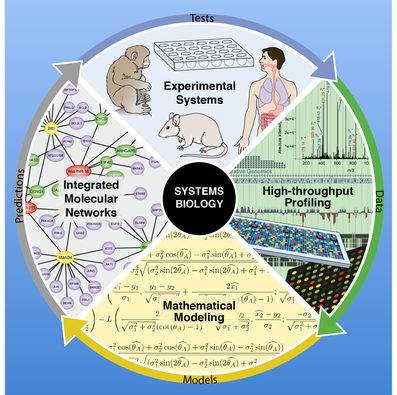
SYSTEMS BIOLOGY, HIV DISEASE, AND RNAs BIG AND SMALL

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INTRODUCTION

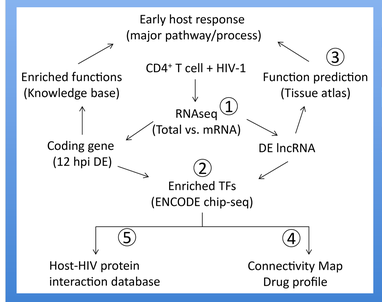


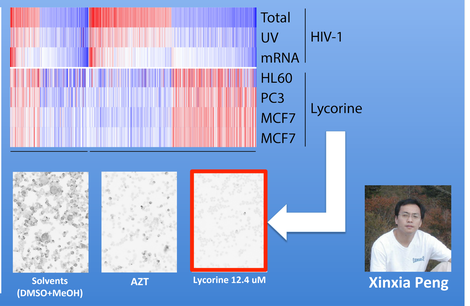
KATZELAB

As high-throughput technologies allow ever broader and deeper views into the intricacies of biological systems, the ability to work with numbers, networks, and vast amounts of data has become essential to deciphering biological complexity. We are using systems biology approaches to understand and model integrated views of virus-host interactions, host-response networks, and viral pathogenesis. This information is being used to identify molecular signatures of disease and to discover new targets and drugs for antiviral therapy as well as identify correlates of immunity in nonhuman primate evaluations of new strategies for HIV vaccines. Here, we describe studies aimed at gaining a systems-level view of SIV vaccine efficacy. We utilize multiple experimental models and new high-throughput technologies, such next-generation RNA sequencing (RNA-seq), in addition to advanced computational and mathematical methodologies for data analysis.

3 SYSTEMATICALLY CATALOGING HIV INFECTION ASSOCIATED TRANSCRIPTS AND USING THE HOST RESPONSE TO PREDICT POTENTIAL THERAPEUTIC CANDIDATES

Polyadenylated mature mRNAs are the focus of standard transcriptome analyses. However, the profiling of nascent transcripts, which often include non-polyadenylated RNAs, can unveil novel insights into transcriptional regulation.





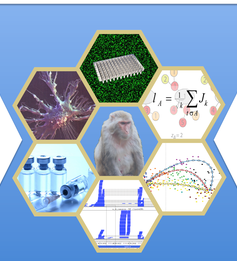
Xinxia Peng

- Many non-polyadenylated RNAs were differentially expressed upon HIV-1 infection
- 8 times more differentially expressed genes at 12 hours post-infection by Total RNAseq than by mRNAseq.
- We identified drugs capable of reversing these HIV-1 induced expression changes.
- Our results show that combined total and mRNA transcriptome analysis can provide a framework for identifying candidate drugs for host-directed therapy against HIV/AIDS.

6 NHP FUNCTIONAL GENOMICS CORE

The mission of the NHP Functional Genomics Core is to ensure standardization and comparability of functional genomics services and assays conducted for the Simian Vaccine Evaluation Unit (SVEU) and other DAIDS-supported preclinical NHP studies.

Nonhuman primate (NHP) models present an opportunity to test a variety of candidate AIDS vaccines, optimizing their ability to elicit immune responses and testing their ability to prevent infection or to control virus replication after virus challenge.

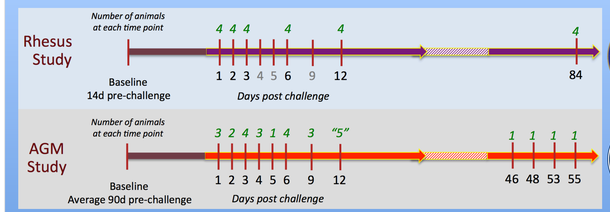


The mission of DAIDS is to ensure an end to the human immunodeficiency virus/ acquired immunodeficiency syndrome (HIV/AIDS) epidemic by supporting research that can lead to the development of therapies, vaccines, and prevention strategies.

We apply high throughput functional genomics approaches in these NHP studies to more effectively evaluate vaccine-induced adaptive and innate immune responses and vaccine efficacy in challenge/protection studies and identify host responses that predict vaccine efficacy.

For more information contact Lynn Law: gllaw@uw.edu

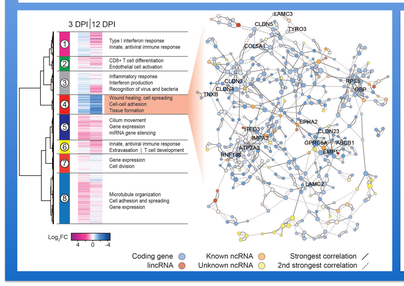
1 SIV ACUTE INFECTION MODEL





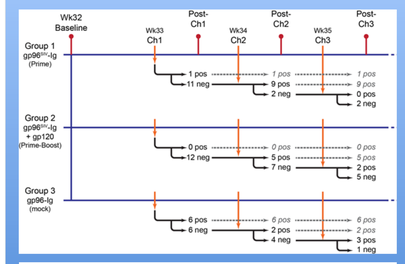
Fredrik Barrenas

- Identification of host response mechanisms that contribute to control of viral replication, and loss thereof
- Next generation sequencing of relevant tissues from pathogenic (rhesus macaques) and natural (African green monkeys) host species of nonhuman primates
- Animals sacrificed at 1, 2, 3, 6, 12 and 84 days post challenge



- Initial study of rhesus rectal mucosa taken during eclipse phase (3 DPI) and peak viremia (12 DPI)
- Strong transcriptional response at 3 DPI, with a market absence of association with immune activation
- Co-expression analysis of coding genes and noncoding RNA showed early down regulation of genes associated with cell morphology and cell adhesion, implying a link with loss of tissue integrity
- These genes were co-expressed with a high number of ncRNA
- Co-expression network analysis implicated genes associated with various pathologies of the gastrointestinal tract

4 gp96-Ig-SIV+gp120 VACCINE STUDY: EVOLVING HOST RESPONSE DURING A CELL-BASED SIV VACCINE TRIAL



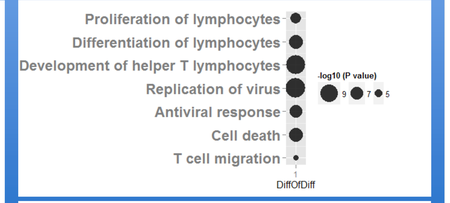
UNIVERSITY OF MIAMI



Christian Selinger

- Why did the (cell based gp96-Ig-SIV +gp120) vaccine trial fail to provide sustained protection over multiple challenges?
- What can whole blood RNA microarrays tell us about possible correlates of protection?

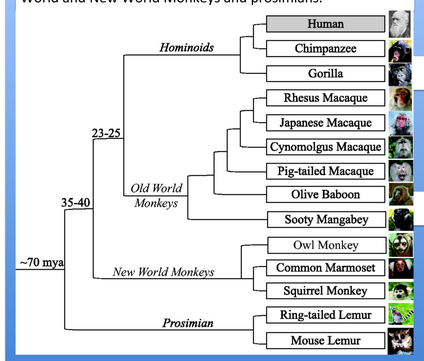
- Using whole blood RNA microarray analysis, we were able to identify vaccine specific pathway enrichment for macrophage activation and B Cell activating factors before challenge.
- Initial strong activation of innate antiviral immunity and subsequent T cell mediated host response with a moderate host cell expansion after consecutive challenges are key features of highly protected animals.
- For infected animals, vaccine specific differences are not apparent at peak viremia.
- The tight schedule of weekly challenges may be an important factor for impairing protection.

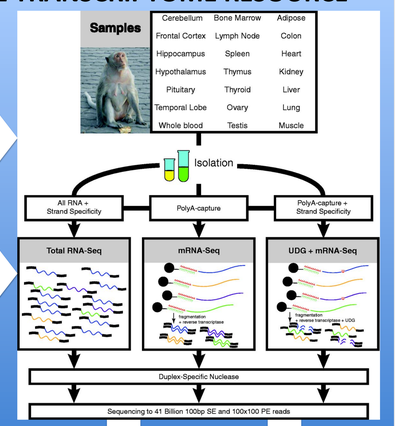


Gene expression analysis reveals that changes in protective host response in between challenges are driven by cell-mediated immune response

7 NONHUMAN PRIMATE REFERENCE TRANSCRIPTOME RESOURCE

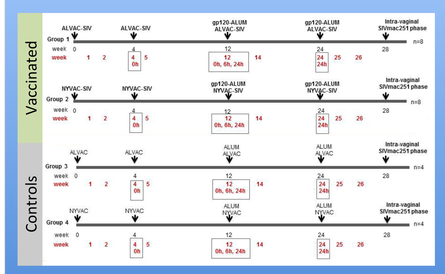
Species of the NHPRTR. Animals were chosen to represent large evolutionary distances, encompassing hominoid, Old World and New World Monkeys and prosimians.





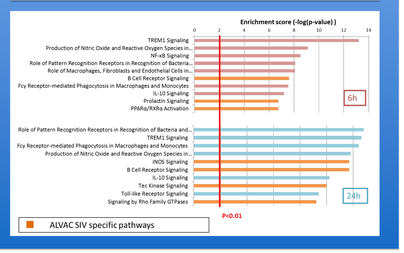
- Systematically derived more complete genome annotations using ultra-deep transcriptome sequencing data. The improved annotation added thousands of novel splicing isoforms to currently annotated genes as well as un-annotated intergenic transcripts.
- The identification of whole or partial un-annotated transcripts highlights the need for more genomic resources.
- Overall these results demonstrate that the ultra-deep RNA-seq data generated by NHPRTR are extremely valuable for improving NHP genome assembly and annotation.

2 ALVAC/NYVAC SIV VACCINE STUDY COMPARISON OF IMMUNOGENECITY AND EFFICACY OF A CANARYPOX VECTOR AND VACCINE VECTOR IN RHESUS



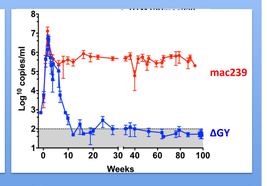
- Using a prime-boost combination of ALVAC-HIV and gp120, RV144 is the first HIV vaccine clinical trial to show efficacy
- To understand the immune correlates and inform future trials, we repeated this regimen in a rhesus model and contrasted two different pox vectors.
- Microarray analysis has been performed on the blood samples from the first day (6h and 24h) after prime immunization
- Efficacy test and comprehensive microarray analysis of the entire immunization phase is in process

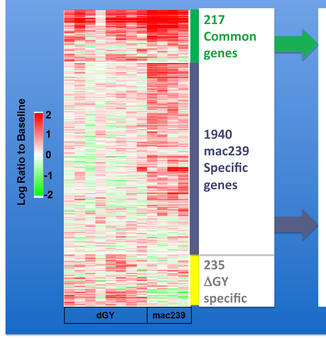
- As early as 6 hours after the initial immunization, the gene expression features of the ALVAC-SIV group were distinct from the animals receiving NYVAC-SIV
- By 24 hours post vaccination, ALVAC-SIV induced more differentially expressed (DE) genes than the other three groups relative to the pre-immunization baseline, and NYVAC-SIV and NYVAC control induced similar numbers of DE genes.
- The impact of these early events on the later outcomes will be determined once the protective efficacies of the two vaccines are determined.

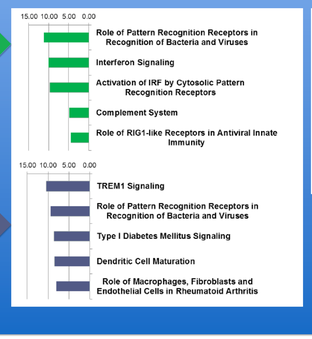


5 COMPARISON OF HOST RESPONSES TO THE INFECTION OF WILDTYPE SIVmac239 AND THE LIVE ATTENUATED VARIANT SIVmac239-ΔGY IN PIGTAIL MACAQUES

- PTMs are known to be highly susceptible to rapid and uniform progression to AIDS following wildtype SIV infection.
- Infection of dGY in PTMs results high acute peak of viremia that is comparable to SIVmac239.
- Most PTMs are elite controllers (19 out of 21)
  - Very low set point
  - Non-progressors
  - Little or no depletion of mucosal CD4+ T cells
  - Acquisition of protection against re-challenges by SIVmac239 and SIVE660.







- Despite comparable peak of viremia, dGY and mac239 induced three different clusters of DE genes at 9d
- Expression of ncRNAs showed opposite trend in PBMC of dGY and mac239 infected animals



Yajie Yang

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- WaNPRC (Brian Agricola, Michael Agy, Randy McLain)
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- Tulane National Primate Research Center, Covington, Louisiana (Andrew Lackner)

6
- **The NHP Functional Genomics Core** is housed in the Katze laboratory at the University of Washington

7
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