“How Can Systems and Computational Biology Impact Medical Counter Measure Development?

March 30\textsuperscript{th}, 2011
Michael G. Katze, Ph.D.
<table>
<thead>
<tr>
<th>Viruses Studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influenza</td>
</tr>
<tr>
<td>Ebola</td>
</tr>
<tr>
<td>Marburg</td>
</tr>
<tr>
<td>Hepatitis C</td>
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<tr>
<td>SARS-CoV</td>
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<tr>
<td>Vaccinia</td>
</tr>
<tr>
<td>Herpes simplex</td>
</tr>
<tr>
<td>West Nile</td>
</tr>
<tr>
<td>HIV-1</td>
</tr>
<tr>
<td>SIV</td>
</tr>
<tr>
<td>Measles</td>
</tr>
<tr>
<td>Lassa</td>
</tr>
<tr>
<td>Chikungunya</td>
</tr>
<tr>
<td>Dengue</td>
</tr>
</tbody>
</table>
Presentation

Exam-Systems Biology-Animal Models-Vaccines-Drugs-Computational Biology
What Pandemics Have We Been Currently Experiencing?

A) HIV/AIDS

B) Hepatitis C

C) Swine Flu (H1N1)

D) All of the above
Answer: D - All of the above**
What Vaccines Are NOT Available?

A) Flu  
B) Polio  
C) Mumps  
D) Measles  
E) AIDS
In 2008, an estimated 33.4 million people were living with HIV/AIDS and greater than 2 million deaths.

1.4 million of the deaths were in Sub-Saharan Africa.
Question

What Event Caused The Greatest Lowering Of Life Expectancy In The U.S. Since 1900?

A) World War I
B) The 1918 influenza pandemic
C) World War II
D) The invention of the BIG MAC in 1901
Answer: B

The 1918 Influenza Pandemic

Courtesy of P. Palese
Question 3
If I sprayed 1918 virus in this room, how many of you would die?
A) We’d probably all die of fright!
B) 60%
C) 17%
D) 2%
E) No one: people today are immune
Answer: D

The case-fatality rate for the 1918 pandemic was about 2.5%, but it may have infected as much as 1/3 of the world’s population.

It’s not just about the virus: How the host responds is important!
VIROLOGIST/IMMUNOLOGIST OF YESTERDAY
To find the “right” keys we may need to examine the connections.
Modern Day Virologists & Immunologists MUST Do Better

Do We REALLY Even Know How A Virus Kills?
Our Systems Biology Paradigm

Experimental systems
- Virus infected:
  - In vitro cell lines
  - Primary cell culture
  - Nonhuman primate model

Virology data
- Samples - multiple time points and conditions

High throughput data
- Genomics
- Proteomics
- Metabolomics
- Nextgen Seq

Application to translational research

Systematic evaluation of the host response
- Genes
- Pathways
- Viruses

Data processing

Data integration

Key genes and pathways

Iterative processing
- host perturbations
- use of viral mutants
The Model Systems

- As you define key nodes/pathways, these results are carried forward and integrated with more complex systems.
- When key findings do not align - is that a problem with your model, or is it telling you something about the other attributes of the host?
Managing High-Throughput Data

IT Infrastructure to support sample management, raw data management, and dissemination

Computational Biology group support functional analysis including:

- Gene Set Enrichment
- Integrative Analysis
- Predictive Modeling
- Advanced Network Analysis

To accommodate the systems biology approach, significant effort goes into maintaining the HARDWARE and SOFTWARE of our computational infrastructure.
Why Should We Care About Systems Biology & Genomics?

- To study global impact of virus infection on host gene expression.
- To discover cellular regulatory pathways targeted by viruses.
- To identify new cellular targets for antiviral therapy.
- To develop vaccines
- To make new discoveries

How can we modulate the host if we don’t have a deep understanding of its behavior?
An Integrated Approach To Infectious Disease

Use of cell culture, primary cells, nonhuman primate and human clinical models to study viral infection

Combine traditional histopathological, virological, and biochemical approaches with functional genomics, proteomics and computational biology

Signatures of virulence and insights into mechanisms of host defense response, viral evasion, and pathogenesis
A Few Quick Examples

Animal Models-
Vaccines-
Drugs-
Computational Biology
SYSTEMS BIOLOGY APPROACH TO UNDERSTANDING 2009 PANDEMIC INFLUENZA ACROSS SPECIES

Sarah E. Belisle, Wenjun Ma, Terrence M. Tumpey, Heinz Feldmann, Juergen A. Richt, Michael G. Katze
Swine act as a reservoir for influenza virus. The ability of these animals to become infected but not die from influenza virus, regardless of strain, is notable and puzzling. A better understanding of response to influenza in swine may lead to greater insights into influenza biology and consequently better vaccines and therapeutics.
2009 Pandemic H1N1 Infection: A/Ca/04/2009

Samples were collected for global transcriptional analysis during ACUTE infection and RESOLUTION. Analysis was limited to transcripts for genes found on all three arrays.
Using Multiple Model Systems To Study Virus-host Interactions

<table>
<thead>
<tr>
<th>Animal Model</th>
<th>Array Type</th>
<th>Representation</th>
<th>Content Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig</td>
<td>Agilent S. scrofa (Pig) Oligo Microarray v1</td>
<td>~43,000 probes (~29,000 transcripts)</td>
<td>Developed from the latest publicly available [RefSeq, UniGene and TIGR]</td>
</tr>
<tr>
<td>Mouse</td>
<td>Agilent Mouse Whole Genome Array</td>
<td>&gt;41,000 genes and transcripts</td>
<td>NCBI Mouse Genome Builds, GenBank, RefSeq, Unigene, Ensembl, NIA, RIKEN, UCSC</td>
</tr>
<tr>
<td>Macaque</td>
<td>Agilent Macaque Expression Array</td>
<td>~ 18,000 genes</td>
<td>Baylor rhesus draft genome sequence, NCBI Rhesus Macaque Genome Builds</td>
</tr>
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Samples were collected for global transcriptional analysis during ACUTE infection and RESOLUTION Pandemic H1N1 Infection: A/Ca/04/2009.

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How does the transcriptional response compare across the three species?

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<tbody>
<tr>
<td>immune response</td>
<td>97</td>
<td>153</td>
<td>94</td>
</tr>
<tr>
<td>activation of normal cells</td>
<td>82</td>
<td>78</td>
<td>81</td>
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<td>binding of normal cells</td>
<td>48</td>
<td>198</td>
<td></td>
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<td>105</td>
<td>93</td>
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<tr>
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<td></td>
<td></td>
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<tr>
<td>cell division process of eukaryotic cells</td>
<td>160</td>
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<tr>
<td>cell movement of blood cells</td>
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<td>58</td>
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<tr>
<td>recruitment of blood cells</td>
<td>34</td>
<td>61</td>
<td>138</td>
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<tr>
<td>quantity of calcium</td>
<td>51</td>
<td></td>
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<tr>
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How does the transcription of immune-related genes compare across the three species?

- Overall functional analysis shows a significant alteration in immune response genes in all (3).
- Although the numbers of genes changing was similar, the precise genes changing were very different.
- Suggests the nature of the immune response within each species may be quite different.
Applying Systems Biology to Vaccine Development

- Can we detect early signatures of efficacy?
- Can we learn why a vaccine fails?
- Can we predict which vaccines could be the most effective?
- Can we better understand the connection between innate immunity and vaccine efficacy?
Microarray Comparisons Show Significant Pre-Challenge Differences In A Vaccine Trial Of Replicating Ad5-HIV/SIV Recombinants Alone Or With Env Boosts

Robert E. Palermo, L. Jean Patterson, Lauri Aicher, Marjorie Robert-Guroff, Michael G. Katze
Vaccine Branch, National Cancer Institute, Bethesda MD
Department of Microbiology University of Washington, Seattle, WA
Expression at Peak Viremia Emphasize Apparent Similarity Between the Two Vaccine Groups – ANOVA Results

- Expression changes for each animal use its Day 0 measurement as its genetically matched control.

i.e. fold-change is relative to the expression level on Day 0

1703 genes!
What Can We Do Once We Identify Host Factors Of Virulence And Pathogenesis?
Applying Systems Biology to Drug Development

- Can we “score” drugs based on the host response to treatment?
- Can we better understand mechanism-of-action through gaining a systems level view?
- How can we leverage and integrate existing data to better design and test candidate therapeutics?
RIG-I-LIKE RECEPTORS AND NOVEL INNATE IMMUNE PATHWAYS FOR ADJUVANT DISCOVERY AND DEVELOPMENT

Michael Gale, Jr.
University of Washington

Shawn Iadonato

Pathway Focused Analysis
Experimental Overview

MRC5 Cells seeded at 5X10^4 Cells/Well
Compounds added in the vehicle DMSO at 10μM concentration

DMSO vehicle control
Compound X negative control
Compound Y
Compound Z

3 replicate wells per condition
Samples harvested at 8 and 20hr post infection in Trizol
Principle Component Analysis - Mock Samples

- PCA analysis reveals a clear difference (principle component 1) between compound Z and the other treatments in uninfected cells.
- A more subtle difference exists between compound Y and the two controls (principle component 2).
The Vehicle Plus Drugs

- DMSO – *the vehicle*
- Compound X – negative control – doesn’t show any activity in RIG-I screening
- Compound Y – shows great activity in RIG-I screen – inhibits influenza replication
- Compound Z – shows great activity in RIG-I screen – inhibits influenza replication
Using Gene Expression to help elucidate mechanism-of-action

There are essentially no significantly differentially expressed genes between the DMSO treatment and treatment with the negative control (X).

Large number of differentially expressed genes between compound Z and the negative control (X).

Relatively few genes differentially expressed between compound Y and the negative control (X).

Both Compound Y and Compound Z inhibit viral replication. Different mechanisms?

Can we use this system to better understand the host response?
Compound Y treatment induces a specific response

Values represented are $\log_{10}(\text{Ratio})$ [ 2.000 = 100 Fold Change ]
Expression patterns of top scoring network

Compound Z treatment does NOT induce this network

Values represented are $\log_{10}(\text{Ratio})$ [ 2.000 = 100 Fold Change ]
As a tool to discover new mechanisms-of-action

Evaluate impact of drug treatment on global gene expression

- Identify alternate signaling pathways impacted by drug treatment
- Identify novel mechanisms of action
- Aid in the elucidation of off-target effects of drug treatment
- Toxicity?
WILL COMPUTATIONAL BIOLOGY BE THE KEY?

BEYOND VISUALIZATION - MODELING/PREDICTION OF THE HOST RESPONSE
New Methods for Analysis – Geometrical Representation (ARNDT BENECKE-PARIS)

- Can we systematically assess “state” change of the host to better understand what makes a high-path virus lethal? Or a drug toxic? Or a vaccine ineffective?
- GOING BEYOND WHAT IS KNOWN TO DEFINE A NEW REFERENCE
Integrating Large Scale Projects

The Connectivity Map: Using Gene-Expression Signatures to Connect Small Molecules, Genes, and Disease

Justin Lamb,1* Emily D. Crawford,1† David Peck,1 Joshua W. Modell,1 Irene C. Blat,1 Matthew J. Wrobel,1 Jim Lerner,1 Jean-Philippe Brunet,1 Aravind Subramanian,1 Kenneth N. Ross,1 Michael Reich,1 Haley Hieronymus,1,2 Guo Wei,1,2 Scott A. Armstrong,2,3 Stephen J. Haggarty,1,4 Paul A. Clemons,1 Ru Wei,1 Steven A. Carr,1 Eric S. Lander,5,6 Todd R. Golub5,2,3,5,7*

SCIENCE VOL 313 29 SEPTEMBER 2006

The Immunological Genome Project: networks of gene expression in immune cells

Nature Immunology Volume 9 number 10 October 2008

Tracy S P Heng, Michio W Painter & The Immunological Genome Project Consortium

The Immunological Genome Project combines immunology and computational biology laboratories in an effort to establish a complete ‘road map’ of gene-expression and regulatory networks in all immune cells
Figure 3 | The Connectivity Map is a tool for the bench researcher. The paths to showing that (a) an uncharacterized small molecule is a heat shock protein 90 (HSP90) inhibitor and (b) sirolimus can reverse glucocorticoid resistance in acute lymphoblastic leukaemia (see text for details) illustrate that the Connectivity Map (cmap) resource is best used in the context of a traditional research project. Indeed, its ultimate value relies on detailed experimental validation and follow-up.
Current Challenges

• The landscape for certain (NEXTGEN-NGS, lipidomics, metabolomics, proteomics) high-throughput technologies is still being defined while it has been worked out for hybridization-based genomics – how can we deal with the constant evolution?

• Communication
  – Getting the right people in the right place talking to the right people
  – Teaching a new generation a new vocabulary as well a new way to view science (local vs. global)
The Technology Is Driving Science

As Scientists we are ill equipped and ill trained to deal with the data
Recommendations

• Early integration of high-throughput data collection in drug and vaccine development as a mechanism to understanding global impact, off-target effects, and biomarkers for efficacy

• *In silico* screening for drug-drug interactions and as a tool for novel drug discovery

• Increased interdisciplinary cross-talk between computational scientists and bench scientists to define standards for study designs
Do We Need Google, Microsoft, and Facebook??

More than NIH, DARPA, BARDA, Merck, etc...
Applying Systems Biology Approaches to Respiratory Virus Research

Valentina Di Francesco

Lynn Law – project manager

www.systemsvirology.org
Our Perspective on the Systems Biology Approach

A Systems Biology Approach to Infectious Disease Research: Innovating the Pathogen-Host Research Paradigm

Alan Aderem, Joshua Adkins, Charles Ansong, James Galagan, Shari Kaiser, Michael Katze, Marcus Korth, Lynn Law, Jason McDermott, Carrie Rosenberger and Gary Schoolnik
A Systems Biology Approach to Emerging Respiratory Viral Diseases

Welcome

Welcome to Systems Virology.org where high-throughput genomic, proteomic, and metabolomic technologies are being combined with mathematical modeling and bioinformatics approaches to comprehensively analyze the host response to emerging viral viruses.

Our studies are focused on two NIAID priority pathogens: H5N1 avian influenza virus and severe acute respiratory syndrome coronavirus (SARS-CoV). We are working to develop and systematically refine network models of the direct and indirect effects of virus infection on cellular signaling pathways and to uncover the role of specific viral and host genes in regulating virus replication and virulence.

Our ultimate goal is to identify differences and commonalities in the host response to these viruses, which may in turn reveal novel targets for therapeutic intervention or suggest alternative vaccine strategies.

Avian Influenza Virus

One of the best known attributes of influenza virus is its pandemics. There is growing concern that the next pandemic, proven to be exceptionally virulent during cases of human-infected birds to humans, have infected nearly 400 people.

SARS Coronavirus

SARS-CoV, successfully controlled by aggressive public health measures three months of emergence. Over 8,000 people became infected and climbing to over 50% among the elderly. The outbreak is significant financial losses associated with disruptions of the world economy, licensed vaccines and drugs do not exist.
SUPPORTING 1918 PANDEMIC INFLUENZA VIRUS RESEARCH

Welcome to pandemicINFLUENZA.org. This site highlights studies by an interdisciplinary group of researchers working to uncover the factors that gave rise to one of the world's deadliest infectious disease outbreaks. With the growing concern that a new pandemic is on the horizon, we believe that a better understanding of the 1918 Spanish flu will help us to be better prepared should a new highly pathogenic virus arise.

The projects and studies described on this site are funded by a grant from the National Institute of Allergy and Infectious Diseases. Please take a look around to learn more about the tactics being used to study the 1918 virus and the scientists behind this exciting research effort.
Systems Biology Approaches to Viral Pathogenesis and Immunity (RCE)
http://www.ohsu.edu/vgti/pnwrce/
WELCOME TO THE NIDA CENTER FOR FUNCTIONAL GENOMICS

This National Institute on Drug Abuse (NIDA) P30 Center supplies the resources needed to apply cutting-edge genomic, proteomic, and bioinformatic technologies to the study of hepatitis C virus (HCV) infection and AIDS, chronic viral infections that are a direct consequence of drug abuse and addiction. We work with a variety of biologic resources, including serial liver biopsies from patients with recurrent HCV after liver transplantation, biopsies from patients co-infected with HCV and HIV-1, and experimental systems for HCV infection such as the SCID-beige/Alb-UPA chimeric mouse model and the HCV 2a in vitro infection system.

The complex and heterogeneous data generated by our genomic and proteomic investigations is organized and maintained in local databases for analysis and knowledge discovery. Ongoing software development is aimed at providing the tools to integrate these diverse types of data and to develop quantitative and predictive models. Our unifying theme is to integrate the use of these technologies to develop a detailed understanding of the host response to virus infection and the molecular mechanisms underlying the progression of HCV-associated liver disease, AIDS, and HDV/HIV-1 dual infection.
WELCOME TO MACAQUE.ORG

Here you can find the latest information on macaque genomic and proteomic resources and see how these tools are providing important new dimensions to research using macaque models of infectious disease. Our research encompasses a number of viruses that pose global threats to human health, including influenza, HIV, and SARS-associated coronavirus.

By combining macaque infection models with gene expression and protein abundance profiling, we are uncovering exciting new insights into the multitude of molecular and cellular events that occur in response to virus infection. A better understanding of these events may provide the basis for innovative antiviral therapies and improvements to vaccine development strategies.

CONFERENCE ANNOUNCEMENT

4TH INTERNATIONAL CONFERENCE ON PRIMATE GENOMICS

APRIL 13-16, 2010 SEATTLE, WASHINGTON
The Katze Lab

MACAQUE.ORG
http://www.macaque.org

NIDAGENOMICS
http://nida.viromics.washington.edu

PANDEMICINFLUENZA.ORG
http://www.pandemicinfluenza.org

KATZELAB
http://viromics.washington.edu

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