ANNOUNCEMENT: Interested members of the University Community are invited to attend the Final Oral Examination for the Degree of Doctor of Philosophy of

Yang Liu

of the Department of Molecular and Cellular Biology on Friday, July 10, 2015 at 9:30 a.m. in SSC 1511

Thesis Title: Functional analysis of the baculovirus AcMNPV me53/ME53

Examination Committee: Dr. A. Bendall, Molecular and Cellular Biology (Chair)
Dr. P. Krell, Molecular and Cellular Biology
Dr. D. Theilmann, Agriculture and Agri-Food Canada
Dr. R. Mosser, Molecular and Cellular Biology
Dr. R. Clem, Kansas State University

ABSTRACT

Yang Liu, M.Sc. Co-Advisors: Dr. Peter Krell / Dr. Éva Nagy

Autographa californica multiple nucleopolyhedrovirus (AcMNPV) is a baculovirus in the family Baculoviridae, genus Alphabaculovirus. The AcMNPV me53 is one of the immediate early genes expressed immediately following AcMNPV infection and is highly conserved in all sequenced lepidopteran baculovirus genomes. Me53 is transcribed both early and late post-infection. Although me53 is not essential for viral DNA synthesis, it greatly attenuates infectious budded virus (BV) production when deleted. ME53 is associated with the nucleocapsid on both budded virus and occlusion-derived virus, but not with the envelope. ME53 co-localizes in plasma membrane foci with the envelope glycoprotein GP64 in a GP64 dependent manner. Despite lack of a reported nuclear localization signal (NLS), ME53 localizes in the cytoplasm early post-infection, and translocates to the nucleus late post-infection, indicating that ME53 itself does not have an intrinsic NLS. To map determinants of ME53 that facilitate its nuclear translocation, recombinant AcMNPV bacmids
containing a series of ME53 truncations, internal deletions and peptides fused with HA or GFP tags were constructed. Intracellular localization identified that residues within amino acids 109 to 137 at the N-terminus of ME53 act as the nuclear translocation sequence (NTS) to facilitate its nuclear transport in the late phase. Since ME53 translocates to the nucleus, and has a conserved zinc-finger domain at its C-terminus which is usually considered to be related to transcriptional regulation, a possible nuclear function of ME53 was also investigated. The transcript levels of select viral early and late genes were quantified by qRT-PCR in both wildtype bacmid transfected cells and me53 knockout bacmid transfected cells. The presence of me53 increased the transcription of viral immediate early genes, genes encoding viral RNA polymerase subunits, and genes required for virus production. Moreover, the most highly up-regulated genes are late genes essential for viral nucleocapsid assembly and egress, suggesting that ME53 may function as a transcription factor in the nucleus to regulate the expression of viral early and late genes. Furthermore, since ME53 translocates to the nucleus and also co-localizes with the viral major envelope protein GP64 at the plasma membrane, this study investigated the possible chaperone proteins ME53 recruits to facilitate its translocation to either site. Either the HA or V5 epitope tag was fused to ME53 to help identify its potential binding partners in immunoprecipitation (IP) assays. Viral major envelope protein GP64 and major capsid protein VP39 were detected as ME53 potential binding partners that might facilitate its translocation to either the plasma membrane or the nucleus.

**CURRICULUM VITAE:**

Yang studied at Sun Yat-sen University (Guangzhou, China), where she received her Bachelor of Science degree in Biotechnology in 2008 and her Master of Science degree in Microbiology in 2010. She began her Ph.D. program in the Fall of 2010 in the lab of Dr. Peter Krell.

**AWARDS:**

- University of Guelph International Graduate Scholarships in 2012 and 2013

**PUBLICATIONS:**

- Yang Liu¹, Jondavid de Jong², Éva Nagy², David A. Theilmann³ and Peter J. Krell¹. Nuclear translocation sequence and regions in AcMNPV ME53 important for optimal baculovirus production (recently submitted to the Journal of Virology)