

**Stony Brook University  
The Graduate School**

Doctoral Defense Announcement

**Abstract**

**Impact of Mutating a Single Conserved Nucleotide between the Cloverleaf and Internal Ribosome Entry Site on Biological Properties of Poliovirus and Coxsackievirus A20**

By

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The chemical synthesis of poliovirus was published in 2002. An unexpected characteristic of synthetic poliovirus type 1 (Mahoney) [sPV1(M)] was its high degree of attenuation in *CD155* transgenic (tg) mice. In a subsequent study by our laboratory, the attenuated phenotype of sPV1(M) in *CD155* tg mice was found to correlate with a marked temperature-sensitive phenotype in human cells of neuronal origin. Genetic analyses clearly indicated that the mutation (A<sub>103</sub>G) – one of 27 mutations originally engineered into the sPV1(M) genome as a means of differentiating it from *wt* PV1(M)— determined both observed phenotypes and maps to a spacer region between the cloverleaf and the internal ribosome entry site in the 5' non-translated region of the genome. Sequence alignment of this spacer region of various polioviruses and other members of the C-cluster of human enteroviruses (HEV-C) revealed complete conservation of the A residue at position 103. This observation prompted us to explore the effect of this single nucleotide substitution (A<sub>103</sub>G) on the growth phenotype *in vitro* and pathogenicity *in vivo* of coxsackievirus A20 (CAV20), the HEV-C strain displaying the greatest sequence homology to the polioviruses. Moreover, the current climate surrounding the eradication of poliovirus combined with the possibility that a closely related virus may fill the niche left vacant by poliovirus led us to expand our analysis of the effect of this single nucleotide mutation to PV1(M)/CAV20 chimeras. Our observations indicate that the A<sub>103</sub>G mutation exerts an effect on the biological properties of CAV20 that is less pronounced than that previously observed for PV1(M). Furthermore, in the context of PV1(M)/CAV20 chimeras, even though the affinity of the various genome segments studied is suggestive of a directionality of compatibility, the effect of the A<sub>103</sub>G mutation remains discernible. Collectively, our findings reveal the importance of the 103 locus in determining the biological properties of PV1(M) and CAV20 and confirm that the overall architecture of the 5' non-translated region significantly influences pathogenesis.

**Date:** March 30<sup>th</sup>, 2007

**Time:** 9:30 AM

**Place:** Life Sciences, Rm. 038

**Program:** Molecular Genetics & Microbiology

**Dissertation Advisor:** Eckard A. Wimmer, Ph.D.