NANYANG TECHNOLOGICAL UNIVERSITY

School of Biological Sciences

SBS Seminar Announcement

Next-Gen virology: Use of microfluidics and live-cell imaging to study poliovirus replication at the single-cell level

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Abstract

For nearly two decades my laboratory has focused on the viral RNA-dependent RNA polymerase (RdRp) as a target for development of antiviral therapeutics with broad-spectrum efficacy and for development of an enzyme mechanism-based strategy for viral attenuation and vaccine development using poliovirus (PV) as our primary model system. Our earliest studies pointed to nucleotide incorporation fidelity of the RdRp as a target for drug and vaccine development. Using a combination of biochemical, biophysical and computational approaches, we have developed a model for nucleotide selection by the RdRp that involves toggling of the nucleotide-binding site between nucleotide binding-occluded and nucleotide binding-competent states. A mutation that favors the binding-competent state creates a mutator polymerase and an attenuated virus capable of eliciting a protective immune response in a small-animal model. Using the plaque-forming unit (pfu) to quantify virus and the one-step growth experiment as a measure of viral fitness, the mutator virus did not exhibit a phenotype when compared to wild-type virus in cell culture. One reason for this observation was that pfu is, itself, a phenotype, with the number of genomes per pfu varying between viruses thereby masking phenotypes. Another reason for this observation was that at a high multiplicity of infection, the conditions used for the one-step growth experiment, complementation occurs, thereby suppressing growth phenotypes. Collectively, these observations highlighted the need for a platform to study single virions infecting single cells to mimic the circumstance encountered by a virus in vivo. We will present the current fruits of our labor using a nano-well, valve-based, on-chip microfluidic device to monitor single, virus-infected cells. This approach reveals phenotypes in vitro masked by conventional methods, as well as a remarkable level of between-cell variability. We will discuss the implications of our findings on the design and execution of virology experiments in the future.

Thursday, 16 Oct 2014 2.00pm to 3.00pm SBS Classroom 2 (SBS-01n-22)

Host: A/Prof Yoon H.S.

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