



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



Moving H5N1 studies into the era of systems biology

Laurence Josset, Jennifer Tisoncik-Go, Michael G. Katze*

Department of Microbiology, School of Medicine, University of Washington, Seattle, WA 98195, United States

ARTICLE INFO

Article history:

Available online 14 March 2013

Keywords:

Influenza virus
Systems biology
Transcriptomic
Proteomic

ABSTRACT

The dynamics of H5N1 influenza virus pathogenesis are multifaceted and can be seen as an emergent property that cannot be comprehended without looking at the system as a whole. In past years, most of the high-throughput studies on H5N1–host interactions have focused on the host transcriptomic response, at the cellular or the lung tissue level. These studies pointed out that the dynamics and magnitude of the innate immune response and immune cell infiltration is critical to H5N1 pathogenesis. However, viral–host interactions are multidimensional and advances in technologies are creating new possibilities to systematically measure additional levels of 'omic data (e.g. proteomic, metabolomic, and RNA profiling) at each temporal and spatial scale (from the single cell to the organism) of the host response. Natural host genetic variation represents another dimension of the host response that determines pathogenesis. Systems biology models of H5N1 disease aim at understanding and predicting pathogenesis through integration of these different dimensions by using intensive computational modeling. In this review, we describe the importance of 'omic studies for providing a more comprehensive view of infection and mathematical models that are being developed to integrate these data. This review provides a roadmap for what needs to be done in the future and what computational strategies should be used to build a global model of H5N1 pathogenesis. It is time for systems biology of H5N1 pathogenesis to take center stage as the field moves toward a more comprehensive view of virus–host interactions.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Highly pathogenic avian influenza (HPAI) H5N1 virus is endemic among wild birds and there are ongoing cases of avian-to-human infection, mostly in Southeast Asia. Since 2003, a total of 358 deaths out of 607 laboratory-confirmed cases have been reported (WHO, 2012). Although human-to-human transmission of H5N1 has been rare so far, recent studies have shown that some avian H5N1 strains only require a few mutations to acquire the capacity for airborne transmission between mammals, thereby constituting a major threat for human health (Herfst et al., 2012; Imai et al., 2012). Given the high mortality associated with H5N1 infection and the risk of an impending influenza pandemic, it is crucial to understand the underlying mechanisms of viral pathogenesis in order to better manage patient care and develop more effective antiviral therapeutics. H5N1 pathogenesis has been extensively studied, but even with the sum of current knowledge, we still lack a quantitative model of molecular events leading to disease at the organismal level. Systems biology allows examination of host–pathogen interactions at several scales, including the whole organism, the target organ, and the cellular level. We believe this

approach holds promise to building models that are able to handle known information about H5N1 and to discover emergent properties of H5N1–host interactions that appear when the system is considered as a whole. The goal of such a model is to reveal major regulators of H5N1 pathogenesis and predict the effect of their disruption on disease outcome, which in turn would accelerate development of novel immunomodulatory therapeutics. In this review, we describe the contribution of 'omic studies to our comprehension of H5N1 pathogenesis, the goal of systems biology in H5N1 research, and the different data and models that need to be developed to help reach that goal.

2. From transcriptomic profiling to systems biology

Human patients with severe H5N1 disease typically develop a viral primary pneumonia progressing rapidly to acute respiratory distress syndrome (ARDS) (Abdel-Ghaffar et al., 2008). Among the mechanisms that contribute to H5N1 pathogenesis, an aberrant immune response is thought to play a significant role in the development of severe respiratory disease that may ultimately lead to death (reviewed in (Peiris et al., 2009)). The term “cytokine storm” is often associated with H5N1, referring to an uncontrolled inflammatory response (Tisoncik et al., 2012). High serum levels of macrophage and neutrophil chemoattractant chemokines (CXCL10, CXCL2, IL-8) and both pro- and anti-inflammatory cytokines (e.g.

* Corresponding author. Tel.: +1 206 732 6135; fax: +1 206 543 8297.
E-mail address: honey@u.washington.edu (M.G. Katze).

IL-6, IL-10, and IFN- γ) were found in human patients infected with H5N1 (To et al., 2001; Peiris et al., 2004; de Jong et al., 2006). Over the past decade, global transcriptional profiling of infected lungs from several mammalian models has been used to characterize the host response to influenza virus at the primary site of viral replication. Here, we focus on lung transcriptomic data for H5N1 infection assessed primarily in the mouse model.

2.1. What have we learned from H5N1 *in vivo* transcriptomic studies?

2.1.1. H5N1 virulence is a function of the level and kinetics of the inflammatory response

The host response to H5N1 has been studied in non-human primate (Baskin et al., 2009; Cillóniz et al., 2009; Shinya et al., 2012), mouse (Cilloniz et al., 2010; Fornek et al., 2009) and ferret models (Cameron et al., 2008). In all three models, extreme virulence of influenza virus has been repeatedly associated with increased host responses, in particular, early and sustained induction of inflammatory responses (summarized in Fig. 1). These studies highlighted the importance of timing and magnitude of inflammatory and innate immune gene expression induced early during infection.

The mouse is the primary model for evaluating host responses to influenza virus, for cost and practical reasons, as well as availability of reagents and extensive data about mouse genetics. The mouse genome was sequenced 10 years ago (Waterston et al., 2002) and is now extremely well annotated, thanks to several international collaborative projects such as FANTOM (Okazaki et al., 2002), the International Knockout Mouse Consortium (Skarnes et al., 2011) and the Collaborative Cross (Churchill et al., 2004). In general, this animal model is thought to reflect H5N1 human disease (Lu et al., 1999; Maines et al., 2005). Human H5N1 isolates are highly pathogenic in mice without prior adaptation, in part due to preferential recognition of avian-type α 2,3 sialic acid (SA) receptors predominantly expressed in the mouse respiratory tract (Ibricevic et al., 2006). Cilloniz et al. (2010) showed that early activation of inflammatory response genes correlate with disease severity during A/Viet Nam/1203/2005 (H5N1) virus (VN1203) infection, including increased expression of inflammasome components and genes associated with viral sensing, neutrophil activation, NF- κ B signaling, and chemokine signaling. In mice, VN1203 disseminates into brain and spleen tissues, and genes associated with extrapulmonary dissemination were associated with sustained activation of inflammatory responses and altered hematological function and lipoxin signaling.

An enhanced and early inflammatory response has been observed in all animal models infected with highly pathogenic H5N1 or 1918 H1N1 influenza viruses (Baskin et al., 2009; Cameron et al., 2008; Chang et al., 2011; Cilloniz et al., 2010; Cillóniz et al., 2009; Shinya et al., 2012), which would argue that the same pathways may be activated after infection with lethal and non-lethal viruses, but only at a higher magnitude and with different kinetics for the lethal virus infections. However, infection with non-lethal viruses may also induce different protective pathways, as cell growth or lipid metabolism pathways, which would then allow the animal to clear the virus and recover from infection (Cameron et al., 2008; Go et al., 2012; Josset et al., 2012a). In a meta-analysis of mouse host responses that considered different mouse genetic backgrounds and different respiratory viruses with varying levels of pathogenicity, Chang et al. (2011) identified two different types of gene signatures differentiating high and low pathogenicity viruses. One gene signature had anti-correlated expression in the two virus groups; genes up-regulated by highly pathogenic viruses were associated with inflammation and apoptosis, while down-regulated genes were associated with cytochrome P450 pathway. The second gene signature involved genes that had magnitude

differences in their levels of expression, with mainly chemokines that were more up-regulated after infection with highly pathogenic viruses. In this study, the 'magnitude' signature differentiated the two virus groups the best and it was also a better predictor for distinguishing lethal vs. non-lethal influenza infection. However, these signatures were derived using the two groups of viruses regardless of time post-infection, and may therefore have missed temporal transitional differences contributing to pathogenesis.

2.1.2. Virulence factors of H5N1 impact the immune response in the lung

The mouse model has also been used to elucidate the impact of specific H5N1 virulence factors on the host response. The PB2 protein is one of three subunits of the viral polymerase and the residue at position 627 is one of the best characterized viral determinants of host range and virulence (Boivin et al., 2010). An H5N1 virus from the 1997 outbreak in Hong Kong possessing lysine at position 627 (627K), A/Hong Kong/483/97 (HK483), was found to be lethal in mice, whereas an H5N1 virus that contained a glutamic acid residue at this position, A/Hong Kong/486/97 (HK486), was non-lethal (Hatta et al., 2001). Fornek et al. (2009) investigated how the presence of lysine at position 627 of the PB2 protein affects the host response to H5N1 in lung and spleen. Increased pathogenicity and dissemination was observed for HK483 and HK486 PB2-E627K viruses, which was associated with enhanced viral replication, activation of immune, inflammatory and apoptosis responses and also the lack of induction of genes required for TCR signaling in the lung. In the spleen, genes involved in NK cell cytotoxicity, antigen presentation and interferon (IFN) signaling were more highly induced in response to HK483 and HK486 PB2-E627K viruses compared to HK486 (Fornek et al., 2009).

The PB1-F2 protein is a small accessory protein expressed by influenza virus, depending on the strain, that was first described in 2001 (Chen et al., 2001) and shown to have pleiotropic effects on apoptosis, inflammation, and regulation of the viral polymerase (reviewed in Krumbholz et al. (2011)). Deletion of PB1-F2 diminished the pathogenicity of mouse-adapted H1N1 in mice (Zamarin et al., 2006), whereas a single N66S mutation in PB1-F2 of Hong Kong/156/1997 (H5N1) resulted in increased virulence of mouse-adapted A/WSN/1933 (H1N1; WSN) virus expressing PB1-F2 N66S of H5N1 virus (Conenello et al., 2007). Global pulmonary transcriptional profiling of C57BL/6J mice infected with reassortant PB1-F2 66N or 66S variants revealed that increased pathogenicity of the 66S variant was associated with a delayed induction of innate immune responses at 1 dpi, and after 3 dpi, increased expression of several pro-inflammatory cytokines could be responsible for the higher trafficking of monocytes and granulocytes into the mouse lung (Conenello et al., 2011). Deletion of PB1-F2 from WSN is associated with decreased virulence in mice and a decrease in host responses, in particular genes linked to cell death, inflammatory response and neutrophil chemotaxis, with minimal impact on viral replication (Le Goffic et al., 2011).

While different functions have been described for PB2 627 and PB1-F2 variants in infected cells, these studies demonstrate that their deletion had a similar impact on host responses *in vivo*, namely by decreasing the inflammatory response (Fig. 1). Attenuation of the inflammatory response observed *in vivo* with these viral mutants could be the result of the deletion of a virulence factor or the modulation of a molecular determinant of pathogenesis, though it may also be the consequence of attenuated fitness of these viruses. Therefore, interest in *in vivo* global 'omics using mutant viruses to better understand the molecular function of virulence factors may be ambiguous, and mechanistic studies or protein-protein interaction screens may bring more information about their role for driving specific interactions with the host.

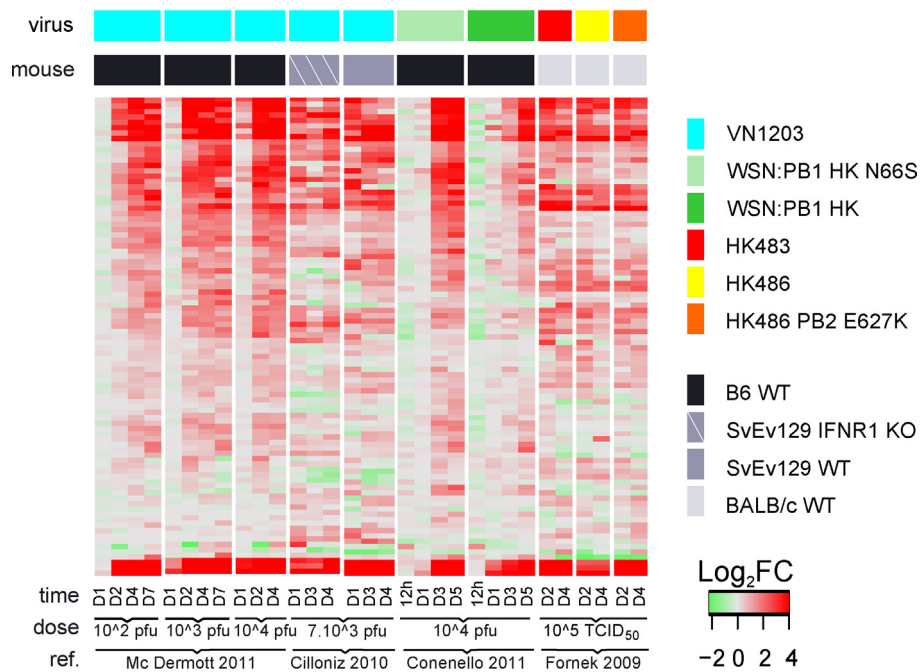


Fig. 1. Induction of host inflammatory response in mice infected with H5N1 depends on the viral strain, viral dosage, and host genetic background, and is associated with pathogenicity. The heatmap represents mean expression levels (in \log_2 fold change, \log_2 FC) for significantly up-regulated inflammatory response genes. Genes were selected based on their GO annotation (GO:0006954, “inflammatory response”) and statistical analysis vs. time-matched mocks ($(|\log_2 \text{FC}| > 1, \text{Student } t\text{-test } q \text{ value} < 0.05 \text{ for at least one biological condition})$). These datasets have been published in Cilloniz et al. (2010), Conenello et al. (2011), Fornek et al. (2009) and McDermott et al. (2011).

2.2. Host response to H5N1 infection is multidimensional

The use of functional genomics in characterizing the global host response to H5N1 virus has revealed that the dynamics and magnitude of the innate immune response to infection, as well as immune cell infiltration, is a crucial aspect of pathogenesis. In addition, because most transcriptomic data are publicly available and easily accessible on Gene Expression Omnibus (GEO, <http://www.ncbi.nlm.nih.gov/geo/>), these data can be re-examined by meta-analysis aimed at identifying host response characteristics of respiratory virus pathogenicity (Chang et al., 2011), or to compare new microarray results of emerging influenza viruses, such as the 2009 pandemic H1N1 influenza virus, with virulence characteristics of other viruses (Josset et al., 2012a).

Despite the advantage of profiling the whole transcriptome, gene expression changes in response to H5N1 represents only a single facet of the host response. Other types of ‘omics might bring different insights on H5N1 pathogenesis. For instance, influenza viruses also modulate post-transcriptional regulation and translation, which cannot be addressed by transcriptomics alone, but requires integration of proteomic data and regulatory RNA profiling. At a lower complexity level, studies at the single molecule level, such as interaction between the viral hemagglutinin (HA) and SA cellular receptors or neuraminidase (NA) enzymatic activity, also provide an understanding of H5N1 virulence (Fig. 2). Aside from the molecular dimension, studies on H5N1–host interactions also need to include the different levels of physiological scales of the host response, from the single infected cell to the lung to the organism. In addition, the time scales that span these physiological levels are very different, from milliseconds for the induction of a signaling cascade or protein–protein interactions, to minutes and hours for the induction of the expression of antiviral genes, to days and weeks for the development of innate immunity and transition to adaptive immune responses. Finally, a fourth dimension that is crucial in shaping the host response to H5N1 infection

is host genetics. This parameter can be studied from the single gene level, using approaches like RNA interference or single gene knock-out (KO) mice, to multifactorial levels of complexity that are present in the human genome and the natural genetic variation can be studied in a mouse resource called the Collaborative Cross (CC). The recognition that we cannot predict the behavior of a living organism by looking at biological properties individually is bringing the field of systems biology to the forefront of infectious disease research.

2.3. What is systems biology?

Systems biology is an evolving field that uses an interdisciplinary approach aimed at understanding and predicting the properties of a living system through systematic quantification of all its components and intensive mathematical and computational modeling to find emergent properties of the system. Emergent properties cannot be entirely explained by the sum of their individual components, that is, at each level, new properties and rules emerge that cannot be predicted by observations and full knowledge of the lower levels (Novikoff, 1945). Virulence is an example of an emergent property applied to H5N1 research, which cannot be explained by looking at each of the viral virulence factors in isolation, nor by examining a narrow range of host factors.

In infectious disease research, the main goal of systems biology is to model and describe in an unbiased manner host–pathogen interactions at every scale of the organism (Tisoncik and Katze, 2010) (Fig. 2). Each component of the system is measured using high-throughput ‘omic techniques, such as transcriptomic, proteomic, and metabolomic, and in theory examined from the cellular level to the whole organism at each temporal scale. These measures have to be integrated with single molecule models of host–virus interactions. Mathematical and computational methods are then developed to analyze these different data

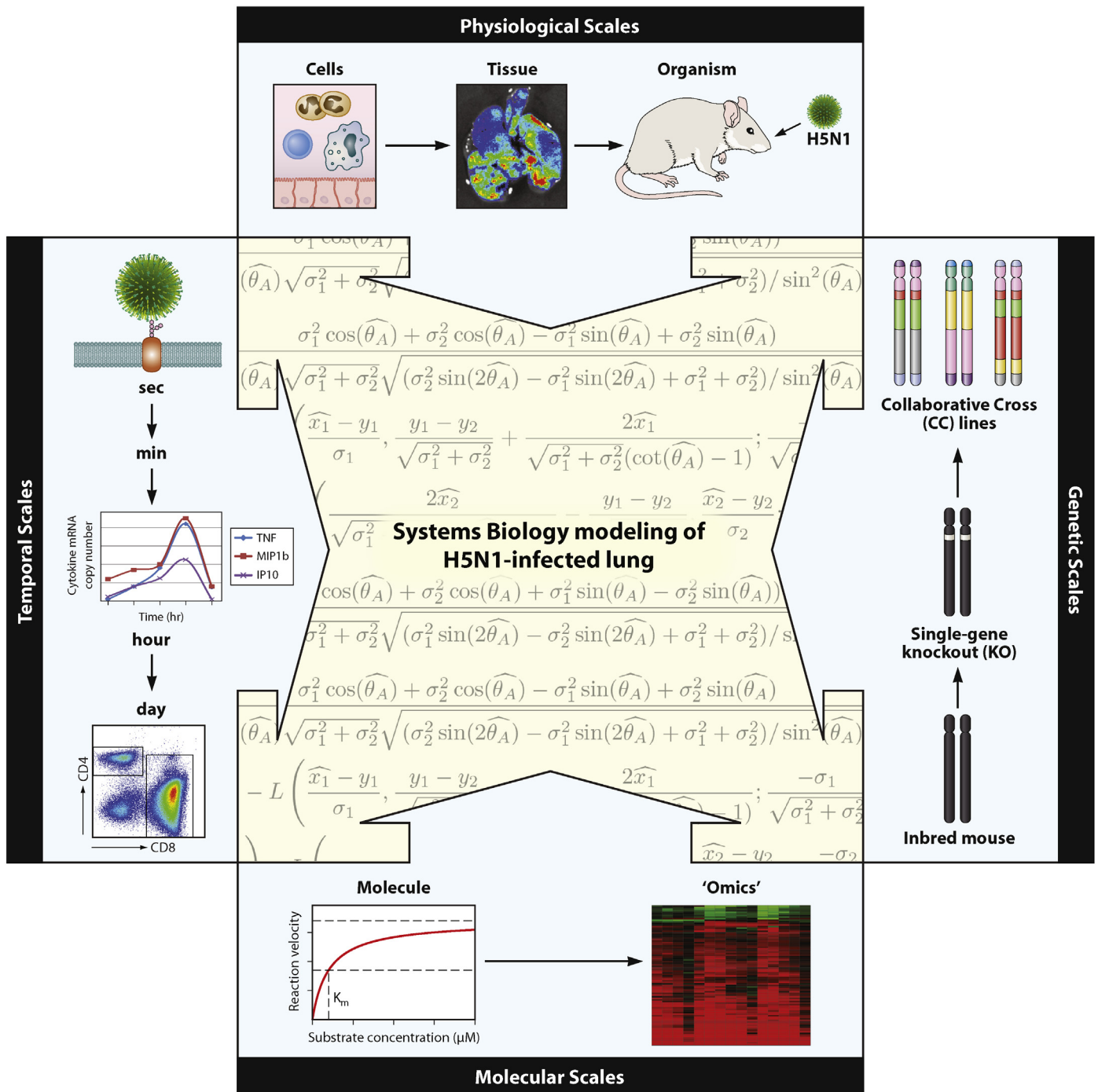


Fig. 2. Systems biology of H5N1-infected lung. Systems biology is an interdisciplinary approach aimed at understanding and predicting the properties of a living system through systematic quantification of all its components and intensive mathematical and computational modeling to find emergent properties of the system. The host response to H5N1 is multidimensional, with several physiological scales involved (from cells to tissue to organism), involved at different temporal scales: from seconds, H5N1 HA SA receptor engagement; minutes representing activation of pathogen recognition receptors (e.g. RIG-I and TLR); hours representing production of cytokines; to days representing adaptive immune responses. Several molecular scales need to be modeled, from the single molecule to increasing systems complexity of diverse 'omics' (e.g. transcriptomics, proteomics, and metabolomics). Finally, the host-genetics dimension has to be integrated and can be studied using KO (knockout) and CC (Collaborative Cross) mouse models. Mathematical and computational methods are developed to analyze these different data types from each of these scales.

types and integrate them in a meaningful model. The aim of this approach is to understand and predict the properties of H5N1 infection, with the overarching goal to find master regulators, such as individual genes, cellular interactions or pathways of the host response that could be targeted for new antiviral therapy.

3. Using 'omics to model the host response to H5N1

To date, an extensive undertaking by the Systems Virology Center (<http://www.systemsvirology.org>) at the University of Washington has generated comprehensive 'omics data sets for H5N1 infected samples, providing access to the

different levels of the host response (Aderem et al., 2011). In parallel, system biology methods are being developed to analyze these data together. However, successful integration of these 'omics has been quite limited in the H5N1 field, mainly because of the challenges to obtain consistent data, as well as the need for powerful mathematical algorithms that can handle the high dimensionality 'omics data.

3.1. Generating different types of 'omics data for H5N1

3.1.1. Proteomic and phosphoproteomic profiling

The proteomic field has been rapidly evolving to allow quantification of a larger number of proteins with greater accuracy (Aebersold and Mann, 2003; Cox and Mann, 2011). However, proteomic technologies still have certain limitations, mainly related to the difficulty to detect low-abundance, hydrophobic or basic proteins (Garbis et al., 2005). Proteomic data usually have incomplete proteome coverage and restricted dynamic range, which makes these data challenging to analyze (Schulze and Usadel, 2010). There are only a few studies about host proteomic changes in response to H5N1 infection, likely due to these limitations, while there is abundant transcriptomic data available.

Consistent with transcriptomic findings, Brown et al. (2010) have shown increased virulence of VN1203 in cynomolgus macaques was associated with elevated pulmonary levels of inflammatory proteins, complement, and proteins reflecting cell proliferation, while proteins related to metabolism were decreased, as compared to animals infected with seasonal H1N1 virus (A/Texas/36/91) or a reassortant H1N1 virus containing r1918 HA and NA (Brown et al., 2010). In an *in vitro* system, proteomics of primary human monocyte-derived macrophages infected with a HPAI A/Vietnam/3212/04 (H5N1) virus revealed significant changes in ribosomal protein abundance and elongation factors at 1 and 3 hpi, which may contribute to efficient viral replication (Cheung et al., 2012). An increase in the level of some ribosomal proteins was also found later during infection (6–12 hpi) of macrophages with H3N2 viruses, together with dramatic changes in the nuclear proteome and an increase in lysosomal and IFN-response proteins (Lietzen et al., 2011).

Other proteomic approaches include studies of interactomics and phosphoproteomics. High-throughput proteomic approaches have been used to identify cellular factors interacting with viral proteins in the cell (Jorba et al., 2008) and in the viral particle (Mayer et al., 2007). A pull-down study of H5N1 PA coupled with mass spectrometry identified new partners of the viral polymerase and in particular, PA was found to be associated with mitochondrial proteins such as the apoptosis-inducing factor (AIFM1), suggesting a potential role of PA during apoptosis (Bradel-Tretheway et al., 2011). Another strategy to identify host factors interacting with viral proteins is to use the yeast two-hybrid system (Shapira et al., 2009; Tafforeau et al., 2011). In particular, Tafforeau et al. (2011) used this system to identify several partners of H5N1 polymerase subunits. However, the approach is limited by a general high rate of false positives and the caveat of protein interactions not taking place in a relevant cellular context. Finally, advances in phosphopeptide enrichment and mass spectrometry now enable high-throughput identification and quantification of protein phosphorylation sites, yet no phosphoproteomic data are published for influenza infected cells. Such data would provide information about signal transduction activated early after infection and would be useful to connect infection to further changes in transcriptional profiles in cells.

3.1.2. Metabolomic and lipidomic profiling

While transcriptomics and proteomics are powerful strategies to measure the global host response to infection, signal

transduction cascades can also involve the synthesis of metabolites for efficient signal transmission. Metabolomics involves the high-throughput characterization of low molecular weight compounds in a biological system. Measurement of extracellular metabolites from central carbon metabolism (glucose, lactate, glutamine and glutamate) and the concentration of 30 intracellular metabolites from the glycolysis, pentose-phosphate-pathway and TCA cycle from MDCK cells infected by a mouse-adapted H1N1 laboratory strain, PR8, showed an increase of glycolysis after infection (Ritter et al., 2010). In a more high-throughput manner, gas chromatography coupled with mass spectrometry (GC/MS) was employed to profile metabolites from cell lines infected with influenza A/Hong Kong/2108/2003 (H9N2) virus, and the results indicated that infection can alter fatty acid biosynthesis and cholesterol metabolism (Lin et al., 2010). Several transcriptomic studies also suggest that lipid metabolism is altered during influenza infection *in vivo* and could be implicated in increased pathogenesis of H1N1 (Josset et al., 2012a; Ma et al., 2011). However there is no metabolic profiling of infected tissue published yet, nor are there metabolomic data for H5N1 virus, though we are currently generating metabolomics data on cell lines infected with VN1203 from similar samples on which transcriptomic and proteomic are available, therefore allowing an integration of these data types. Given the importance of the inflammatory response in H5N1 pathogenesis, the profiling of lipids with pro- or anti-inflammatory effects could be important for better characterizing viral-host interactions and for developing potential immunotherapies.

3.1.3. Genome-wide screening

Several studies using RNA interference have identified cellular factors required for influenza replication (Hao et al., 2008; Brass et al., 2009; Karlas et al., 2010; König et al., 2010; Shapira et al., 2009). Viruses used for these screens included PR8 and a second mouse-adapted H1N1 influenza virus, WSN, as well as a modified influenza virus in which HA was replaced with vesicular stomatitis virus glycoprotein G and NA was replaced with a reporter for use in an orthologous system (Hao et al., 2008). There was little overlap of identified factors among the different screens, which could be explained by differences in systems and readouts. Nevertheless, considering these factors as a whole could be important in modeling H5N1-host interactions. Several reviews and a commentary have been dedicated to understanding the importance of these results and provide more detail on host factors regulating influenza virus replication (Mehle and Doudna, 2010; Stertz and Shaw, 2011; Watanabe et al., 2010).

3.1.4. Regulatory non-coding RNA profiling

Non-protein-coding RNAs (ncRNAs) are potentially important host factors in the antiviral response, but their functions remain largely unexplored. There is an increasing number of different classes of regulatory RNA that have been described in the past few years, including small interfering RNA (siRNA), microRNA (miRNA), PIWI-interacting RNA (piRNA), promoter-associated small RNA (PASRs), small nucleolar RNA (snoRNA) and long non-coding RNAs (lncRNAs) (Taft et al., 2010). Accurate and global quantification of these different RNAs has been made possible by the recently developed RNA sequencing (RNA-Seq) technique. This method is based on next-generation sequencing (NGS) platforms that allow parallel sequencing of all RNA molecules present in the sample without relying on pre-defined sequences as in microarrays or traditional PCR.

MicroRNAs are becoming increasingly recognized as important players in host-pathogen interactions. For example, the analysis of lung tissue from macaques infected with VN1203 revealed changes in the expression of numerous miRNAs (Li et al., 2011). Among these differentially expressed miRNAs, 23 were regulated with

similar trends in mice infected with lethal 1918 virus. Predicted target genes of these miRNAs encoded cell death and inflammatory factors involved in influenza pathogenesis, which suggest that their regulation could play crucial roles in influenza virulence (Li et al., 2011). In mice, 45 miRNAs were differentially expressed in lung samples from different mouse strains during severe acute respiratory syndrome coronavirus (SARS-CoV) (MA15) or influenza virus (PR8) infection and expression of 6 miRNAs was confirmed to change after VN1203 infection (Peng et al., 2011). These miRNAs, such as miR155 that was previously implicated in lymphocyte function (Vigorito et al., 2007), represent potential important regulators of the host response and provide an interesting target to validate in studies focused on understanding their mechanisms.

Among other non-protein-coding RNAs with a potential role in H5N1 pathogenesis are long ncRNAs (lncRNA), endogenous cellular RNAs that are larger than 200 nucleotides in length and that lack positive-strand open reading frames longer than 30 amino acids. Recent studies suggest that lncRNAs may play a role in the host response to pathogens. Pang et al. (2009) showed expression of many lymphocyte-specific lncRNAs changed with CD8+ T cell differentiation. In a separate study, Guttman et al. (2009) identified 20 lncRNAs highly upregulated after stimulation of Toll-like receptor TLR4 in dendritic cells, and Peng et al. (2010) observed 500 annotated and 1000 nonannotated lncRNAs were differentially expressed in mice after SARS-CoV infection. Differential expression of lncRNAs was also observed in mice and mouse embryonic fibroblasts (MEF) infected with PR8. As we probe deeper into the identification of ncRNAs and the diverse species that may have a regulatory role during infection, it will be important to profile these ncRNAs, particularly lncRNAs, in H5N1 infected cells and animals to better understand host-pathogen interactions.

3.1.5. Virus profiling

To date, 'omic studies have focused almost exclusively on the host response. However, RNA-Seq offers new exciting opportunities to sequence both viral and host RNA that may conciliate virus-centric and host-centric views. While this dual RNA-Seq has been mostly used for bacterial or parasite sequencing, together with the host (Westermann et al., 2012); it is directly applicable for influenza and cellular mRNA profiling, as influenza mRNA is polyadenylated. On the other hand, directional total RNA-Seq allows access to both vRNA and viral mRNA sequences, together with host RNA. An illustration of the importance of considering both viral sequence evolution and host response to fully model pathogenesis comes from studying the PB2-627E viral mutant. Performing total RNA-Seq analysis of lung from mice infected with 10^4 pfu of VN1203 carrying the PB2-627E mutation revealed that around half of the viral population had reverted back to the wild-type sequence by day 2 pi (unpublished data). These data are crucial to interpret the host-transcriptome response. Differences in magnitude and kinetic of induction of the inflammatory response by the wild-type and mutant virus may likely be more a function of difference in viral dosage and time than related to the specific PB2-627E mutation (Tchitchek et al., submitted for publication).

3.2. Finding emergent properties of the H5N1-host system from 'omics data

We and others have been generating different types of data to understand H5N1 pathogenesis. However, most of the studies described in the previous section are still focused on a single type of 'omics data. Integrating different types of 'omic' data is indeed very challenging because it requires consistent data. That is, profiling of several 'omic' datatypes from either the same or related samples, as well as the development of new methods for data integration. Graphical models are one option to integrate different

types of data (for a didactic review about network modeling in systems biology see Ma'ayan, 2011) and can also be used for predicting regulatory mechanisms of cells or mice infected by H5N1 (Li et al., 2011; McDermott et al., in review; Mitchell et al., submitted for publication).

3.2.1. Co-regulation network analysis of transcriptomic data reveals key regulators of the host response

Statistical analysis of microarray data is conventionally used to determine lists of differentially expressed genes between two conditions. In contrast, co-expression network analysis explores all pairwise relationships among genes and analyzes the structure of these relationships to predict the behavior of the system. Pairwise relations between 2 genes can be estimated by different methods, such as correlation (in WGCNA (Zhang and Horvath, 2005)), or mutual information concept from information theory (in ARACNE (Margolin et al., 2006) or CLR (Faith et al., 2007)). These relations are then represented by using co-regulation networks with nodes being genes and edges representing the strength of the relation. Network topology analysis allows identification of modules of co-expressed genes, and key points of the network such as "bottlenecks", bridges between modules, and "hubs", highly connected genes inside a module (Yu et al., 2007). It has been shown that genes belonging to the same pathway or biological function are usually co-expressed, that is their tight expression levels vary concomitantly, and that hubs and bottlenecks are essential in protein-protein interaction networks and regulatory protein networks, respectively.

Co-expression network analysis of human Calu-3 lung epithelial cells infected with VN1203 identified 12 different modules of co-regulated genes, including one module that mainly contained downregulated genes related to metabolism and cell cycle, and an 'inflammatory' module that contained up-regulated genes, including a large number of chemokines genes and genes known to play a role in influenza pathogenesis (Li et al., 2011). Of note, there were a significant number of genes with no known functions among the inflammatory module. On the basis of "guilt-by-association," these unknown genes could be involved in H5N1 pathogenesis and are interesting targets to validate by biological experiments.

Recently, network analysis was used to model the host response of mice infected with H5N1 (Fig. 3) or SARS-CoV (McDermott et al., in review). Two methods were used for network construction (WGCNA and CLR), and genes were ranked based on scores identifying bottlenecks and hubs. Some highly ranked genes were then validated using KO mice to assess their importance on host response organization. For H5N1, two genes predicted to be of high importance included *Tnfrsf1b* and *Ido1* (Fig. 3). The infection of knockout mice containing a single deletion of either gene resulted in decreased weight loss compared with wild-type mice. Importantly, transcriptomic analysis of infected lungs from KO mice confirmed the structure of the inferred network, as expression of neighbor genes of the two targets were significantly differentially regulated in the KO mice relative to infection of wild-type mice.

These co-regulation networks successfully pointed out targets related to H5N1 pathogenesis. However, these approaches have several limits: (i) they only consider marginal correlation which might lead to false positive relationships between genes that are correlated though influence other variables, (ii) they do not model dynamic relationships between genes; and (iii) they infer only correlation, not causation. Additional graphical models, like graphical Gaussian models (Witten and Tibshirani, 2009), dynamic Bayesian Networks (Rau et al., 2010), and causation networks (Maathuis et al., 2010) represent computationally intensive but attractive

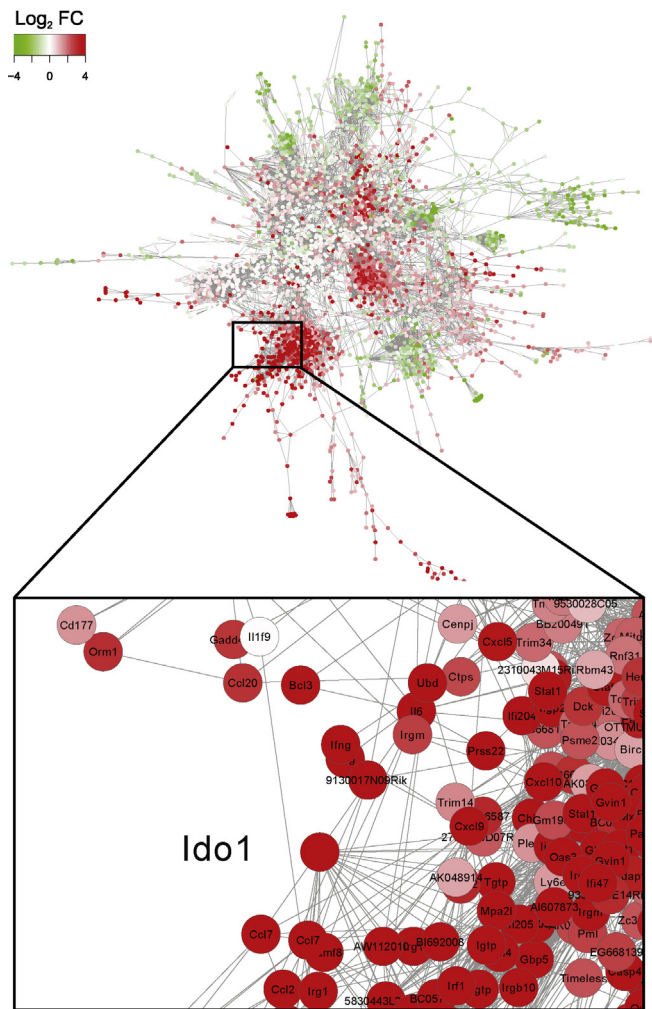


Fig. 3. Network analysis of pulmonary response to H5N1 infection. The context likelihood of relatedness (CLR) method was used to infer associations between differentially expressed genes in mice after infection with VN1203 across 4 time points (days 1, 2, 4 and 7) and 3 dosages (10^2 , 10^3 and 10^4 pfu). Each node corresponds to a gene and edges represent co-expression associations among genes. Genes are colored by their expression levels (in \log_2 FC) 4 days after infection with 10^3 pfu of VN1203. Based on topological analysis, *Ido1* was predicted to be a significant regulator of the host-response. Its location is enlarged to show associations with neighbor genes. *Ido1* belongs to a module of highly induced genes related to the innate immune response.

models that could be applied to some extent to H5N1 disease to overcome these limitations.

3.2.2. Co-regulation network analysis for integration of different types of data

Most published regulatory networks are based on transcriptomic data, though this approach can also be used to integrate different types of data. We recently applied this method to both transcriptomic and proteomic data of Calu3 cells infected with VN1203 or SARS-CoV. The co-expression network was inferred from transcriptomic data, and proteomic data was then used to add edges between genes when the corresponding proteins were co-expressed (Mitchell et al., submitted for publication). As such, the network topology changed to take into account information held at the protein level.

Co-regulation network analysis is also an attractive approach to integrate phenotypic and transcriptomic data. Turan et al. (2011) used ARACNE to model the relationships between muscular gene expression, serum levels of cytokines and physiological

measurements, including specific respiratory functions (VO_2 max, VO_2 peak, PaO_2) from healthy individuals or patients with chronic obstructive pulmonary disease (COPD), which showed uncoupling of tissue remodeling and bioenergetics modules was a specific hallmark of COPD-diseased tissues. This method could be used for H5N1 transcriptomic and phenotypic data to define how gene-regulatory networks are associated with particular phenotypes. Phenotypic data usually measured in mice to evaluate influenza infection include weight loss, histology and viral lung titers. Because lung pathology is an important determinant of H5N1 outcome, longitudinal measurements of lung function during experimental infection by plethysmography represents one valuable technique to measure respiratory variability. Such measurements were used to monitor mice after 2009 H1N1 infection and were found to correlate with lung pathology (Julander et al., 2011). Assessing associations between such phenotypic parameters and 'omic data could bring meaningful insights into how H5N1 pathogenesis is regulated.

3.2.3. Additional methods for 'omics integration

Most studies using both proteomic and transcriptomic data have analyzed each data type separately and then aggregated the results in a *post hoc* manner. For example, functional pathway or network analyses of derived gene and protein lists were compared to identify common regulators among the two datasets (Piruzian et al., 2010), or networks inferred from differentially expressed proteins were scored for enrichment in differentially expressed genes (Imielinski et al., 2012).

Others have tried to analyze correlation between mRNA and protein abundance and found only modest concordance between transcript and protein levels in yeast (Hack, 2004), mammalian cells (Zhao et al., 2009), or tissue (Ghazalpour et al., 2011). Attempting a direct correlation between proteomic and transcriptomic data is extremely challenging because of multiple layers of possible discrepancies. These include distinct sensitivities of cDNA array hybridization and peptide measurement, different biases in the two technologies (Ghazalpour et al., 2011), timing differences between transcription and translation, or post-transcriptional and translational modifications. It is interesting to note that influenza virus modifies both transcriptional and translational regulation by cap-snatching (Plotch et al., 1981), interaction with the cellular RNA polymerase II (Engelhardt et al., 2005) and spliceosome components (Wolff et al., 1998), and by ribosomal recruitment (Kash et al., 2002, 2006). Therefore, looking at the correlation between transcriptomic and proteomic data could provide additional value into influenza virus-mediated translational control. In Calu-3 cells infected with VN1203, Pearson correlation between transcriptomic and proteomic data revealed that very early (0–3 hpi) up-regulated proteins and late (12–24 hpi) downregulated proteins changed in abundance without regulation of their transcript (Eisfeld et al., in review). These anti-correlations could be driven by viral targeting of the mRNA or translation machinery; however, consideration of additional levels of mRNA regulation, such as miRNA and isoform quantification, would be necessary to fully model the system.

3.3. Prior knowledge that can be used to model infections

Can we use previously published reductionist data about H5N1 in systems biology? A recent review argues that use of prior biological knowledge is crucial to increase signal detection in systems biology (Ideker and Krogan, 2012). However, if prior assumptions on signal distribution can lead to development of effective statistics for integrating 'omics (for example Kislinger et al., 2006), most of the reductionist knowledge about H5N1 cannot be directly integrated into systems biology models. Why might this be the case? Let us consider a viral factor V interacting with a host protein H,

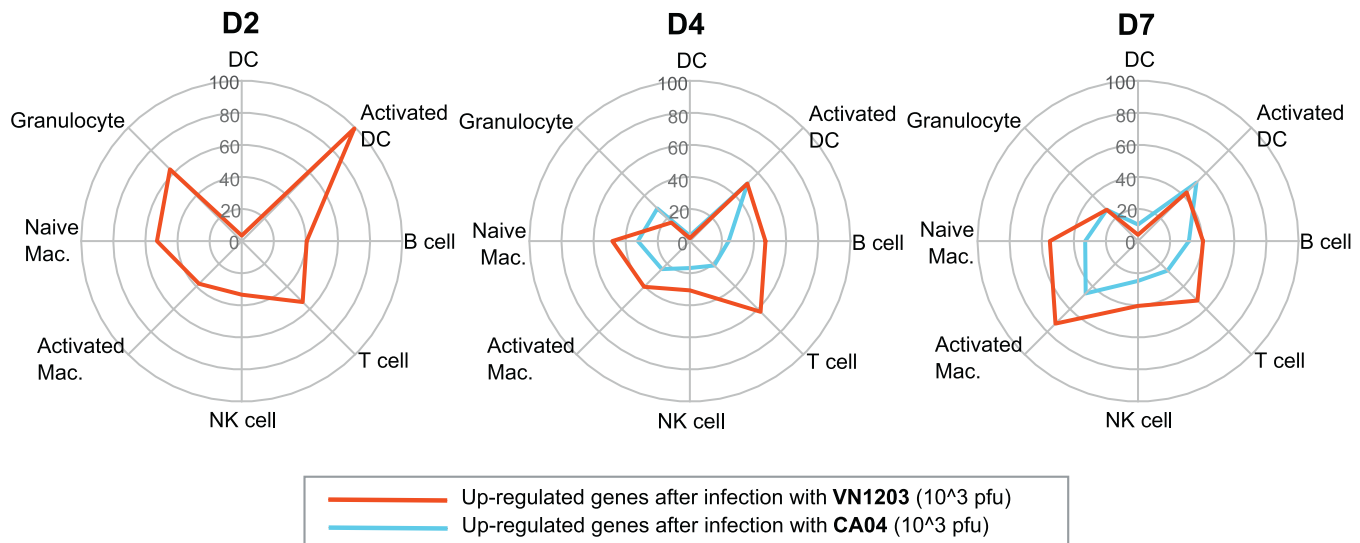


Fig. 4. Induced pulmonary genes after VN1203 infection are related to stimulated DC, macrophages and granulocytes. The radial plots represent enrichment scores for immune cell genes induced in mouse lung after infection with H1N1 A/California/04/09 (CA04) or VN1203 at day 2, 4 and 7 post-infection. Enrichment scores were calculated as a \log_{10} p -value, using a right-tailed Fisher's exact test. Genes specific to each immune cell subset were determined using ImmGen database (GSE15907), as genes significantly overexpressed in one cell subset compared to all other cells from this database. DC, dendritic cell; Mac., macrophage.

this interaction may have been published in a paper, but, most of the time, it is described in qualitative terms and for a specific biological condition. However, we need to know precisely when this interaction V–H occurs, with what affinity and which system. An example of cases of such known interactions between viral and host-factors are the binding of HA or NA and cellular SA receptors, which affinities can be modeled using Michaelis–Menten equations. Even if some models can consider known dependencies between molecules in terms of qualitative interaction as prior knowledge to integrate 'omics' (Choi and Pavelka, 2012), these data still need to be grouped in a database that can be queried automatically. Quantitative data relevant for influenza pathogenesis include microarray data, siRNA screen data, and virus–host protein–protein interactions (PPI), which can be downloaded from the literature, or public repositories (e.g. GEO or SRA). Given the importance of immune cell infiltration into the lung toward H5N1 pathogenesis, datasets from other sources to consider include the Immunological Genome Project (ImmGen) for immune cells subsets (Heng et al., 2008), the Mouse Gene Atlas for specific mouse tissues (Su et al., 2004), and the Immune Response In Silico (IRIS) database for humans (Abbas et al., 2005).

In addition to specific knowledge about H5N1, several databases supporting mammalian systems analyses provide general information on known biological pathways, PPIs, and protein functions. Biological pathways are sets of biochemical events that drive cellular processes and are listed in several repositories, including Reactome (D'Eustachio, 2011), KEGG (Kanehisa and Goto, 2000), Biocarta (<http://www.biocarta.com>), Gene Set Enrichment Analysis (Subramanian et al., 2005), and several commercial tools (e.g. Ingenuity Systems and GeneGo MetaCore). Biological ontologies, such as gene ontology (GO), characterize and describe gene products in a collection of three hierarchical ontologies, cellular component, biological process, and molecular function, that offers a much higher coverage of the genome (Ashburner et al., 2000). As most proteins act in complexes to regulate biological processes, it is of great interest to incorporate PPI data available in many public databases, such as STRING (Szklarczyk et al., 2011), BioGrid, or the Human Protein Reference Database (HPRD) (Keshava Prasad et al., 2009).

Several methods are available to analyze new experimental data in the context of known biology including Bayesian networks

(Husmeier and Werhli, 2007), Steiner trees (Huang and Fraenkel, 2009), and flux balance analysis (FBA) (Orth et al., 2010), but have yet to be applied to model H5N1 pathogenesis. Gene lists derived from statistical analysis have traditionally been used for functional enrichment analysis. More recently, we and others have used datasets from immune cell subsets to relate genes differentially expressed after vaccination (Nakaya et al., 2011), or infection (Josset et al., 2012a,b) with specific immune cells. By using a similar analysis, we show in Fig. 4 that up-regulated genes after VN1203 infection were related to infiltration with specific immune cell subsets. Especially, VN1203 induced early expression of genes associated with activated DC and granulocytes, and genes specific to T cell and activated macrophages later in infection. In contrast, genes induced by the lower pathogen A/California/04/09 (H1N1) were associated with activated DC and macrophages only after day 4, and to a more limited extent. Moreover, prior biological knowledge could also be used to integrate 'omics data to better model H5N1 infection, as performed with phosphoproteomic and transcriptomic data in a yeast system (Huang and Fraenkel, 2009).

3.4. Model refinement and validation

How robust is the system under different conditions? What part of the system is changing when viral mutations are introduced, or when host genes are disrupted? Can the model be used to predict other host–pathogen systems? These are just a few of the questions that arise and need to be addressed in the iterative cycles of systems biology.

One of the systems biology paradigms is that inferred models and prediction should be validated by biological experiments so that iterative cycles of modeling, prediction and perturbation would result in model refinement and future predictions (Kitano, 2002). This process has been successfully applied to murine primary dendritic cells to reconstruct regulatory networks controlling pathogen–host responses (Amit et al., 2009). Amit et al. (2009) identified a set of potential regulators from transcriptomic data of Toll-like receptor-treated cells, which were experimentally validated using lentiviral shRNA, and used to construct a model associating the regulators to their targets. This study identified core and fine-tuning regulators of inflammatory and antiviral programs.

As discussed earlier, using topological analysis of co-expression networks of transcriptomic profiles derived from mouse lungs infected with VN1203, we predicted potential regulators of the system, including *Tnfrsf1b* and *Ido1* genes that were validated using KO mice (McDermott et al., *in review*). Gene expression changes in KO mice compared to wild-type mice were then used to validate the global structure of the network. These results could be further analyzed to determine which parts of the predicted network are stable and if new regulators are found.

4. Challenges for building a global model of H5N1 pathogenesis

4.1. Host response dynamics are crucial: we need to model how host pathways change with time

Transcriptomic profiling of the host response to H5N1 infection has made it clear that both timing and magnitude of induction of the inflammatory response is a determinant of disease outcome. However, even if gene regulatory networks that have been inferred for H5N1 infected cells or infected tissue intrinsically hold information about temporal gene expression changes, they do not describe dependency between genes as a function of time. In addition, these models cannot predict dynamics of cellular pathway activation.

In modeling, description and simulation of changes in number or concentration of molecules with time is typically performed using equation-based models (EBM). Simple systems of ordinary differential equations (ODEs) have been used to describe *in vitro* influenza viral growth (Beauchemin et al., 2008; Heldt et al., 2012; Möhler et al., 2005; Sidorenko and Reichl, 2004). While these models gave important insight into viral replication properties, they did not consider activation of cellular pathways. Quantitatively assessing cellular pathway activation together with viral replication is difficult and has not extensively been studied to date. The activation of the P58^{IPK} pathway and its effect on viral replication have been described using a simple set of ODEs, but this model was limited due to variations in 3 host proteins (Goodman et al., 2011). Describing the dynamical behavior of several (or ideally, all) cellular pathways is crucial for modeling H5N1 disease, yet extremely challenging as it requires high-quality data on kinetic parameters. Dynamical modeling it has been applied to only a few simple systems, such as the bacterial SOS DNA repair system (Ronen et al., 2002). Recently, methods combining co-regulation network inference and ODE have been applied to simulate cell-wide regulatory network dynamics for *Escherichia coli* and *Saccharomyces cerevisiae* (Wang et al., 2011); but whether such methods are applicable to more complex organisms remains to be shown. Modelers will have to take a more prominent role in driving experimental designs to be able to generate sufficient data for building dynamical models. In addition to constructing transcriptomic-based models, collecting quantitative proteomic and phosphoproteomic data for H5N1-infected cells will be important for inferring dynamic representation of signaling pathways, similar to what was done for modeling the MAPK signaling pathway for example (Schoeberl et al., 2002; Hornberg et al., 2005).

At the level of the single infected cell, use of single-cell analysis (SCA) may be necessary to model pathway temporal activation. Arguably, the potential importance for performing SCA of infected cells is based on studies of NF- κ B activation patterns after TNF α stimulation. Notably, SCA revealed that NF- κ B activation follows an oscillation pattern (Nelson et al., 2004) and is heterogeneous with a digital process at the single-cell level (Tay et al., 2010). This dynamic is not apparent when analyzing NF- κ B activation averaged over the entire population. As NF- κ B is a major transcription factor

controlling the host response to H5N1 infection (Schmolke et al., 2009), analysis of infected single cells could be important to more precisely model the virus-host dynamic. In theory, recent advances in sequencing and MS technologies have made it possible to analyze the transcriptome, proteome and metabolome from a single cell (reviewed in Fritzsche et al., 2012). In the near future, performing SCA on H5N1 infected cells will most likely reveal important insights in specific dynamics of the host response to this virus.

At a higher scale (organism), it should be noted that several equation-based models have been used to describe influenza viral load evolution with time, without considering the host response (Baccam et al., 2006; Handel et al., 2007; Smith et al., 2011), or including the immune host response at different levels of complexity (Hancioglu et al., 2007; Handel et al., 2010; Lee et al., 2009a; Miao et al., 2010). Recently, Canini and Carrat (2011) combined structural equations and a statistical model fitted to H1N1 human data to characterize the dynamics of the infection, immune response, and illness in humans. These studies provide a quantitative understanding of the host immune response in controlling influenza virus replication, though the approach has not been applied specifically to H5N1 datasets. It would be particularly interesting to compare parameters derived from data from low-pathogenic viruses with data from H5N1 viruses, which could suggest molecular events responsible for the excessive virulence of H5N1. Experimental data in mice suggests the rapid replication of H5N1 overwhelms the immune response, especially the CD8+ T cell response that is otherwise protective, and in turn, kills the host by direct cytopathic effects (Hatta et al., 2010). It would be informative to model the relations between viral replication, CD8+ T cell responses and pathology in mice to understand these events more quantitatively.

4.2. Interactions between different cell types determine lung pathogenesis: modeling their behavior and localization is essential

During H5N1 infection, pathogenic events in the lung that contribute to acute respiratory distress syndrome (ARDS) include infection of type II pneumocytes and alveolar macrophages (van Riel et al., 2006), immune cell infiltration into the lung (Perrone et al., 2008), and bystander effects of cytokines and oxidative stress on lung cells (Peiris et al., 2009). However, the basis for communication between immune cells and structural elements of the lung during infection remain largely unknown, as well as the impact of infection on lung structure-function.

How does cell localization and lung microenvironment influence the response of similar cell types to infection? It is clear from studies of H5N1 pathogenesis that viral tropism (lower vs. upper respiratory tract) is crucial to determine virulence (Nicholls et al., 2007). In addition, host response after infection with H5N1 depends on the type and differentiation state of respiratory cells (Chan et al., 2010). Currently, *in vivo* omic studies have almost solely focused on analyzing whole lung tissue. From these studies, it is difficult to determine which cell types largely contribute to the host response and whether the microenvironment impacts this response. Similar cells from precise infectious foci in lung could be selected by using laser capture microdissection (LCM), an effective technique for harvesting pure cell populations from tissue sections. For instance, microarray profiling of LCM-selected stromal and epithelial compartments during breast cancer progression was used to study how the tumor environment drives tumorigenesis (Ma et al., 2009). Moreover, protocols for performing microproteomics on LCM-selected cells are available (Roulhac et al., 2011). Applied to H5N1 studies, LCM coupled with the sensitivity of RNA-Seq could allow transcriptomic profiling of infected cells, as well as surrounding immune and epithelial cells. Such data would open modeling

approaches to analysis of cell-to-cell interactions and better define the impact of each cell type during H5N1 pathogenesis.

Where are foci of infection in lung? At what rate do immune cells infiltrate the lung? Are these parameters related to outcome? Recent technology advances in live-animal imaging make it possible to analyze in real-time immune cell circulation and cellular movement in the mouse lung. Two-photon imaging of live mouse lung has shown trafficking of immune cells in pulmonary capillaries in real-time (Looney et al., 2010). This technique was able to measure the transit and velocity of neutrophils and naïve and activated T cells in normal lung and after intratracheal challenge with MIP-2 or LPS. The migratory activity measures represent important parameters that could be used to model behavior of immune cells in mouse lung. Manicassamy et al. (2010) have reported on the use of a recombinant PR8 NS1-GFP virus to monitor whole lung dynamics of influenza infection. They showed infection in the respiratory tract starts in areas close to large conducting airways and later spreads to deeper sections of the lungs. However, this imaging was done on excised lung at 4 dpi and not in real-time on live animals due to high background fluorescence (Manicassamy et al., 2010). This virus could be used with the two-photon imaging system described by Looney et al. (2010) to determine more in-depth infection rate parameters of different parts of the respiratory tract and ultimately, the traffick patterns of infected cells throughout the lung and in the periphery of the living organism. Alternatively, as signal-to-background is notably greater with bioluminescence than fluorescence, bioluminescence imaging (BLI) could be used to determine dynamics of influenza infection, given that construction of influenza virus expressing the luciferase reporter gene is possible, as demonstrated with Sindbis virus (Cook and Griffin, 2003). Interestingly, BLI has been used to quantify NF- κ B activation (Le Goffic et al., 2011) or IFN response (Pulverer et al., 2010) in live mouse lung after influenza infection. Through the use of these imaging techniques in live animals after H5N1 infection, we could begin to generate maps of whole mouse lung showing in real-time the localization and extent of infection within the lung and the dynamics of the immune response more precisely, which could then be used to model spatial and temporal responses to H5N1 virus.

Rule-based computational models, such as agent-based models (ABM) and cellular automata (CA), represent interesting models that could be used to investigate spatial aspects and cell-to-cell communication during H5N1 infection. CA models are dynamic simulation models where cell transitions are based on the state of the current cell and the states of neighboring cells, while in ABM, the model consists of a set of autonomous agents that interact with each other and the environment, taking into account actions according to a set of logical rules. Studies by Beauchemin et al. (2005) and Baccam et al. (2006) used a simple two-dimensional CA model consisting of fixed epithelial cells and mobile immune cells to model an infection with influenza A virus. In this model, initial spatial distribution of infected cells had an important effect on outcome of infection. Recently, a CA model was used together with ODEs fitted to viral titers observed in primary normal human bronchial epithelial (NHBE) cells to estimate virus productivity per cell and viral spread through the cell monolayer (Mitchell et al., 2011). These models, however, did not model how the different infiltrating immune cells interact with each other and with infected epithelial cells. ABM and CA have been largely used in immunology to represent cell-to-cell interactions that occur during the immune response (for a review, see Chavali et al., 2008). For instance, Folcik et al. (2011) have developed an ABM of the immune system with representations of the immune and tissue cells as agents, cytokines, chemokines, or pathogens as signals, and parenchymal tissue, secondary lymphoid tissue and the lymphatic circulation as three virtual zones. Using this model the authors

found that dendritic cells acted as hubs in the immune system network after challenge. Such a model has not been developed for influenza virus to date, but developing a model for H5N1 may help to elucidate which cytokines determine immune cell infiltration into the lung as well as which immune cells act as key players of immunopathology. Such a model could also simulate the effect of disrupting a specific signal or cell on the infection dynamics.

Another important parameter that has not yet been taken into account in our models of infection is the structure of the lung. How does H5N1 virus affect lung structure and function? Currently, there is no model of lung structure-function modification as a determinant of disease outcome. And yet, several hallmarks of respiratory infection, including hyaline membrane formation resulting from coagulation activation and immune cell infiltration, are disrupting the architecture of the lung, leading to acute lung injury and ARDS. It is crucial that we start modeling infection in terms of modification of the lung structure. In particular, this effort could support the framework of the Lung Physiome project that has developed integrative models of lung structure-function at different levels of biological organization (reviewed in Burrowes et al., 2008; Tawhai et al., 2011).

4.3. Each physiological scale of the host response is contributing to disease: multiscale approaches have to be developed.

As described in Fig. 2, the host response to infection displays a large range of temporal (from seconds to days) and spatial scales (from molecules to the whole organism). Most of the work on host responses to H5N1 infection has been focused on two main spatial scales, the cellular *in vitro* dimension and the whole lung *in vivo* dimension. Models focused on the single molecule level are important for decoding the function of specific virulence factors, but have been developed, to date, mostly to describe NA enzymatic activity (Ilyushina et al., 2010) or the interaction between HA and cellular SA receptors (Richard et al., 2012), as described earlier. Such models are useful in order to quantifiably assess how variations in virus sequence impact virus-host dynamics, which will be necessary to develop for other virulence factors.

At the cellular levels, *in vitro* profiling of the host response to H5N1 has been performed using models such as homogeneous epithelial cell lines (Josset et al., 2010; Li et al., 2011), primary endothelial or epithelial cells (Chan et al., 2010; Schmolke et al., 2009), or primary macrophages (Cheung et al., 2012, 2002; Lee et al., 2009c). However, immune cells and resident pulmonary cells also contribute to H5N1-induced lung disease and it is important to determine their responses during infection. Experimentally, one could sort the main types of lung cells by FACS or other sorting methods (e.g. magnetic bead-based sorting methods) and perform 'omic analysis on sorted cell populations. Transcriptomic profiling of sorted cells from blood has been done by several laboratories (Nakaya et al., 2011; Novershtern et al., 2011). It is more challenging to sort cells from a tissue compared to blood because RNA, metabolites or peptides can be sensitive to sampling-related manipulations and several approaches have been proposed to limit degradation (Rubakhin et al., 2011).

At the organismal level, the host response to H5N1 has been extensively studied in the whole lung. One current challenge is to elucidate how signaling networks studied in an *in vitro* cell culture system relate to *in vivo* organ networks, as well as whether models trained on data from infected cells can accurately predict outcome of the whole organism. This has been suggested from a multivariate modeling approach that identified similarities in transcriptional responses to H5N1 virus in the lungs of mice and macaques infected with H5N1 virus, and human lung epithelial cells (McDermott et al., 2011). Given H5N1 systemic dissemination, studying the response

of other organs and modeling systemic inflammation might also be necessary to predict disease outcome.

To bridge *in vivo* and *in vitro* models and build a global model of H5N1 infection, we need to apply methods that can link models at different spatial scales and over different temporal scales. Multiscale models have received greater interest in the past few years (Meier-Schellersheim et al., 2009) but they have not been applied to H5N1 systems to date. A few modeling platforms have been developed that can handle the coordination of coupled models whose component modules may be expressed using different formalisms (e.g., EBM, CA, ABM, or other discrete approaches) (see the review of Sloom and Hoekstra (2010) for multiscale models). Several studies concerning other respiratory diseases could be of interest for future H5N1 applications. As an example of disruption of lung function, one model that bridges three different spatial scales has been developed for simulating pulmonary gas exchange during hepatopulmonary syndrome (Chakraborty et al., 2007). As an example of multiscale modeling of host-pathogen interaction in the lung, Fallahi-Sichani et al. (2011) developed a model that combined ABM to represent cellular and tissue scale events and ODE to represent the single-cell molecular scale during granuloma formation in lung after *Mycobacterium tuberculosis* (Mtb) infection. This 2D model does not take into account the structure of the lung and considers only a set of cells relevant for Mtb infection, but was able nevertheless to identify key processes that control TNF and bacterial level interplays in a granuloma. Together with bridging *in vivo* and *in vitro* models, multiscale models for H5N1 disease could address important specific questions, as, for instance, how many infected cells in the upper or lower respiratory tract are necessary for efficient viral transmission?, or is there a threshold in viral replication rate in the lower respiratory tract that determines fatal outcome?

4.4. Host genetics shapes disease severity: integrating genetics into the H5N1 disease model

It is becoming increasingly clear that host genetics influences the pathogenesis of infectious disease (Aouizerat et al., 2011; Newport and Finan, 2011; Wurfel, 2008). Clinical disease associated with influenza infection can range from asymptomatic infection to severe respiratory disease, including the onset of ARDS. The basis for a genetic predisposition to fatal influenza was demonstrated through a genealogical assessment of a Utah Population Database (Albright et al., 2008). Familial aggregation can be a hallmark of genetically determined disease that has led some to postulate a heritable contribution explaining H5N1 infection observed among blood relatives for three Indonesian clusters in 2005 (Kandun et al., 2008; Olsen et al., 2005; WHO, 2010). A compilation of confirmed H5N1 cases worldwide found that, on average, 22% of cases occurred in clusters, and only 6% of cases within the clusters were not genetically related to other cluster members (Horby et al., 2010). In addition, acute encephalopathy is a rare complication of influenza infection, which is more commonly reported in Japan and East-Asia, and with some family cases (de Jong et al., 2005; Prasun and Stockton, 2012). This complication has been associated with a missense mutation in TLR3 (Hidaka et al., 2006) and with the observation of reduced enzyme activity of carnitine palmitoyltransferase II (CPT-II) related to a polymorphism in the CPT-II gene in Japanese (Chen et al., 2005) and Chinese patients (Mak et al., 2011). Moreover, several human genetic variants have been recently associated with severe pneumonia caused by 2009 H1N1 infection (Antonopoulou et al., 2012; Everitt et al., 2012; Zhou et al., 2012; Zúñiga et al., 2012). GWAS studies have supported a genetic basis of infectious disease susceptibility in humans (Chapman and Hill, 2012), and international collaborative projects like the 1000 Genomes Project

initiative aim to provide further data on sequence variation and haplotype structure of multiple human populations (Consortium, 2010).

Studies using inbred laboratory mouse strains have also highlighted significant variation in clinical disease and immune responses observed during influenza infection, depending on the mouse genetic background. Differential host responses have been investigated using a variety of inbred laboratory strains showing differences in susceptibility phenotypes (Alberts et al., 2010; Boon et al., 2011; Otte et al., 2011; Srivastava et al., 2009). Boon et al. (2011) showed H5N1 infection of a panel of 21 inbred mouse strains results in variable disease phenotypes, and further inspection of 6 H5N1 virus-infected inbred mouse strains, DBA/2, 129/SvIm, A/J, SM, C57BL/6, and BALB/c, revealed SM, C57BL/6 and BALB/c mice were more resistant to H5N1 infection, whereas DBA/2, 129/SvIm and A/J mouse strains were more susceptible to H5N1 infection. The strains that were more susceptible to infection had higher viral loads in the lung and increased production of pro-inflammatory mediators, such as CCL2, IFN β , and TNF. In contrast, the resistant mouse strains had reduced virus titers in the lung that resulted in lower production of pro-inflammatory mediators and less lung immunopathology. Boon et al. (2011) concluded that the genetic component of susceptible hosts controlling disease severity is primarily influencing viral replication, though it can also be argued that the genetic component of susceptible hosts causes mice to mount an uncontrolled or overly aggressive immune response with pathologic consequences.

As complex genetic traits are involved in the host response to influenza infection, new resources such as the Collaborative Cross (CC) mouse resource are providing unique opportunities to probe the contribution of host genetics to infectious disease susceptibility in greater detail (Churchill et al., 2004). The CC is a recombinant inbred mouse resource designed to capture the genetic heterogeneity of the human population, supporting systems genetics studies. The mouse resource is being utilized to identify host genetic determinants regulating resistance or susceptibility to respiratory virus infection among other phenotypic variances (Aylor et al., 2011). Host genetic components underlying complex traits associated with infection can be explored by quantitative trait loci (QTL) and expression QTL (eQTL) analysis. Initial studies in pre-CC mice, incompletely inbred animals from CC lines, showed that there was wide phenotypic variation in influenza and SARS-CoV-associated disease, such as significant differences in weight loss, viral lung titers, and immune cell infiltration at 4 dpi (Ferris et al., 2013). Bottomly et al. (2012) used pre-CC mice to elucidate the genetic control of eQTL in mice with extreme response to influenza infection. Significant eQTL were identified that allowed examination of genes associated with regulation of host response to infection. The pattern of allele effects across the eight founder animal haplotypes used in conjunction with whole-genome sequences (Keane et al., 2011) will likely reveal candidate SNPs that can be validated experimentally in fully recombinant inbred intercrosses (RIX) that are developed in the future.

Susceptibility allele identification is only a starting point, the next challenge will be to understand and model the roles these genomic variations may have in shaping severe influenza infection. The CC resource will be instrumental toward this goal, allowing for large-scale and targeted modeling efforts, which should benefit from other methods developed in system genetics (as in Lee et al., 2009b or Ayroles et al., 2009). These methods focus on understanding how genetic information is integrated and coordinated from the molecular scale to the phenotypic scales. In addition to genetics, environmental and co-morbidity factors also need to be taken into account in order to build more comprehensive and predictive models of H5N1 disease in humans.

4.5. Causation over correlation: the necessity for predictive models

The ultimate overarching goal of systems biology models for H5N1 is to lead to new antiviral therapies or patient care management strategies that would prevent severe cases of H5N1 infection. Analysis of transcriptomic data has led to the testing of several drugs that target the cellular response in an effort to identify immunomodulatory drugs that potentially inhibit H5N1 replication (Cameron et al., 2008; Josset et al., 2010). These studies were based on the simple idea that reverting or stopping the host response induced by the virus will inhibit its replication. However, targeting the genes that are associated with infection does not always prevent infection. For instance, H5N1 lethality is associated with high induction of cytokines, but deletion of specific cytokine genes, including IL-6, MIP1 α , IL1-R and TNF-R1 genes, quite unexpectedly displayed different phenotypes in knock-out (KO) mice after infection with H5N1 viruses (Szretter et al., 2007). While IL-6 and MIP1 α KO mice had no difference in morbidity, IL1-R KO mice exhibited increased mortality and TNF-R1 KO mice showed reduced mortality, protecting animals from lethal infection. This study illustrates the well-known maxim that “correlation is not causation” and that cellular pathways are redundant and might have unknown facets. Redundancy in cellular pathways was especially obvious by looking at the host response of IFN β KO mice to VN1203 (Fig. 1). Even in the absence of the IFN receptor, these mice elicited steady and sustained activation of type I IFN-related genes, leading to a high inflammation that was associated with death (Cilloniz et al., 2010).

For drug development, we need to build models that are able to predict the effect of disrupting one or more cellular signaling pathways or genes. For now, models that have been inferred by profiling the transcriptional response from whole mouse lung have predicted several significant regulators of the host response to H5N1 infection (McDermott et al., *in review*); however, these models are unable to predict the effect of their disruption on outcome. For example, both Ido1 (Fig. 3) and Kepi genes were identified as regulators of H5N1 pathogenesis, yet deletion of Ido1 protected mice against VN1203 and SARS-CoV, while Kepi disruption increased SARS-CoV susceptibility. To be able to predict the behavior of a system from a gene regulatory network, causal relationships are needed. In terms of reverse-engineering gene regulatory networks, perturbations of network variables are required to infer causation networks (Maathuis et al., 2010). These perturbations could result from KO (*in vivo*) or siRNA (*in vitro*) experiments. However, single gene deletion may miss or under-estimate the role of a gene in the case of functional redundancy and synergy between genes. As a result, we need multiple perturbations of the system. Genetic polymorphisms in a segregating population are ideal settings for multifactorial perturbations of a living system, particularly as each allele is a potential source of perturbation (Rockman, 2008). Several modeling approaches, such as Bayesian networks (Vignes et al., 2011; Xing et al., 2011; Zhu et al., 2007) and structural equation modeling (SEM) (Aten et al., 2008; Liu et al., 2008), have been developed to infer causal networks from system genetics experiments. Applying these methods to ‘omics’ data from CC mice infected with H5N1 will not only link susceptibility alleles to phenotypes, but it will also infer a better predictive model that could be used to orient new treatment and predict therapeutic efficacy.

5. Conclusions

Our understanding of H5N1 pathogenesis has benefited from extensive studies at the molecular, genomic, physiologic and histologic levels. The major challenge that we are now facing is how

to integrate this information in a quantitative and predictive model of H5N1 disease that will inform patient care and accelerate drug development. Systems biology for H5N1 aims to systematically quantify and integrate every level of the host response to infection from scales spanning the single cell to the whole organism, in an effort to build global models of H5N1 disease. We expect that an integrative H5N1 model will utilize several of the approaches outlined in this review, such as causality networks or multiscale modeling, and that this will require new types of data that are being generated through advancement of technologies. For example, single-cell analysis could be used to model events occurring at the level of one or more virus particles and the target cell. At higher spatial levels, efforts such as the Lung Physiome have modeled the relationships between lung function and the organ structure. This information could, in turn, be used as a scaffold to model the location of H5N1 infection and pathologic events leading to ARDS, such as immune cell infiltration and hyaline membrane formation. Finally, the CC mice represent multi-factorial perturbations of the systems that could be used to refine our models.

Current capabilities of computational biology are illustrated by the first whole-cell computational model that simulates the entire cell cycle of a living organism (Karr et al., 2012). We envision that systems biology will be able to provide computational models of H5N1 disease and reveal targets for intervention that would most likely impact the course of clinical disease for a given patient and virus strain. This would be a large step toward the direction of personalized medicine.

Acknowledgments

The authors thank Marcus Korth and Stewart Chang for valuable feedback on the manuscript. We also thank Patrick Lane of ScEYence Studios for his illustration (Fig. 2), and Ilhem Messaoudi and Balaji Manicassamy for contributing images to the illustration. We thank Nicolas Tchitcheck and Sean Proll for their contribution to figures. This project was funded in part by federal funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, under contract HHSN272200800060C (M.G.K.).

References

- Abbas, A.R., Baldwin, D., Ma, Y., Ouyang, W., Gurney, A., Martin, F., Fong, S., Campagne, M.V.L., Godowski, P., Williams, M., Chan, A.C., Clark, H.F., 2005. Immune response in silico (IRIS): immune-specific genes identified from a compendium of microarray expression data. *Genes and Immunity* 6 (4), 319–331.
- Abdel-Ghaffar, A.N., Chotpitayasunondh, T., Gao, Z., Hayden, F.G., Nguyen, D.H., de Jong, M.D., Naghdaliyev, A., Peiris, J.S., Shindo, N., Soeroso, S., Uyeki, T.M., 2008. Update on avian influenza A (H5N1) virus infection in humans. *New England Journal of Medicine* 358 (3), 261–273.
- Aderem, A., Adkins, J.N., Ansong, C., Galagan, J., Kaiser, S., Korth, M.J., Law, G.L., McDermott, J.G., Proll, S.C., Rosenberger, C., Schoolnik, G., Katze, M.G., 2011. A systems biology approach to infectious disease research: innovating the pathogen-host research paradigm. *MBio* 2 (1), e00325–10. <http://dx.doi.org/10.1128/mBio.00325-10>.
- Aebersold, R., Mann, M., 2003. Mass spectrometry-based proteomics. *Nature* 422 (6928), 198–207.
- Alberts, R., Srivastava, B., Wu, H., Viegas, N., Geffers, R., Klawonn, F., Novoselova, N., do Valle, T.Z., Panthier, J.J., Schughart, K., 2010. Gene expression changes in the host response between resistant and susceptible inbred mouse strains after influenza A infection. *Microbes and Infection* 12 (4), 309–318.
- Albright, F.S., Orlando, P., Pavia, A.T., Jackson, G.G., Cannon Albright, L.A., 2008. Evidence for a heritable predisposition to death due to influenza. *Journal of Infectious Diseases* 197 (1), 18–24.
- Amit, I., Garber, M., Chevrier, N., Leite, A.P., Donner, Y., Eisenhaure, T., Guttman, M., Grenier, J.K., Li, W., Zuk, O., Schubert, L.A., Birditt, B., Shay, T., Goren, A., Zhang, X., Smith, Z., Deering, R., McDonald, R.C., Cabili, M., Bernstein, B.E., Rinn, J.L., Meissner, A., Root, D.E., Hacohen, N., Regev, A., 2009. Unbiased reconstruction of a mammalian transcriptional network mediating pathogen responses. *Science* 326 (5950), 257–263.
- Antonopoulou, A., Baziaka, F., Tsaganos, T., Raftogiannis, M., Koutoukas, P., Spyridaki, A., Mouktaroudi, M., Kotsaki, A., Savva, A., Georgitsi, M., Giamarellos-Bourboulis, E.J., 2012. Role of tumor necrosis factor gene single nucleotide polymorphisms

- in the natural course of 2009 influenza A H1N1 virus infection. *International Journal of Infectious Diseases* 16 (3), e204–e208.
- Aouizerat, B.E., Pearce, C.L., Miaszkowski, C., 2011. The search for host genetic factors of HIV/AIDS pathogenesis in the post-genome era: progress to date and new avenues for discovery. *Current HIV/AIDS Reports* 8 (1), 38–44.
- Ashburner, M., Ball, C.A., Blake, J.A., Botstein, D., Butler, H., Cherry, J.M., Davis, A.P., Dolinski, K., Dwight, S.S., Eppig, J.T., Harris, M.A., Hill, D.P., Issel-Tarver, L., Kasarskis, A., Lewis, S., Matese, J.C., Richardson, J.E., Ringwald, M., Rubin, G.M., Sherlock, G., 2000. Gene ontology: tool for the unification of biology. *Nature Genetics* 25 (1), 25–29.
- Aten, J.E., Fuller, T.F., Lusic, A.J., Horvath, S., 2008. Using genetic markers to orient the edges in quantitative trait networks: the NEO software. *BMC Systems Biology* 2 (1), 34.
- Aylor, D.L., Valdar, W., Foulds-Mathes, W., Buus, R.J., Verdugo, R.A., Baric, R.S., Ferris, M.T., Frelinger, J.A., Heise, M., Frieman, M.B., Gralinski, L.E., Bell, T.A., Didion, J.D., Hua, K., Nehrenberg, D.L., Powell, C.L., Steigerwalt, J., Xie, Y., Kelada, S.N., Collins, F.S., Yang, I.V., Schwartz, D.A., Branstetter, L.A., Chesler, E.J., Miller, D.R., Spence, J., Liu, E.Y., McMillan, L., Sarkar, A., Wang, J., Wang, W., Zhang, Q., Broman, K.W., Korstanje, R., Durrant, C., Mott, R., Iraqi, F.A., Pomp, D., Threadgill, D., de Villena, F.P., Churchill, G.A., 2011. Genetic analysis of complex traits in the emerging Collaborative Cross. *Genome Research* 21 (8), 1213–1222.
- Ayroles, J.F., Carbone, M.A., Stone, E.A., Jordan, K.W., Lyman, R.F., Magwire, M.M., Rollmann, S.M., Duncan, L.H., Lawrence, F., Anholt, R.R., Mackay, T.F., 2009. Systems genetics of complex traits in *Drosophila melanogaster*. *Nature Genetics* 41 (3), 299–307.
- Baccam, P., Beauchemin, C., Macken, C.A., Hayden, F.G., Perelson, A.S., 2006. Kinetics of Influenza A virus infection in humans. *Journal of Virology* 80 (15), 7590–7599.
- Baskin, C.R., Bielefeldt-Ohmann, H., Tumpey, T.M., Sabourin, P.J., Long, J.P., García-Sastre, A., Tolnay, A.-E., Albrecht, R., Pyles, J.A., Olson, P.H., Aicher, L.D., Rosenzweig, E.R., Murali-Krishna, K., Clark, E.A., Kotur, M.S., Fornek, J.L., Proll, S., Palermo, R.E., Sabourin, C.L., Katze, M.G., 2009. Early and sustained innate immune response defines pathology and death in nonhuman primates infected by highly pathogenic influenza virus. *Proceedings of the National Academy of Sciences of the United States of America* 106 (9), 3455–3460.
- Beauchemin, C., Samuel, J., Tuszyński, J., 2005. A simple cellular automaton model for influenza A viral infections. *Journal of Theoretical Biology* 232 (2), 223–234.
- Beauchemin, C.A.A., McSharry, J.J., Drusano, G.L., Nguyen, J.T., Went, G.T., Ribeiro, R.M., Perelson, A.S., 2008. Modeling amantadine treatment of Influenza A virus in vitro. *Journal of Theoretical Biology* 254 (2), 439–451.
- Boivin, S., Cusack, S., Ruigrok, R.W.H., Hart, D.J., 2010. Influenza A virus polymerase: structural insights into replication and host adaptation mechanisms. *Journal of Biological Chemistry* 285 (37), 28411–28417.
- Boon, A.C.M., Finkelstein, D., Zheng, M., Liao, G., Allard, J., Klumpp, K., Webster, R., Peltz, G., Webby, R.J., 2011. H5N1 influenza virus pathogenesis in genetically diverse mice is mediated at the level of viral load. *mBio* 2 (5).
- Bottomly, D., Ferris, M.T., Aicher, L.D., Rosenzweig, E., Whitmore, A., Aylor, D.L., Haagsmans, B.L., Gralinski, L.E., Bradel-Tretheway, B.G., Bryan, J.T., Threadgill, D.W., de Villena, F.P.-M., Baric, R.S., Katze, M.G., Heise, M., McWeeney, S.K., 2012. Expression quantitative trait loci for extreme host response to Influenza A in pre-collaborative cross mice. *G3: Genes/Genomes/Genetics* 2 (2), 213–221.
- Bradel-Tretheway, B.G., Mattiaccio, J.L., Krasnoselsky, A., Stevenson, C., Purdy, D., Dewhurst, S., Katze, M.G., 2011. Comprehensive proteomic analysis of influenza virus polymerase complex reveals a novel association with mitochondrial proteins and RNA polymerase accessory factors. *Journal of Virology* 85 (17), 8569–8581.
- Brass, A.L., Huang, I.C., Benita, Y., John, S.P., Krishnan, M.N., Feeley, E.M., Ryan, B.J., Weyer, J.L., van der Weyden, L., Fikrig, E., Adams, D.J., Xavier, R.J., Farzan, M., Elledge, S.J., 2009. The IFITM proteins mediate cellular resistance to influenza A H1N1 virus, West Nile virus, and dengue virus. *Cell* 139 (7), 1243–1254.
- Brown, J.N., Palermo, R.E., Baskin, C.R., Gritsenko, M., Sabourin, P.J., Long, J.P., Sabourin, C.L., Bielefeldt-Ohmann, H., García-Sastre, A., Albrecht, R., Tumpey, T.M., Jacobs, J.M., Smith, R.D., Katze, M.G., 2010. Macaque proteome response to highly pathogenic avian influenza and 1918 reassortant influenza virus infections. *Journal of Virology* 84, 12058–12068.
- Burrowes, K.S., Swan, A.J., Warren, N.J., Tawhai, M.H., 2008. Towards a virtual lung: multi-scale, multi-physics modelling of the pulmonary system. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences* 366 (1879), 3247–3263.
- Cameron, C.M., Cameron, M.J., Bermejo-Martin, J.F., Ran, L., Xu, L., Turner, P.V., Ran, R., Danesh, A., Fang, Y., Chan, P.-K.M., Mytelle, N., Sullivan, T.J., Collins, T.L., Johnson, M.G., Medina, J.C., Rowe, T., Kelvin, D.J., 2008. Gene expression analysis of host innate immune responses during lethal H5N1 infection in ferrets. *Journal of Virology* 82 (22), 11308–11317.
- Canini, L., Carrat, F., 2011. Population modeling of influenza A/H1N1 virus kinetics and symptom dynamics. *Journal of Virology* 85 (6), 2764–2770.
- Chakraborty, S., Balakotaiah, V., Bidani, A., 2007. Multiscale model for pulmonary oxygen uptake and its application to quantify hypoxemia in hepatopulmonary syndrome. *Journal of Theoretical Biology* 244 (2), 190–207.
- Chan, R.W.Y., Yuen, K.M., Yu, W.C.L., Ho, C.C.C., Nicholls, J.M., Peiris, J.S.M., Chan, M.C.W., 2010. Influenza H5N1 and H1N1 virus replication and innate immune responses in bronchial epithelial cells are influenced by the state of differentiation. *PLoS ONE* 5 (1), e8713. <http://dx.doi.org/10.1371/journal.pone.008713>.
- Chang, S.T., Tchitchek, N., Ghosh, D., Bencke, A., Katze, M.G., 2011. A chemokine gene expression signature derived from meta-analysis predicts the pathogenicity of viral respiratory infections. *BMC Systems Biology* 5, 202.
- Chapman, S.J., Hill, A.V., 2012. Human genetic susceptibility to infectious disease. *Nature Reviews Genetics* 13 (3), 175–188.
- Chavali, A.K., Gianchandani, E.P., Tung, K.S., Lawrence, M.B., Peirce, S.M., Papin, J.A., 2008. Characterizing emergent properties of immunological systems with multi-cellular rule-based computational modeling. *Trends in Immunology* 29 (12), 589–599.
- Chen, W., Calvo, P.A., Malide, D., Gibbs, J., Schubert, U., Bacik, I., Basta, S., O'Neill, R., Schickli, J., Palese, P., Henklein, P., Bennink, J.R., Yewdell, J.W., 2001. A novel influenza A virus mitochondrial protein that induces cell death. *Nature Medicine* 7 (12), 1306–1312.
- Chen, Y., Mizuguchi, H., Yao, D., Ide, M., Kuroda, Y., Shigematsu, Y., Yamaguchi, S., Yamaguchi, M., Kinoshita, M., Kido, H., 2005. Thermolabile phenotype of carnitine palmitoyltransferase II variations as a predisposing factor for influenza-associated encephalopathy. *FEBS Letters* 579 (10), 2040–2044.
- Cheung, C.Y., Chan, E.Y., Krasnoselsky, A., Purdy, D., Navare, A.T., Bryan, J.T., Leung, C.K., Hui, K.P., Peiris, J.S., Katze, M.G., 2012. H5N1 virus causes significant perturbations in host proteome very early in influenza virus-infected primary human monocyte-derived macrophages. *Journal of Infectious Diseases* 206 (5), 640–645.
- Cheung, C.Y., Poon, L.L.M., Lau, A.S., Luk, W., Lau, Y.L., Shortridge, K.F., Gordon, S., Guan, Y., Peiris, J.S.M., 2002. Induction of proinflammatory cytokines in human macrophages by influenza A (H5N1) viruses: a mechanism for the unusual severity of human disease? *Lancet* 360 (9348), 1831–1837.
- Choi, H., Pavelka, N., 2012. When one and one gives more than two: challenges and opportunities of integrative omics. *Frontiers in Genetics* 2, 00105.
- Churchill, G.A., Airey, D.C., Allayee, H., Angel, J.M., Attie, A.D., Beatty, J., Beavis, W.D., Belknap, J.K., Bennett, B., Berrettini, W., Bleich, A., Bogue, M., Broman, K.W., Buck, K.J., Buckler, E., Burmeister, M., Chesler, E.J., Cheverud, J.M., Clapcote, S., Cook, M.N., Cox, R.D., Crabbe, J.C., Crusio, W.E., Darvas, A., Deschepper, C.F., Doerge, R.W., Farber, C.R., Forejt, J., Gaile, D., Garlow, S.J., Geiger, H., Gershenfeld, H., Gordon, T., Gu, J., Gu, W., de Haan, G., Hayes, N.L., Heller, C., Himmelbauer, H., Hitzemann, R., Hunter, K., Hsu, H.C., Iraqi, F.A., Ivandic, B., Jacob, H.J., Jansen, R.C., Jepsen, K.J., Johnson, D.K., Johnson, T.E., Kempermann, G., Kendziorski, C., Kotb, M., Kooy, R.F., Llamas, B., Lammert, F., Lassalle, J.M., Lowenstein, P.R., Lu, L., Lusic, A., Manly, K.F., Marcucio, R., Matthews, D., Medrano, J.F., Miller, D.R., Mittleman, G., Mock, B.A., Mogil, J.S., Montagutelli, X., Morahan, G., Morris, D.G., Mott, R., Nadeau, J.H., Nagase, H., Nowakowski, R.S., O'Hara, B.F., Osadchuk, A.V., Page, G.P., Paigen, B., Paigen, K., Palmer, A.A., Pan, H.J., Peltonen-Palotie, L., Peirce, J., Pomp, D., Pravenec, M., Prows, D.R., Qi, Z., Reeves, R.H., Roder, J., Rosen, G.D., Schadt, E.E., Schalkwyk, L.C., Seltzer, Z., Shimomura, K., Shou, S., Sillanpaa, M.J., Siracusa, L.D., Snoeck, H.W., Spearow, J.L., Svenson, K., et al., 2004. The Collaborative Cross, a community resource for the genetic analysis of complex traits. *Nature Genetics* 36 (11), 1133–1137.
- Cilloniz, C., Pantin-Jackwood, M.J., Ni, C., Goodman, A.G., Peng, X., Proll, S.C., Carter, V.S., Rosenzweig, E.R., Szretter, K.J., Katz, J.M., Korth, M.J., Swayne, D.E., Tumpey, T.M., Katze, M.G., 2010. Lethal dissemination of H5N1 influenza virus is associated with dysregulation of inflammation and lipoxin signaling in a mouse model of infection. *Journal of Virology* 84 (15), 7613–7624.
- Cillóniz, C., Shinya, K., Peng, X., Korth, M.J., Proll, S.C., Aicher, L.D., Carter, V.S., Chang, J.H., Kobasa, D., Feldmann, F., Strong, J.E., Feldmann, H., Kawaoka, Y., Katze, M.G., 2009. Lethal influenza virus infection in macaques is associated with early dysregulation of inflammatory related genes. *PLoS Pathogens* 5 (10).
- Conenello, G.M., Tisoncik, J.R., Rosenzweig, E., Varga, Z.T., Palese, P., Katze, M.G., 2011. A single N665 mutation in the PB1-F2 protein of Influenza A virus increases virulence by inhibiting the early interferon response in vivo. *Journal of Virology* 85 (2), 652–662.
- Conenello, G.M., Zamarin, D., Perrone, L.A., Tumpey, T., Palese, P., 2007. A single mutation in the PB1-F2 of H5N1 (HK/97) and 1918 Influenza A viruses contributes to increased virulence. *PLoS Pathogens* 3 (10), e141.
- Consortium, G.P., 2010. A map of human genome variation from population-scale sequencing. *Nature* 467 (7319), 1061–1073.
- Cook, S.H., Griffin, D.E., 2003. Luciferase imaging of a neurotropic viral infection in intact animals. *Journal of Virology* 77 (9), 5333–5338.
- Cox, J., Mann, M., 2011. Quantitative, high-resolution proteomics for data-driven systems biology. *Annual Review of Biochemistry* 80, 273–299.
- D'Eustachio, P., 2011. Reactome knowledgebase of human biological pathways and processes. *Methods in Molecular Biology* (Clifton, NJ) 694, 49–61.
- de Jong, M.D., Bach, V.C., Phan, T.Q., Vo, M.H., Tran, T.T., Nguyen, B.H., Beld, M., Le, T.P., Truong, H.K., Nguyen, V.V.C., Tran, T.H., Do, Q.H., Farrar, J., 2005. Fatal avian influenza A (H5N1) in a child presenting with diarrhea followed by coma. *The New England Journal of Medicine* 352 (7), 686–691.
- de Jong, M.D., Simmons, C.P., Thanh, T.T., Hien, V.M., Smith, G.J.D., Chau, T.N.B., Hoang, D.M., Chau, N.V.V., Khanh, T.H., Dong, V.C., Qui, P.T., Cam, B.V., Ha, D.Q., Guan, Y., Peiris, J.S.M., Chinh, N.T., Hien, T.T., Farrar, J., 2006. Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia. *Nature Medicine* 12 (10), 1203–1207.
- Eisfeld, A.J., Teuton, J.R., Li, C., Heegel, R.A., Luna, M.L., Schepmoes, A.A., Monroe, M.E., Jacobs, J.M., Matzke, M.M., Webb-Robertson, B.-J.M., Tilton, S.C., Law, L., Neumann, G., Katze, M.G., Kawaoka, Y., Smith, R.D., Waters, K.M., Metz, T.O. Integrated proteomics of epithelial cells infected with highly pathogenic avian influenza virus reveals novel inflammatory and cytopathic mechanisms. *PLoS Pathogens*, in review.
- Engelhardt, O.G., Smith, M., Fodor, E., 2005. Association of the Influenza A virus RNA-dependent RNA polymerase with cellular RNA polymerase II. *Journal of Virology* 79 (9), 5812–5818.

- Everitt, A.R., Clare, S., Pertel, T., John, S.P., Wash, R.S., Smith, S.E., Chin, C.R., Feeley, E.M., Sims, J.S., Adams, D.J., Wise, H.M., Kane, L., Goulding, D., Digard, P., Anttila, V., Baillie, J.K., Walsh, T.S., Hume, D.A., Palotie, A., Xue, Y., Colonna, V., Tyler-Smith, C., Dunning, J., Gordon, S.B., Investigators, T.G., Investigators, T.M., Smyth, R.L., Openshaw, P.J., Dougan, G., Brass, A.L., Kellam, P., 2012. IFITM3 restricts the morbidity and mortality associated with influenza. *Nature* 484 (7395), 519–523.
- Faith, J.J., Hayete, B., Thaden, J.T., Mogno, I., Wierzbowski, J., Cottarel, G., Kasif, S., Collins, J.J., Gardner, T.S., 2007. Large-scale mapping and validation of *Escherichia coli* transcriptional regulation from a compendium of expression profiles. *PLoS Biology* 5, 1.
- Fallahi-Sichani, M., El-Kebir, M., Marino, S., Kirschner, D.E., Linderman, J.J., 2011. Multi-scale computational modeling reveals a critical role for TNF receptor 1 dynamics in tuberculous granuloma formation. *Journal of Immunology* (Baltimore, MD: 1950) 186 (6), 3472–3483.
- Ferris, M.T., Aylor, D.L., Bottomly, D., Whitmore, A.C., Aicher, L.D., Bell, T.A., Bradel-Trethewey, B., Bryan, J.T., Buus, R.J., Gralinski, L.E., Haagmans, B.L., McMillan, L., Miller, D.R., Rosenzweig, E., Valdar, W., Wang, J., Churchill, G.A., Threadgill, D.W., McWeeny, S.K., Katze, M.G., Pardo-Manuel de Villena, F., Baric, R.S., Heise, M.T., 2013. Modeling host genetic regulation of influenza pathogenesis in the collaborative cross. *PLoS Pathogens* 9 (2), e1003196. <http://dx.doi.org/10.1371/journal.ppat.1003196>.
- Folcik, V.A., Broderick, G., Mohan, S., Block, B., Ekbote, C., Doolittle, J., Khoury, M., Davis, L., Marsh, C.B., 2011. Using an agent-based model to analyze the dynamic communication network of the immune response. *Theoretical Biology and Medical Modelling* 8, 1.
- Fornek, J.L., Gillim-Ross, L., Santos, C., Carter, V., Ward, J.M., Cheng, L.I., Proll, S., Katze, M.G., Subbarao, K., 2009. A single-amino-acid substitution in a polymerase protein of an H5N1 influenza virus is associated with systemic infection and impaired T-cell activation in mice. *Journal of Virology* 83 (21), 11102–11115.
- Fritsch, F.S.O., Dusny, C., Frick, O., Schmid, A., 2012. Single-cell analysis in biotechnology, systems biology, and biocatalysis. *Annual Review of Chemical and Biomolecular Engineering* 3 (1), 129–155.
- Garbis, S., Lubec, G., Fountoulakis, M., 2005. Limitations of current proteomics technologies. *Journal of Chromatography A* 1077 (1), 1–18.
- Ghazalpour, A., Bennett, B., Petyuk, V.A., Orozco, L., Hagopian, R., Mungrue, I.N., Farber, C.R., Sinsheimer, J., Kang, H.M., Furlotte, N., Park, C.C., Wen, P.-Z., Brewer, H., Weitz, K., Camp, D.G., Pan, C., Yordanova, R., Neuhaus, I., Tilford, C., Siemers, N., Gargalovic, P., Eskin, E., Kirchgessner, T., Smith, D.J., Smith, R.D., Lusis, A.J., 2011. Comparative analysis of proteome and transcriptome variation in mouse. *PLoS Genetics* 7 (6), e1001393.
- Go, J.T., Belisle, S.E., Tchitchek, N., Tumpey, T.M., Ma, W., Richt, J.A., Safronetz, D., Feldmann, H., Katze, M.G., 2012. 2009 pandemic H1N1 influenza virus elicits similar clinical course but differential host transcriptional response in mouse, macaque, and swine infection models. *BMC Genomics* 13, 627. <http://dx.doi.org/10.1186/1471-2164-13-627>.
- Goodman, A.G., Tanner, B.C.W., Chang, S.T., Esteban, M., Katze, M.G., 2011. Virus infection rapidly activates the P58IPK pathway, delaying peak kinase activation to enhance viral replication. *Virology* 417 (1), 27–36.
- Guttman, M., Amit, I., Garber, M., French, C., Lin, M.F., Feldser, D., Huarte, M., Zuk, O., Carey, B.W., Cassady, J.P., Cabili, M.N., Jaenisch, R., Mikkelsen, T.S., Jacks, T., Hacohen, N., Bernstein, B.E., Kellis, M., Regev, A., Rinn, J.L., Lander, E.S., 2009. Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. *Nature* 458 (7235), 223–227.
- Hack, C.J., 2004. Integrated Transcriptome and Proteome Data: The Challenges Ahead. *Briefings in Functional Genomics & Proteomics* 3 (3), 212–219.
- Hancioglu, B., Swigon, D., Clermont, G., 2007. A dynamical model of human immune response to influenza A virus infection. *Journal of Theoretical Biology* 246 (1), 70–86.
- Handel, A., Longini, I.M., Antia, R., 2007. Neuraminidase inhibitor resistance in influenza: assessing the danger of its generation and spread. *PLoS Computational Biology* 3 (12).
- Handel, A., Longini, I.M., Antia, R., 2010. Towards a quantitative understanding of the within-host dynamics of influenza A infections. *Journal of the Royal Society Interface* 7 (42), 35–47.
- Hao, L., Sakurai, A., Watanabe, T., Sorensen, E., Nidom, C.A., Newton, M.A., Ahlquist, P., Kawaoka, Y., 2008. Drosophila RNAi screen identifies host genes important for influenza virus replication. *Nature* 454 (7206), 890–893.
- Hatta, M., Gao, P., Halfmann, P., Kawaoka, Y., 2001. Molecular basis for high virulence of Hong Kong H5N1 influenza A viruses. *Science* 293 (5536), 1840–1842.
- Hatta, Y., Hershberger, K., Shinya, K., Proll, S.C., Dubielzig, R.R., Hatta, M., Katze, M.G., Kawaoka, Y., Suresh, M., 2010. Viral replication rate regulates clinical outcome and CD8T cell responses during highly pathogenic H5N1 influenza virus infection in mice. *PLoS Pathogens* 6 (10).
- Heldt, F.S., Frensing, T., Reichl, U., 2012. Modeling the intracellular dynamics of influenza virus replication to understand the control of viral RNA synthesis. *Journal of Virology* 86 (15), 7806–7817.
- Heng, T.S.P., Painter, M.W., Elpek, K., Lukacs-Kornek, V., Mauermann, N., Turley, S.J., Koller, D., Kim, F.S., Wagers, A.J., Asinowski, N., Davis, S., Fassett, M., Feuerer, M., Gray, D.H.D., Haxhinasto, S., Hill, J.A., Hyatt, G., Laplace, C., Leatherbee, K., Mathis, D., Benoist, C., Jianu, R., Laidlaw, D.H., Best, J.A., Knell, J., Goldrath, A.W., Jarjoura, J., Sun, J.C., Zhu, Y., Lanier, L.L., Ergun, A., Li, Z., Collins, J.J., Shinton, S.A., Hardy, R.R., Friedline, R., Sylvia, K., Kang, J., 2008. The immunological genome project: networks of gene expression in immune cells. *Nature Immunology* 9 (10), 1091–1094.
- Herfst, S., Schrauwen, E.J., Linster, M., Chutinimitkul, S., de Wit, E., Munster, V.J., Sorrell, E.M., Bestebroer, T.M., Burke, D.F., Smith, D.J., Rimmelzwaan, G.F., Osterhaus, A.D., Fouchier, R.A., 2012. Airborne transmission of influenza A/H5N1 virus between ferrets. *Science* 336 (6088), 1534–1541.
- Hidaka, F., Matsuo, S., Muta, T., Takeshige, K., Mizukami, T., Nunoi, H., 2006. A missense mutation of the Toll-like receptor 3 gene in a patient with influenza-associated encephalopathy. *Clinical Immunology* 119 (2), 188–194.
- Horby, P., Sudoyo, H., Viprakasit, V., Fox, A., Thai, P.Q., Yu, H., Davila, S., Hibberd, M., Dunstan, S.J., Monteerarat, Y., Farrar, J.J., Marzuki, S., Hien, N.T., 2010. What is the evidence of a role for host genetics in susceptibility to influenza A/H5N1? *Epidemiology and Infection* 138 (11), 1550–1558.
- Hornberg, J.J., Binder, B., Bruggeman, F.J., Schoeberl, B., Heinrich, R., Westerhoff, H.V., 2005. Control of MAPK signalling: from complexity to what really matters. *Oncogene* 24 (36), 5533–5542.
- Huang, S.-S.C., Fraenkel, E., 2009. Integrating proteomic, transcriptional, and interactome data reveals hidden components of signaling and regulatory networks. *Science Signaling* 2 (81), ra40.
- Husmeier, D., Werhli, A.V., 2007. Bayesian integration of biological prior knowledge into the reconstruction of gene regulatory networks with Bayesian networks. In: *Computational Systems Bioinformatics/Life Sciences Society. Computational Systems Bioinformatics Conference*, vol. 6, pp. 85–95.
- Ibricevic, A., Pekosz, A., Walter, M.J., Newby, G., Battaie, J.T., Brown, E.G., Holtzman, M.J., Brody, S.L., 2006. Influenza virus receptor specificity and cell tropism in mouse and human airway epithelial cells. *Journal of Virology* 80 (15), 7469–7480.
- Ideker, T., Krogan, N.J., 2012. Differential network biology. *Molecular Systems Biology* 8 (1).
- Ilyushina, N.A., Seiler, J.P., Rehg, J.E., Webster, R.G., Govorkova, E.A., 2010. Effect of neuraminidase inhibitor-resistant mutations on pathogenicity of clade 2.2 A/Turkey/15/06 (H5N1) influenza virus in ferrets. *PLoS Pathogens* 6 (5), e1000933.
- Imai, M., Watanabe, T., Hatta, M., Das, S.C., Ozawa, M., Shinya, K., Zhong, G., Hanson, A., Katsura, H., Watanabe, S., Li, C., Kawakami, E., Yamada, S., Kiso, M., Suzuki, Y., Maher, E.A., Neumann, G., Kawaoka, Y., 2012. Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets. *Nature* 486 (7403), 420–428.
- Imielski, M., Cha, S., Rejtar, T., Richardson, E.A., Karger, B.L., Sgroi, D.C., 2012. Integrated proteomic, transcriptomic, and biological network analysis of breast carcinoma reveals molecular features of tumorigenesis and clinical relapse. *Molecular and Cellular Proteomics* 11, 6.
- Jorba, N., Juarez, S., Torreira, E., Gastaminza, P., Zamarreño, N., Albar, J.P., Ortín, J., 2008. Analysis of the interaction of influenza virus polymerase complex with human cell factors. *Proteomics* 8 (10), 2077–2088.
- Josset, L., Belser, J.A., Pantin-Jackwood, M.J., Chang, J.H., Chang, S.T., Belisle, S.E., Tumpey, T.M., Katze, M.G., 2012a. Implication of inflammatory macrophages, nuclear receptors, and interferon regulatory factors in increased virulence of pandemic 2009 H1N1 Influenza A virus after host adaptation. *Journal of Virology* 86 (13), 7192–7206.
- Josset, L., Engelmann, F., Habarth, K., Kelly, S., Park, B., Kawaoka, Y., Garcia-Sastre, A., Katze, M.G., Messaoudi, I., 2012b. Increased viral loads and exacerbated innate host responses in aged macaques infected with the 2009 pandemic H1N1 influenza A virus. *Journal of Virology* 86 (20), 11115–11127.
- Josset, L., Textoris, J., Loriod, B., Ferraris, O., Moules, V., Lina, B., N'Guyen, C., Diaz, J.-J., Rosa-Calatrava, M., 2010. Gene expression signature-based screening identifies new broadly effective influenza A antivirals. *PLoS ONE* 5 (10), e13169.
- Julander, J.G., Hagloch, J., Latimer, S., Motter, N., Dagley, A., Barnard, D.L., Smee, D.F., Morrey, J.D., 2011. Use of plethysmography in assessing the efficacy of antivirals in a mouse model of pandemic influenza A virus. *Antiviral Research* 92 (2), 228–236.
- Kandun, I.N., Tresnaningsih, E., Purba, W.H., Lee, V., Samaan, G., Harun, S., Soni, E., Septiawati, C., Setiawati, T., Sariwati, E., Wandura, T., 2008. Factors associated with case fatality of human H5N1 virus infections in Indonesia: a case series. *Lancet* 372 (9640), 744–749.
- Kanehisa, M., Goto, S., 2000. KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Research* 28 (1), 27–30.
- Karlas, A., Machuy, N., Shin, Y., Pleissner, K.-P., Artarini, A., Heuer, D., Becker, D., Khalil, H., Ogilvie, L.A., Hess, S., Maurer, A.P., Müller, E., Wolff, T., Rudel, T., Meyer, T.F., 2010. Genome-wide RNAi screen identifies human host factors crucial for influenza virus replication. *Nature* 463 (7282), 818–822.
- Karr, J.R., Sanghvi, J.C., Macklin, D.N., Gutschow, M.V., Jacobs, J.M., Bolival Jr., B., Assad-Garcia, N., Glass, J.I., Covert, M.W., 2012. A whole-cell computational model predicts phenotype from genotype. *Cell* 150 (2), 389–401.
- Kash, J.C., Cunningham, D.M., Smit, M.W., Park, Y., Fritz, D., Wilusz, J., Katze, M.G., 2002. Selective translation of eukaryotic mRNAs: functional molecular analysis of GRSF-1, a positive regulator of influenza virus protein synthesis. *Journal of Virology* 76 (20), 10417–10426.
- Kash, J.C., Goodman, A.G., Korth, M.J., Katze, M.G., 2006. Hijacking of the host-cell response and translational control during influenza virus infection. *Virus Research* 119 (1), 111–120.
- Keane, T.M., Goodstadt, L., Danecek, P., White, M.A., Wong, K., Yalcin, B., Heger, A., Agam, A., Slater, G., Goodson, M., Furlotte, N.A., Eskin, E., Nellaker, C., Whitley, H., Cleak, J., Janowitz, D., Hernandez-Piiego, P., Edwards, A., Belgard, T.G., Oliver, P.L., McIntyre, R.E., Bhomra, A., Nicod, J., Gan, X., Yuan, W., van der Weyden, L., Steward, A.A., Bala, S., Stalker, J., Mott, R., Durbin, R., Jackson, I.J., Czechanski, P., Guerra-Assuncao, J.A., Donahue, L.R., Reinholdt, L.G., Payseur, B.A., Ponting, C.P.,

- Birney, E., Flint, J., Adams, D.J., 2011. Mouse genomic variation and its effect on phenotypes and gene regulation. *Nature* 477 (7364), 289–294.
- Keshava Prasad, T.S., Goel, R., Kandasamy, K., Keerthikumar, S., Kumar, S., Mathivanan, S., Telikicherla, D., Raju, R., Shafreen, B., Venugopal, A., Balakrishnan, L., Marimuthu, A., Banerjee, S., Somanathan, D.S., Sebastian, A., Rani, S., Ray, S., Harrys Kishore, C.J., Kanth, S., Ahmed, M., Kashyap, M.K., Mohmood, R., Ramachandra, Y.L., Krishna, V., Rahiman, B.A., Mohan, S., Ranganathan, P., Ramabadrana, S., Chaerkady, R., Pandey, A., 2009. Human protein reference database—2009 update. *Nucleic Acids Research* 37 (Database issue), D767–D772.
- Kislinger, T., Cox, B., Kannan, A., Chung, C., Hu, P., Ignatchenko, A., Scott, M.S., Gramolini, A.O., Morris, Q., Hallett, M.T., Rossant, J., Hughes, T.R., Frey, B., Emili, A., 2006. Global survey of organ and organelle protein expression in mouse: combined proteomic and transcriptomic profiling. *Cell* 125 (1), 173–186.
- Kitano, H., 2002. Systems biology: a brief overview. *Science* 295 (5560), 1662–1664.
- König, R., Stertz, S., Zhou, Y., Inoue, A., Heinrich Hoffmann, H., Bhattacharyya, S., Alamares, J.G., Tscherne, D.M., Ortigoza, M.B., Liang, Y., Gao, Q., Andrews, S.E., Bandyopadhyay, S., De Jesus, P., Tu, B.P., Pache, L., Shih, C., Orth, A., Bonamy, G., Miraglia, L., Ideker, T., García-Sastre, A., Young, J.A.T., Palese, P., Shaw, M.L., Chanda, S.K., 2010. Human host factors required for influenza virus replication. *Nature* 463 (7282), 813–817.
- Krumbholz, A., Philipps, A., Oehring, H., Schwarzer, K., Eitner, A., Wutzler, P., Zell, R., 2011. Current knowledge on PB1–F2 of influenza A viruses. *Medical microbiology and immunology* 200 (2), 69–75.
- Le Goffic, R., Leymarie, O., Chevalier, C., Rebours, E., Da Costa, B., Vidic, J., Descamps, D., Sallenave, J.-M., Rauch, M., Samson, M., Delmas, B., 2011. Transcriptomic analysis of host immune and cell death responses associated with the influenza A virus PB1–F2 protein. *PLoS Pathogens* 7 (8).
- Lee, H.Y., Topham, D.J., Park, S.Y., Hollenbaugh, J., Treanor, J., Mosmann, T.R., Jin, X., Ward, B.M., Miao, H., Holden-Wiltse, J., Perelson, A.S., Zand, M., Wu, H., 2009a. Simulation and prediction of the adaptive immune response to influenza A virus infection. *Journal of Virology* 83 (14), 7151–7165.
- Lee, S.I., Dudley, A.M., Drubin, D., Silver, P.A., Krogan, N.J., Pe'er, D., Koller, D., 2009b. Learning a prior on regulatory potential from eQTL data. *PLoS Genetics* 5 (1), e1000358.
- Lee, S.M.Y., Gardy, J.L., Cheung, C.Y., Cheung, T.K.W., Hui, K.P.Y., Ip, N.Y., Guan, Y., Hancock, R.E.W., Peiris, J.S.M., 2009c. Systems-level comparison of host-responses elicited by avian H5N1 and seasonal H1N1 influenza viruses in primary human macrophages. *PLoS ONE* 4 (12), e8072.
- Li, C., Bankhead, A., Einfeld, A.J., Hattar, Y., Jeng, S., Chang, J.H., Aicher, L.D., Proll, S., Ellis, A.L., Law, G.L., Waters, K.M., Neumann, G., Katze, M.G., McWeeney, S., Kawaoka, Y., 2011. Host regulatory network response to infection with highly pathogenic H5N1 avian influenza virus. *Journal of Virology* 85 (21), 10955–10967.
- Lietzen, N., Ohman, T., Rintahaka, J., Julkunen, I., Aittokallio, T., Matikainen, S., Nyman, T.A., 2011. Quantitative subcellular proteome and secretome profiling of influenza A virus-infected human primary macrophages. *PLoS Pathogens* 7 (5), e1001340.
- Lin, S., Liu, N., Yang, Z., Song, W., Wang, P., Chen, H., Lucio, M., Schmitt-Kopplin, P., Chen, G., Cai, Z., 2010. GC/MS-based metabolomics reveals fatty acid biosynthesis and cholesterol metabolism in cell lines infected with influenza A virus. *Talanta* 83 (1), 262–268.
- Liu, B., de la Fuente, A., Hoeschele, I., 2008. Gene network inference via structural equation modeling in genetical genomics experiments. *Genetics* 178 (3), 1763–1776.
- Looney, M.R., Thornton, E.E., Sen, D., Lamm, W.J., Glenn, R.W., Krummel, M.F., 2010. Stabilized imaging of immune surveillance in the mouse lung. *Nature Methods* 8 (1), 91–96.
- Lu, X., Tumpey, T.M., Morken, T., Zaki, S.R., Cox, N.J., Katz, J.M., 1999. A mouse model for the evaluation of pathogenesis and immunity to influenza A (H5N1) viruses isolated from humans. *Journal of Virology* 73 (7), 5903–5911.
- Ma, W., Belisle, S.E., Mosier, D., Li, X., Stigger-Rosser, E., Liu, Q., Qiao, C., Elder, J., Webby, R., Katze, M.G., Richt, J.A., 2011. 2009 pandemic H1N1 influenza virus causes disease and upregulation of genes related to inflammatory and immune responses, cell death, and lipid metabolism in pigs. *Journal of Virology* 85 (22), 11626–11637.
- Ma, X.-J., Dahiya, S., Richardson, E., Erlander, M., Sgroi, D.C., 2009. Gene expression profiling of the tumor microenvironment during breast cancer progression. *Breast Cancer Research* 11 (1), R7.
- Ma'ayan, A., 2011. Introduction to network analysis in systems biology. *Science Signaling* 4 (190), tr5.
- Maathuis, M.H., Colombo, D., Kalisch, M., Bühlmann, P., 2010. Predicting causal effects in large-scale systems from observational data. *Nature Methods* 7 (4), 247–248.
- Maines, T.R., Lu, X.H., Erb, S.M., Edwards, L., Guarner, J., Greer, P.W., Nguyen, D.C., Szretter, K.J., Chen, L.-M., Thawatsupha, P., Chittaganpitch, M., Waicharoen, S., Nguyen, D.T., Nguyen, T., Nguyen, H.H.T., Kim, J.-H., Hoang, L.T., Kang, C., Phuong, L.S., Lim, W., Zaki, S., Donis, R.O., Cox, N.J., Katz, J.M., Tumpey, T.M., 2005. Avian influenza (H5N1) viruses isolated from humans in Asia in 2004 exhibit increased virulence in mammals. *Journal of Virology* 79 (18), 11788–11800.
- Mak, C.M., Lam, C.-w., Fong, N.-c., Siu, W.-k., Lee, H.-c.H., Siu, T.-s., Lai, C.-k., Law, C.-y., Tong, S.-f., Poon, W.-t., Lam, D.S.-y., Ng, H.-l., Yuen, Y.-p., Tam, S., Que, T.-l., Kwong, N.-s., Chan, A.Y.-w., 2011. Fatal viral infection-associated encephalopathy in two Chinese boys: a genetically determined risk factor of thermolabile carnitine palmitoyltransferase II variants. *Journal of Human Genetics* 56 (8), 617–621.
- Manicassamy, B., Manicassamy, S., Belicha-Villanueva, A., Pisanelli, G., Pulendran, B., García-Sastre, A., 2010. Analysis of in vivo dynamics of influenza virus infection in mice using a GFP reporter virus. *Proceedings of the National Academy of Sciences* 107, 11531–11536.
- Margolin, A.A., Nemenman, I., Basso, K., Wiggins, C., Stolovitzky, G., Favera, R.D., Califano, A., 2006. ARACNE: an algorithm for the reconstruction of gene regulatory networks in a mammalian cellular context. *BMC Bioinformatics* 7 (Suppl. 1), S7.
- Mayer, D., Molawi, K., Martínez-Sobrido, L., Ghanem, A., Thomas, S., Baginsky, S., Grossmann, J., García-Sastre, A., Schwemmle, M., 2007. Identification of cellular interaction partners of the influenza virus ribonucleoprotein complex and polymerase complex using proteomic-based approaches. *Journal of Proteome Research* 6 (2), 672–682.
- McDermott, J.E., Gralinski, L.E., Einfeld, A.J., Mitchell, H.D., Bankhead, A., Josset, L., Tchitchek, N., Chang, J., Neumann, G., Tilton, S.C., Li, C., Fan, S., McWeeney, S., Kawaoka, Y., Baric, R.S., Waters, K.M., Katze, M.G. Identification and validation of common regulators of pathogenesis in highly pathogenic respiratory viruses using a systems biology approach. *Molecular System Biology*, in review.
- McDermott, J.E., Shankaran, H., Einfeld, A.J., Belisle, S.E., Neuman, G., Li, C., McWeeney, S., Sabourin, C., Kawaoka, Y., Katze, M.G., Waters, K.M., 2011. Conserved host response to highly pathogenic avian influenza virus infection in human cell culture, mouse and macaque model systems. *BMC Systems Biology* 5, 190.
- Mehle, A., Doudna, J.A., 2010. A host of factors regulating influenza virus replication. *Viruses* 2 (2), 566–573.
- Meier-Schellersheim, M., Fraser, I.D.C., Klauschen, F., 2009. Multi-scale modeling in cell biology. *Wiley interdisciplinary reviews. Systems Biology and Medicine* 1 (1), 4–14.
- Miao, H., Hollenbaugh, J.A., Zand, M.S., Holden-Wiltse, J., Mosmann, T.R., Perelson, A.S., Wu, H., Topham, D.J., 2010. Quantifying the early immune response and adaptive immune response kinetics in mice infected with influenza A virus. *Journal of Virology* 84 (13), 6687–6698.
- Mitchell, H., Levin, D., Forrest, S., Beauchemin, C.A., Tipper, J., Knight, J., Donart, N., Layton, R.C., Pyles, J., Gao, P., Harrod, K.S., Perelson, A.S., Koster, F., 2011. Higher level of replication efficiency of 2009 (H1N1) pandemic influenza virus than those of seasonal and avian strains: kinetics from epithelial cell culture and computational modeling. *Journal of Virology* 85 (2), 1125–1135.
- Mitchell, H., Einfeld, A.J., Sims, A.C., McDermott, J.E., Matzke, M.M., Webb-Robertson, B.J., Tilton, S.C., Tchitchek, N., Josset, L., Chengjun, L., Ellis, A.L., Chang, J., Heegel, R.A., Luna, M.L., Schepmoes, A.A., Shukla, A.K., Metz, T.O., Neuman, G., Bencke, A., Smith, R.D., Baric, R.S., Kawaoka, Y., Katze, M.G., Waters, K.M. A network integration approach to identify highly conserved regulators related to pathogenicity of influenza and SARS-CoV respiratory viruses. *PLoS One*, submitted for publication.
- Möhler, L., Flockerzi, D., Sann, H., Reichl, U., 2005. Mathematical model of influenza A virus production in large-scale microcarrier culture. *Biotechnology and Bioengineering* 90 (1), 46–58.
- Nakaya, H.I., Wrarmert, J., Lee, E.K., Racioppi, L., Marie-Kunze, S., Haining, W.N., Means, A.R., Kasturi, S.P., Khan, N., Li, G.-M., McCausland, M., Kanchan, V., Kokko, K.E., Li, S., Elbein, R., Mehta, A.K., Adorem, A., Subbarao, K., Ahmed, R., Pulendran, B., 2011. Systems biology of seasonal influenza vaccination in humans. *Nature Immunology* 12 (8), 786–795.
- Nelson, D.E., Ihekweaba, A.E.C., Elliott, M., Johnson, J.R., Gibney, C.A., Foreman, B.E., Nelson, G., See, V., Horton, C.A., Spiller, D.G., Edwards, S.W., McDowell, H.P., Unitt, J.F., Sullivan, E., Grimley, R., Benson, N., Broomhead, D., Kell, D.B., White, M.R.H., 2004. Oscillations in NF-kappaB signaling control the dynamics of gene expression. *Science (New York, NY)* 306 (5696), 704–708.
- Newport, M.J., Finan, C., 2011. Genome-wide association studies and susceptibility to infectious diseases. *Briefings in Functional Genomics* 10 (2), 98–107.
- Nicholls, J.M., Chan, M.C., Chan, W.Y., Wong, H.K., Cheung, C.Y., Kwong, D.L., Wong, M.P., Chui, W.H., Poon, L.L., Tsao, S.W., Guan, Y., Peiris, J.S., 2007. Nature of avian influenza A (H5N1) in the upper and lower respiratory tract. *Tropical Medicine* 13 (2), 147–149.
- Novershtern, N., Subramanian, A., Lawton, L.N., Mak, R.H., Haining, W.N., McConkey, M.G., Habib, N., Yosef, N., Chang, C.Y., Shay, T., Frampton, G.M., Drake, A.C.B., Leskov, I., Nilsson, B., Preffer, F., Dombkowski, D., Evans, J.W., Liefeld, T., Smutko, J.S., Chen, J., Friedman, N., Young, R.A., Golub, T.R., Regev, A., Ebert, B.L., 2011. Densely interconnected transcriptional circuits control cell states in human hematopoiesis. *Cell* 144 (2), 296–309.
- Novikoff, A.B., 1945. The concept of integrative levels and biology. *Science* 101 (2618), 209–215.
- Okazaki, Y., Furuno, M., Kasukawa, T., Adachi, J., Bono, H., Kondo, S., Nikaido, I., Osato, N., Saito, R., Suzuki, H., Yamanaka, I., Kiyosawa, H., Yagi, K., Tomaru, Y., Hasegawa, Y., Nogami, A., Schonbach, C., Gojobori, T., Baldarelli, R., Hill, D.P., Bult, C., Hume, D.A., Quackenbush, J., Schriml, L.M., Kanapin, A., Matsuda, H., Batalov, S., Beisel, K.W., Blake, J.A., Bradt, D., Brusica, V., Chothia, C., Corbani, L.E., Cousins, S., Dalla, E., Dragani, T.A., Fletcher, C.F., Forrester, A., Frazer, K.S., Gaasterland, T., Gariboldi, M., Gissi, C., Godzik, A., Gough, J., Grimmond, S., Gustincin, S., Hirokawa, N., Jackson, I.J., Jarvis, E.D., Kanai, A., Kawaji, H., Kawasawa, Y., Kedzierski, R.M., King, B.L., Konagaya, A., Kurochkin, I.V., Lee, Y., Lenhard, B., Lyons, P.A., Maglott, D.R., Maltais, L., Marchionni, L., McKenzie, L., Miki, H., Nagashima, T., Numata, K., Okido, T., Pavan, W.J., Perlea, G., Pesole, G., Petrovsky, N., Pillai, R., Pontius, J.U., Qi, D., Ramachandran, S., Ravasi, T., Reed, J.C., Reed, D.J., Reid, J., Ring, B.Z., Ringwald, M., Sandelin, A., Schneider, C., Semple, C.A., Setou, M., Shimada, K., Sultana, R., Takenaka, Y., Taylor, M.S., Teasdale, R.D., Tomita, M., Verardo, R., Wagner, L., Wahlestedt, C., Wang, Y., Watanabe, Y., Wells, C., Wilming, L.G., Wynshaw-Boris,

- A., Yanagisawa, M., et al., 2002. Analysis of the mouse transcriptome based on functional annotation of 60,770 full-length cDNAs. *Nature* 420 (6915), 563–573.
- Olsen, S.J., Ungchusak, K., Sovann, L., Uyeki, T.M., Dowell, S.F., Cox, N.J., Aldis, W., Chunsuttiwat, S., 2005. Family clustering of avian influenza A (H5N1). *Emerging Infectious Diseases* 11 (11), 1799–1801.
- Orth, J.D., Thiele, I., Palsson, B.O., 2010. What is flux balance analysis? *Nature Biotechnology* 28 (3), 245–248.
- Otte, A., Sauter, M., Alleve, L., Baumgarte, S., Klingel, K., Gabriel, G., 2011. Differential host determinants contribute to the pathogenesis of 2009 pandemic H1N1 and human H5N1 influenza A viruses in experimental mouse models. *American Journal of Pathology* 179 (1), 230–239.
- Pang, K.C., Dinger, M.E., Mercer, T.R., Malquori, L., Grimmond, S.M., Chen, W., Mattick, J.S., 2009. Genome-wide identification of long noncoding RNAs in CD8+ T cells. *Journal of Immunology* 182 (12), 7738–7748.
- Peiris, J.S.M., Cheung, C.Y., Leung, C.Y.H., Nicholls, J.M., 2009. Innate immune responses to influenza A H5N1: friend or foe? *Trends in Immunology* 30 (12), 574–584.
- Peiris, J.S.M., Yu, W.C., Leung, C.W., Cheung, C.Y., Ng, W.F., Nicholls, J.M., Ng, T.K., Chan, K.H., Lai, S.T., Lim, W.L., Yuen, K.Y., Guan, Y., 2004. Re-emergence of fatal human influenza A subtype H5N1 disease. *The Lancet* 363 (9409), 617–619.
- Peng, X., Gralinski, L., Armour, C.D., Ferris, M.T., Thomas, M.J., Proll, S., Bradel-Tretheway, B.G., Korth, M.J., Castle, J.C., Biery, M.C., Bouzek, H.K., Haynor, D.R., Frieman, M.B., Heise, M., Raymond, C.K., Baric, R.S., Katze, M.G., 2010. Unique signatures of long noncoding RNA expression in response to virus infection and altered innate immune signaling. *mBio* 1 (5).
- Peng, X., Gralinski, L., Ferris, M.T., Frieman, M.B., Thomas, M.J., Proll, S., Korth, M.J., Tisoncik, J.R., Heise, M., Luo, S., Schroth, G.P., Tumpey, T.M., Li, C., Kawaoka, Y., Baric, R.S., Katze, M.G., 2011. Integrative deep sequencing of the mouse lung transcriptome reveals differential expression of diverse classes of small RNAs in response to respiratory virus infection. *mBio* 2 (6).
- Perrone, L.A., Plowden, J.K., Garcia-Sastre, A., Katz, J.M., Tumpey, T.M., 2008. H5N1 and 1918 pandemic influenza virus infection results in early and excessive infiltration of macrophages and neutrophils in the lungs of mice. *PLoS Pathogens* 4 (8), e1000115.
- Piruzian, E., Bruskin, S., Ishkin, A., Abdeev, R., Moshkovskii, S., Melnik, S., Nikolsky, Y., Nikolskaya, T., 2010. Integrated network analysis of transcriptomic and proteomic data in psoriasis. *BMC Systems Biology* 4 (1), 41.
- Plotch, S.J., Bouloy, M., Ulmanen, I., Krug, R.M., 1981. A unique cap(m7GpppXm)-dependent influenza virion endonuclease cleaves capped RNAs to generate the primers that initiate viral RNA transcription. *Cell* 23 (3), 847–858.
- Prasun, P., Stockton, D.W., 2012. Fatal acute encephalopathy in two siblings: a distinct hereditary entity? *Journal of the Neurological Sciences* 314 (1–2), 155–157.
- Pulverer, J.E., Rand, U., Lienenklaus, S., Kugel, D., Ziętara, N., Kochs, G., Naumann, R., Weiss, S., Staeheli, P., Hauser, H., Köster, M., 2010. Temporal and spatial resolution of Type I and III interferon responses in vivo. *Journal of Virology* 84 (17), 8626–8638.
- Rau, A., Jaffrézic, F., Foulley, J.-L., Doerge, R.W., 2010. An empirical Bayesian method for estimating biological networks from temporal microarray data. *Statistical Applications in Genetics and Molecular Biology* 9 (1).
- Richard, M., Erny, A., Care, B., Traversier, A., Barthelemy, M., Hay, A., Lin, Y.P., Ferraris, O., Lina, B., 2012. Rescue of a H3N2 influenza virus containing a deficient neuraminidase protein by a hemagglutinin with a low receptor-binding affinity. *PLoS ONE* 7 (5), e33880.
- Ritter, J.B., Wahl, A.S., Freund, S., Genzel, Y., Reichl, U., 2010. Metabolic effects of influenza virus infection in cultured animal cells: intra- and extracellular metabolite profiling. *BMC Systems Biology* 4 (1), 61.
- Rockman, M.V., 2008. Reverse engineering the genotype–phenotype map with natural genetic variation. *Nature* 456 (7223), 738–744.
- Ronen, M., Rosenberg, R., Shraiman, B.L., Alon, U., 2002. Assigning numbers to the arrows: parameterizing a gene regulation network by using accurate expression kinetics. *Proceedings of the National Academy of Sciences of the United States of America* 99 (16), 10555–10560.
- Roulhac, P.L., Ward, J.M., Thompson, J.W., Soderblom, E.J., Silva, M., Moseley, M.A., Jarvis, E.D., 2011. Microproteomics: quantitative proteomic profiling of small numbers of laser-captured cells. *Cold Spring Harbor Protocols* 2011 (2), pdb.prot5573.
- Rubakhin, S.S., Romanova, E.V., Nemes, P., Sweedler, J.V., 2011. Profiling metabolites and peptides in single cells. *Nature Methods* 8 (4s), S20–S29.
- Schmolke, M., Viemann, D., Roth, J., Ludwig, S., 2009. Essential Impact of NF- κ B signaling on the H5N1 influenza A virus-induced transcriptome. *Journal of Immunology* 183 (8), 5180–5189.
- Schoeberl, B., Eichler-Jonsson, C., Gilles, E.D., Müller, G., 2002. Computational modeling of the dynamics of the MAP kinase cascade activated by surface and internalized EGF receptors. *Nature Biotechnology* 20 (4), 370–375.
- Schulze, W.X., Usadel, B., 2010. Quantitation in mass-spectrometry-based proteomics. *Annual Review of Plant Biology* 61 (1), 491–516.
- Shapira, S.D., Gat-Viks, I., Shum, B.O.V., Dricot, A., de Grace, M.M., Wu, L., Gupta, P.B., Hao, T., Silver, S.J., Root, D.E., Hill, D.E., Regev, A., Hacohen, N., 2009. A physical and regulatory map of host–influenza interactions reveals pathways in H1N1 infection. *Cell* 139 (7), 1255–1267.
- Shinya, K., Gao, Y., Cilloniz, C., Suzuki, Y., Fujie, M., Deng, G., Zhu, Q., Fan, S., Makino, A., Muramoto, Y., Fukuyama, S., Tamura, D., Noda, T., Eisefeld, A.J., Katze, M.G., Chen, H., Kawaoka, Y., 2012. Integrated clinical, pathologic, virologic, and transcriptomic analysis of H5N1 influenza virus-induced viral pneumonia in the rhesus macaque. *Journal of Virology* 86 (11), 6055–6066.
- Sidorenko, Y., Reichl, U., 2004. Structured model of influenza virus replication in MDCK cells. *Biotechnology and Bioengineering* 88 (1), 1–14.
- Skarnes, W.C., Rosen, B., West, A.P., Koutourakis, M., Bushell, W., Iyer, V., Mujica, A.O., Thomas, M., Harrow, J., Cox, T., Jackson, D., Severin, J., Biggs, P., Fu, J., Nefedov, M., de Jong, P.J., Stewart, A.F., Bradley, A., 2011. A conditional knockout resource for the genome-wide study of mouse gene function. *Nature* 474 (7351), 337–342.
- Sloot, P.M.A., Hoekstra, A.G., 2010. Multi-scale modelling in computational biomedicine. *Briefings in Bioinformatics* 11 (1), 142–152.
- Smith, A.M., Adler, F.R., McAuley, J.L., Gutenkunst, R.N., Ribeiro, R.M., McCullers, J.A., Perelson, A.S., 2011. Effect of 1918 PB1-F2 expression on influenza A virus infection kinetics. *PLOS Computational Biology* 7 (2), e1001081.
- Srivastava, B., Błażejewska, P., Heßmann, M., Bruder, D., Geffers, R., Mauel, S., Gruber, A.D., Schughart, K., 2009. Host genetic background strongly influences the response to influenza A virus infections. *PLoS ONE* 4 (3).
- Stertz, S., Shaw, M.L., 2011. Uncovering the global host cell requirements for influenza virus replication via RNAi screening. *Microbes and Infection/Institut Pasteur* 13 (5), 516–525.
- Su, A.I., Wiltschko, T., Batalov, S., Lapp, H., Ching, K.A., Block, D., Zhang, J., Soden, R., Hayakawa, M., Kreiman, G., Cooke, M.P., Walker, J.R., Hogenesch, J.B., 2004. A gene atlas of the mouse and human protein-encoding transcriptomes. *Proceedings of the National Academy of Sciences of the United States of America* 101 (16), 6062–6067.
- Subramanian, A., Tamayo, P., Mootha, V.K., Mukherjee, S., Ebert, B.L., Gillette, M.A., Paulovich, A., Pomeroy, S.L., Golub, T.R., Lander, E.S., Mesirov, J.P., 2005. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proceedings of the National Academy of Sciences of the United States of America* 102 (43), 15545–15550.
- Szklarczyk, D., Franceschini, A., Kuhn, M., Simonovic, M., Roth, A., Minguez, P., Doerks, T., Stark, M., Müller, J., Bork, P., Jensen, L.J., von Mering, C., 2011. The STRING database in 2011: functional interaction networks of proteins, globally integrated and scored. *Nucleic Acids Research* 39 (Database issue), D561–D568.
- Szretter, K.J., Gangappa, S., Lu, X., Smith, C., Shieh, W.-J., Zaki, S.R., Sambhara, S., Tumpey, T.M., Katz, J.M., 2007. Role of host cytokine responses in the pathogenesis of avian H5N1 influenza viruses in mice. *Journal of Virology* 81 (6), 2736–2744.
- Tafforeau, L., Chantier, T., Pradezynski, F., Pellet, J., Mangeot, P.E., Vidalain, P.-O., Andre, P., Rabourdin-Combe, C., Lotteau, V., 2011. Generation and comprehensive analysis of an influenza virus polymerase cellular interaction network. *Journal of Virology* 85 (24), 13010–13018.
- Taft, R.J., Pang, K.C., Mercer, T.R., Dinger, M., Mattick, J.S., 2010. Non-coding RNAs: regulators of disease. *Journal of Pathology* 220 (2), 126–139.
- Tawhai, M., Clark, A., Donovan, G., Burrows, K., 2011. Computational modeling of airway and pulmonary vascular structure and function: development of a “lung physiome”. *Critical Reviews in Biomedical Engineering* 39 (4), 319–336.
- Tay, S., Hughey, J.J., Lee, T.K., Lipniacki, T., Quake, S.R., Covert, M.W., 2010. Single-cell NF- κ B dynamics reveal digital activation and analog information processing in cells. *Nature* 466 (7303), 267–271.
- Tchitchek, N., Eisefeld, A.J., Tisoncik-Go, J., Josset, L., Gralinski, L.E., Bécavin, C., Tilton, S.C., Webb-Robertson, B.-J., Ferris, M.T., Totura, A.L., Li, C., Neumann, G., Metz, T.O., Smith, R.D., Waters, K.M., Baric, R., Kawaoka, Y., Katze, M.G. Specific mutations in H5N1 mainly impact the magnitude and velocity of the host response in mice. *BMC Syst Biol*, submitted for publication.
- Tisoncik, J.R., Katze, M.G., 2010. What is systems biology? *Future Microbiology* 5 (2), 139–141.
- Tisoncik, J.R., Korth, M.J., Simmons, C.P., Farrar, J., Martin, T.R., Katze, M.G., 2012. Into the eye of the cytokine storm. *Microbiology and Molecular Biology Reviews* 76 (1), 16–32.
- To, K.-F., Chan, P.K.S., Chan, K.-F., Lee, W.-K., Lam, W.-Y., Wong, K.-F., Tang, N.L.S., Tsang, D.N.C., Sung, R.Y.T., Buckley, T.A., Tam, J.S., Cheng, A.F., 2001. Pathology of fatal human infection associated with avian influenza A H5N1 virus. *Journal of Medical Virology* 63 (3), 242–246.
- Turan, N., Kalko, S., Stincone, A., Clarke, K., Sabah, A., Howlett, K., Curnow, S.J., Rodriguez, D.A., Cascante, M., O'Neill, L., Egginton, S., Roca, J., Falciani, F., 2011. A systems biology approach identifies molecular networks defining skeletal muscle abnormalities in chronic obstructive pulmonary disease. *PLOS Computational Biology* 7 (9), e1002129.
- van Riel, D., Munster, V.J., de Wit, E., Rimmelzwaan, G.F., Fouchier, R.A.M., Osterhaus, A.D.M.E., Kuiken, T., 2006. H5N1 virus attachment to lower respiratory tract. *Science* 312 (5772), 399.
- Vignes, M., Vandel, J., Allouche, D., Ramadan-Alban, N., Cierco-Ayrolles, C., Schiex, T., Mangin, B., de Givry, S., 2011. Gene regulatory network reconstruction using Bayesian networks, the Dantzig selector, the Lasso and their meta-analysis. *PLoS ONE* 6 (12), e29165.
- Vigorito, E., Perks, K.L., Abreu-Goodger, C., Bunting, S., Xiang, Z., Kohlhaas, S., Das, P.P., Miska, E.A., Rodriguez, A., Bradley, A., Smith, K.G., Rada, C., Enright, A.J., Toellner, K.M., MacLennan, I.C., Turner, M., 2007. microRNA-155 regulates the generation of immunoglobulin class-switched plasma cells. *Immunity* 27 (6), 847–859.
- Wang, X.D., Qi, Y.X., Jiang, Z.L., 2011. Reconstruction of transcriptional network from microarray data using combined mutual information and network-assisted regression. *IET Systems Biology* 5 (2), 95–102.
- Watanabe, T., Watanabe, S., Kawaoka, Y., 2010. Cellular networks involved in the influenza virus life cycle. *Cell Host and Microbe* 7 (6), 427–439.
- Waterston, R.H., Lindblad-Toh, K., Birney, E., Rogers, J., Abril, J.F., Agarwal, P., Agarwala, R., Ainscough, R., Alexandersson, M., An, P., Antonarakis, S.E., Attwood, J., Baertsch, R., Bailey, J., Barlow, K., Beck, S., Berry, E., Birren, B., Bloom, T., Bork,

- P., Botcherby, M., Bray, N., Brent, M.R., Brown, D.G., Brown, S.D., Bult, C., Burton, J., Butler, J., Campbell, R.D., Carninci, P., Cawley, S., Chiaromonte, F., Chinwalla, A.T., Church, D.M., Clamp, M., Clee, C., Collins, F.S., Cook, L.L., Copley, R.R., Coulson, A., Couronne, O., Cuff, J., Curwen, V., Cutts, T., Daly, M., David, R., Davies, J., Delehaanty, K.D., Deri, J., Dermitzakis, E.T., Dewey, C., Dickens, N.J., Diekhans, M., Dodge, S., Dubchak, I., Dunn, D.M., Eddy, S.R., Elnitski, L., Emes, R.D., Esvara, P., Eyas, E., Felsenfeld, A., Fewell, G.A., Fliecek, P., Foley, K., Frankel, W.N., Fulton, L.A., Fulton, R.S., Furey, T.S., Gage, D., Gibbs, R.A., Glusman, G., Gnerre, S., Goldman, N., Goodstadt, L., Grafham, D., Graves, T.A., Green, E.D., Gregory, S., Guigo, R., Guyer, M., Hardison, R.C., Haussler, D., Hayashizaki, Y., Hillier, L.W., Hinrichs, A., Hlavina, W., Holzer, T., Hsu, F., Hua, A., Hubbard, T., Hunt, A., Jackson, I., Jaffe, D.B., Johnson, L.S., Jones, M., Jones, T.A., Joy, A., Kamal, M., Karlsson, E.K., et al., 2002. Initial sequencing and comparative analysis of the mouse genome. *Nature* 420 (6915), 520–562.
- Westermann, A.J., Gorski, S.A., Vogel, J., 2012. Dual RNA-seq of pathogen and host. *Nature Reviews Microbiology* 10 (9), 618–630.
- WHO, 2010. Summary of human infection with highly pathogenic avian influenza A (H5N1) virus reported to WHO, January 2003–March 2009: cluster-associated cases. *Relevé épidémiologique hebdomadaire/Section d'hygiène du Secrétariat de la Société des Nations (Weekly epidemiological record/Health Section of the Secretariat of the League of Nations)*. 85 (3), 13–20.
- WHO, 2012. Cumulative number of confirmed human cases of avian influenza A (H5N1) reported to WHO.
- Witten, D.M., Tibshirani, R., 2009. Covariance-regularized regression and classification for high-dimensional problems. *Journal of the Royal Statistical Society Series B: Statistical Methodology* 71 (3), 615–636.
- Wolff, T., O'Neill, R.E., Palese, P., 1998. NS1-binding protein (NS1-BP): a novel human protein that interacts with the influenza A virus nonstructural NS1 protein is relocalized in the nuclei of infected cells. *Journal of Virology* 72 (9), 7170–7180.
- Wurfel, M.M., 2008. Genetic insights into sepsis: what have we learned and how will it help? *Current Pharmaceutical Design* 14 (19), 1900–1911.
- Xing, H., McDonagh, P.D., Bienkowska, J., Cashorali, T., Runge, K., Miller, R.E., Decaprio, D., Church, B., Roubenoff, R., Khalil, I.G., Carulli, J., 2011. Causal modeling using network ensemble simulations of genetic and gene expression data predicts genes involved in rheumatoid arthritis. *PLOS Computational Biology* 7 (3), e1001105.
- Yu, H., Kim, P.M., Sprecher, E., Trifonov, V., Gerstein, M., 2007. The importance of bottlenecks in protein networks: correlation with gene essentiality and expression dynamics. *PLOS Computational Biology* 3 (4), e59.
- Zamarin, D., Ortigoza, M.B., Palese, P., 2006. Influenza A virus PB1-F2 protein contributes to viral pathogenesis in mice. *Journal of Virology* 80 (16), 7976–7983.
- Zhang, B., Horvath, S., 2005. A general framework for weighted gene co-expression network analysis. *Statistical Applications in Genetics and Molecular Biology* 4, Article 17.
- Zhao, C., Zhang, H., Wong, W.-C., Sem, X., Han, H., Ong, S.-M., Tan, Y.-C., Yeap, W.-H., Gan, C.-S., Ng, K.-Q., Koh, M.B.-C., Kourilsky, P., Sze, S.-K., Wong, S.-C., 2009. Identification of novel functional differences in monocytic subsets using proteomic and transcriptomic methods. *Journal of Proteome Research* 8 (8), 4028–4038.
- Zhou, J., To, K.K.-W., Dong, H., Cheng, Z.-S., Lau, C.C.-Y., Poon, V.K.M., Fan, Y.-H., Song, Y.-Q., Tse, H., Chan, K.-H., Zheng, B.-J., Zhao, G.-P., Yuen, K.-Y., 2012. A functional variation in CD55 increases the severity of 2009 pandemic H1N1 influenza A virus infection. *Journal of Infectious Diseases* 206 (4), 495–503.
- Zhu, J., Wiener, M.C., Zhang, C., Fridman, A., Minch, E., Lum, P.Y., Sachs, J.R., Schadt, E.E., 2007. Increasing the power to detect causal associations by combining genotypic and expression data in segregating populations. *PLOS Computational Biology* 3 (4), e69.
- Zúñiga, J., Buendía-Roldán, I., Zhao, Y., Jiménez, L., Torres, D., Romo, J., Ramírez, G., Cruz, A., Vargas-Alarcon, G., Sheu, C.C., Chen, F., Su, L., Tager, A.M., Pardo, A., Selman, M., Christiani, D.C., 2012. Genetic variants associated with severe pneumonia in A/H1N1 influenza infection. *European Respiratory Journal* 39 (3), 604–610.