereby submit for the degree Ph.D. at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at another university.

JJacobs

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SUMMARY

Rabies is a highly fatal nervous disease of humans and all other warm-blooded vertebrates, and is generally transmitted by the bite of diseased animals, most commonly dogs and other carnivores. The aim of this investigation was to investigate the functional role that nucleoprotein phosphorylation might play in the ability of this protein to bind single-stranded RNA and to form complexes with the phosphoprotein (P). Towards achieving these goals, full-length cDNA copies of the N and P genes of a South African viverrid rabies virus isolate were cloned and characterized by nucleotide sequencing. Comparison to cognate rabies virus sequences indicated high levels of homology and a high degree of conservation with regard to functional domains. Analysis of the N and P proteins furthermore indicated the presence of potential consensus recognition sites for both protein kinase C and casein kinase II.

Both the N and P genes were subsequently expressed in the BAC-to-BAC™ baculovirus expression system. Expression of the P protein by the recombinant baculovirus yielded a soluble protein, but the recombinant baculovirus expressed N protein was insoluble. During dual expression of the N and P proteins, which has been reported to aid in N protein solubility by N-P protein complex formation, no complex formation could be shown with the assays used. A contributing factor may have been the low expression

levels for the respective proteins. To overcome this, the N and P genes were consequently expressed in *Escherichia coli* using recombinant pGEX expression vectors. Using this approach, both the N and P proteins were expressed as soluble GST fusion proteins that could be purified by glutathione affinity chromatography to a level of homogeneity.

By making use of a commercially available PKC enzyme an *in vitro* phosphorylation kinase assay was developed whereby the bacterial-expressed unphosphorylated recombinant rabies virus proteins could specifically be phosphorylated. This is the first report of the rabies virus N protein being phosphorylated by PKC as previously identified phosphorylated amino acid residues are located within the consensus recognition motifs correspond to casein kinase II.

The ability of the *in vitro* phosphorylated rabies virus N protein to bind to ssRNA was investigated by poly(U)- and poly(A)-Sepharose binding assays. It was shown that the *in vitro* phosphorylated N protein displayed a higher affinity for ssRNA when compared to the unphosphorylated version of the protein. The phosphorylated rabies virus N protein did not exhibit a preference towards the A-rich artificial ssRNA substrate. To investigate the role of N protein phosphorylation on its ability to bind to P protein, different combinations of phosphorylated and unphosphorylated rabies virus N and P proteins were used in a protein-protein binding assay. The results indicated that unphosphorylated versions of these proteins formed complexes having a 2:1 stoichiometry of N to P protein. *In vitro* phosphorylation of the P protein resulted in the N-P protein complexes with a stoichiometry of 1:2 of N to P protein, whether the N protein independent of the phosphorylation status of the N protein. In the event of unphosphorylated P protein complexed to the phosphorylated N protein, a N:P ratio of 1:1 was found.

COMMUNICATIONS

Congress contributions:

1. J. Theron, J.A. Jacobs and L.H. Nel. The role of phosphorylation in RNA binding of the rabies virus nucleoprotein and in nucleoprotein-phosphoprotein interaction. *XIth Congress of Virology,* Sydney, Australia, August 1999. (Poster).

2. J.A. Jacobs, L.H. Nel and J.Theron. Characterization of the phosphorylation of rabies virus Nucleo- and Phosphoproteins. *17th Congress of the South African Genetics Society*, Pretoria, South Africa, June 2000. (Paper).

3. J.A. Jacobs, L.H. Nel and J. Theron. Genetic characterization of the Nucleo- and Phosphoprotein of rabies virus. *BioY2K Millennium Congress*, Grahamstown, South Africa, January 2000. (Poster).

4. J.A. Jacobs, L.H. Nel and J. Theron. Analysis of the *in vitro* phosphorylation of the Nucleoprotein and Phosphoprotein of rabies virus. *BioY2K Millennium Congress*, Grahmstown, South Africa, January 2000 (Poster).

5. J.A. Jacobs, J. Theron and L.H. Nel. RNA- and protein-binding characteristics of the phosphorylated Nucleoprotein of rabies virus. *Journal of Virology.* (In preparation).