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Antiviral Innate Immunity through the lens of Systems Biology

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Abstract

Cellular innate immunity poses the first hurdle against invading viruses in their attempt to establish infection. This antiviral response is manifested with the detection of viral components by the host cell, followed by transduction of antiviral signals, transcription and translation of antiviral effectors and leads to the establishment of an antiviral state. These events occur in a rather branched and interconnected sequence than a linear path. Traditionally, these processes were studied in the context of a single virus and a host component. However, with the advent of rapid and affordable OMICS technologies it has become feasible to address such questions on a global scale. In the discipline of Systems Biology¹, extensive omics datasets are assimilated using computational tools and mathematical models to acquire deeper understanding of complex biological processes. In this review we have catalogued and discussed the application of Systems Biology approaches in dissecting the antiviral innate immune responses.

Introduction

Antiviral innate immunity studies; performed extensively in recent years, revealed that it is executed through multiple overlapping pathways and involves multi-functional components which engage in elaborate cross talk (Schneider et al., 2014). The outcome of this response can be in favor of the host or the virus, depending on the dynamics of the interactions between virus and host factors. Complexity of the innate immune response arises, both, with the host where different cell types respond differently to viral infections and with the viruses, where differences in genetic makeup, cell tropism and replication kinetics elicit variable host responses (Zak et al., 2014). Such complex and dynamic nature of antiviral innate immunity makes it a perfect subject to tackle with the tools of systems biology. Here we have reviewed the current knowledge of anti-viral innate immune responses, described

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the system biology approaches to study these biological processes and compiled systems studies on anti-viral innate immunity, with special focus on influenza A viruses.

Anti-viral innate immunity

Viruses are obligate intracellular parasites and infect a broad range of living organisms, from prokaryotes to humans. In this review however, we will limit our discussion to human viral pathogens. The infecting viruses possess or generate pathogen associated molecular patterns (PAMPs) which can be viral genomic material, transcripts, replication intermediates or glycoproteins (Iwasaki, 2012). These PAMPs are detected by cellular pattern recognition receptors (PRRs), which then initiate a cascade of antiviral signaling. PRRs include Toll-like receptors (TLRs), retinoic acid-inducible gene I (RIG-I) like receptors (RLRs), AIM2 like receptors (ALRs), Nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) and a growing list of DNA sensors. TLRs are either present in endosomes where they detect viral nucleic acid (TLR 3/ 7/ 8/ 9) or on the cell membrane where they detect viral glycoproteins (TLR2/ TLR4) (O'Neill et al., 2013). The RLRs comprise of RIG-I, melanoma differentiation associated gene 5 (MDA5) and laboratory of genetics and physiology 2 (LGP2), all of which are present in the cytosol and detect viral RNA or transcription intermediates (Yoneyama et al., 2015). There are several known DNA sensing mechanisms against viruses (DAI, AIM2, IFI16, DHX9, DDX41) and new sensors are being discovered regularly (Dempsey and Bowie, 2015). Most recent addition is cGAMP synthase (cGAS), which detects viral DNA and produces cGMP, which in turn binds to STING and activates anti-viral signaling (Ablasser et al., 2013). Viral DNA and RNA sensing mechanisms cross paths at several junctions, for example RNA Pol III is a DNA sensor, which detects and transcribes viral DNA and resulting RNA feeds back into the RIG-I pathway (Chiu et al., 2009). NLRs primarily detect bacterial pathogens, but can also detect viruses. Among NLRs, NOD2 has been reported to detect viral RNA whereas NLRP3 was shown to be activated by viral infection of dendritic cells (Allen et al., 2009; Sabbah et al., 2009). PAMP-PRR engagement leads to activation of interferon regulatory factors (IRF3/ IRF7/ IRF5) and NFkB transcription factors, which translocate to the nucleus and drive expression of secreted signaling molecules called interferons (IFN) and cytokines. This requires specific kinases (TBK1, IKK α / IKK β and MAPKs) and adaptor molecules MAVS (for RLRs), STING (for DNA sensors), TRIF and MYD88 (for TLRs) (Ishikawa and Barber, 2008; Ishikawa et al., 2009; Kawai and Akira, 2010).

Interferons are the backbone of antiviral innate immune response. There are 3 classes of IFN among which Type I IFN and Type III IFN are primary contributors to antiviral innate immunity. Once secreted out of the infected cell, primary IFNs bind to IFN receptors on the same or bystander cells leading to activation of JAK-STAT pathway. In this pathway, transcription factors STAT1, STAT2 and IRF9 form interferon stimulated gene factor 3 complex (ISGF3), which translocates in to the nucleus and binds to interferon stimulated response elements (ISREs) and drives expression of hundreds of genes collectively known as interferon stimulated genes (ISGs). Many of these ISGs have specific or broad spectrum antiviral effectors functions such as Mx, IFITIMs, Viperin and Tetherin (see detailed review by Schneider et al 2014). Among ISGs are also RNA-sensing executors such as OAS and PKR, which can detect viral RNA and induce general RNA degradation mediated by OAS-

RNaseL, and shut down mRNA translation (PKR-eIF2 α) as a blanket strategy to restrict virus replication. ISGs also include IRFs, STATs and other positive regulators of IFN induction, which further potentiate the antiviral IFN response. Antiviral IFN signaling also leads to secretion of several cytokines and chemokines, which activate immune effector cells and prime the adaptive immune response against viral infections. Finally, IFN signaling is also reported to induce the expression of specific miRNAs, long non coding RNAs (lnc RNAs) and splice variants of specific transcripts which contribute to antiviral host response (Schneider et al., 2014). To keep the IFN signaling in check some ISGs also perform negative regulatory functions such as USP18 (Ritchie et al., 2004) and SOCS (Baetz et al., 2004).

In a parallel pathway, PRR activation (NLRs and ALRs) can lead to formation and activation of inflammasomes which activate caspase 1 to potentiate cytokine production and can eventually initiate cell death (Hornung et al., 2009; Martinon et al., 2009). The components of antiviral signaling are often regulated through, post translational modifications such as phosphorylation, ubiquitination, sumoylation, and ISGylation. These modifications are frequently carried out by ISGs, thus adding several activation and inhibition loops to the antiviral signaling cascades (Ivashkiv and Donlin, 2014).

Systems biology approach to study biological systems

Systems biology can be defined as the study of complex biological processes through orthogonal integration of varied 'omics' datasets obtained temporally under different conditions from the biological system in question (Ideker et al., 2001). The goal of a systems approach usually is to develop models which can predict the behavior of a biological system under specific conditions. It is a multidisciplinary field of science where biology, computation, mathematics and engineering come together to solve complex scientific problems. At the core of systems biology approaches are the high throughput methodologies for global data acquisition of different biological properties, computational tools for data analysis and mathematical algorithms to generate probabilistic models. The human genome project can be credited for bringing the systems biology approach to the research forefront (Aderem and Smith, 2004). It was the first major attempt to get global readouts of biological traits using high throughput sequencing technologies. This approach was further developed and employed to sequence the genomes of other important organisms. The vast amount of data generated through these projects required development of databases and software tools for analysis. This brought together researchers from biology and computer sciences and led to the emergence of the field of 'Genomics'. With that 'omics' became the catch all phrase to describe all high throughput approaches to analyze biological systems. The knowledge of complete genome sequences of organisms allowed the design of oligonucleotide arrays to get genome level transcriptional readouts. With the advances in next generation sequencing, in addition to analyzing whole genome transcript levels, one can also detect splice variants, miRNAs and other non-coding RNAs under the discipline of 'Transcriptomics'. Meanwhile the mass spectrometry based methods to study proteins saw major advances under the field of 'Proteomics'. It became possible to study protein-protein and protein nucleic acid interactions, protein abundance and post translational modifications at whole genome level. The most complex and still in its infancy is the discipline of 'Metabolomics'. The genomics,

transcriptomics and proteomics datasets complement each other well, however metabolomics datasets are difficult to assimilate due to extremely complex range of metabolites produced in biological systems, which are unknown to large extent (Nicholson and Wilson, 2003). Nevertheless improved methods to study certain classes of metabolites such as lipids (Lipidomics) and sugars (Glycomics) have started contributing to systems studies.

Typically, a systems biology experiment involves measurement of different biological properties such as transcript and protein levels at global scale over a period of time under different conditions. During the experiment, the biological system is perturbed by different means to induce differences in behavior of the system. So far RNAi has been the preferred technology for introducing gene specific or genome level perturbations. With emergence of CRISPR technology scientists have a powerful off the shelf tool for studying the role of genetic elements in biological processes (Sternberg and Doudna, 2015). The advantage of CRISPR technology over RNAi lies in its ability to abolish target gene expression completely, modify parts of it or target non coding regulatory regions of the genome. With emerging applications and variants of CRISPR technology, it may replace RNAi in future systems level studies. The data generated during systems experiments is analyzed using computational methods to identify peculiar enrichment of any class of molecules. A network analysis of the enriched components is usually carried out to identify direct and indirect molecular interactions which may contribute to the change in the behavior of the biological system (Ng et al., 2006). There are several open source and commercial computational tools available to study large scale datasets, which are compiled in Table 1 according to their specific applications. Further, different classes of omics datasets are subjected to Meta-analysis where they are overlapped, integrated and assimilated. This allows identification of key regulators of biological processes, which behave in similar way across varied datasets and are more likely to be true hits. The true power of a systems approach lies however, in its ability to observe ‘emergent properties’ of a system which arise due to combinatorial action of its components (Zak et al., 2014). A familiar example of an emergent property is the sound of music, which emerges when several instruments are played together and it cannot be appreciated by listening to the sound of individual instruments. Once the key components of a biological process are identified, mathematical algorithms are applied to predict a functioning model for the system under study (Azhar and Vodovotz, 2014). The hypothesis put forward through this model is tested again on the biological system through selective perturbations, data acquisition and analysis. This cycle of experiments is reiterated to refine and validate the model until it can predict behavior of the biological system robustly (Fig 2).

Systems studies on anti-viral innate immunity

In addition to being complex and dynamic, there are other characteristics of the innate immune system which make it amenable to be studied using systems approaches. It is possible to isolate different immune effector cell populations and study their specific contribution to innate immunity. Advances in viral reverse genetics allow introduction of desired mutations in the viral genome and suitable animal models are available for many viral pathogens (Ye et al., 2014). Traditionally the antiviral innate immunity has been studied in a reductionist approach focusing on small set of host and virus factors at a time.

With advances in high-throughput omics technologies it became feasible to study cellular processes during viral infection at global scale. The genetically tractable invertebrate model organisms such as *C. elegans* and *Drosophila melanogaster* were used first to study innate immunity and contributed significantly to the knowledge, such as the discovery of TLRs (Irazoqui et al., 2010). In mammalian systems RNAi has been the mainstay for genome wide perturbations in systems analysis of innate immunity. It has been used in combination with transcriptional, proteomic and recently with lipidomic profiling to get systems level understanding of anti-viral innate immune responses. One of the early applications of systems approaches to mammalian systems was done by Bouwmeester *et al* to map the components of the TNF- α /NF- κ B pathway (Bouwmeester et al., 2004). Extensive work has been done at the Institute for systems biology (ISB, Seattle), initially to understand TLR signaling in macrophages and dendritic cells in response to bacterial pathogens (Gilchrist et al., 2006; Litvak et al., 2009). Later, in the context of anti-viral response they used systems approach to discover a negative regulatory action of FOXO3 on IRF7-Type I IFN antiviral signaling pathway (Litvak et al., 2012). Chevrier *et al* used transcriptional profiling, genetic and small-molecule perturbations, and phosphoproteomics to identify novel regulators of anti-viral TLR signaling in dendritic cells (Chevrier et al., 2011). In a similar study, the same group identified mediators of antiviral signaling in response to cytosolic DNA and retroviruses (Lee et al., 2013). In another study, Cho *et al* used genome wide RNAi approach to discover regulators of virus induced necrotic cell death (Martinon et al., 2009). Using a more proteomics inclined approach Li *et al* characterized the human innate immunity interactome, and its genetic gain and loss of function validation led to identification of mind bomb (MIB) E3 ligases which controlled ubiquitination of TBK1 (Li et al., 2011). Using proteomic methods to screen DNA binding proteins which are also induced by IFN, Bürckstümmer *et al* identified AIM2 as cytosolic sensor of double-stranded DNA viruses, which recruits the inflammasome and triggers IL-1 β production (Burckstummer et al., 2009). At the genome level innate immunity is regulated by epigenetic modifications and interaction between transcription regulators (Stender and Glass, 2013). In a comprehensive unbiased study Amit *et al* revealed a transcriptional circuit involving 125 factors (transcription factors, chromatin modifiers, RNA binding proteins) which regulated or fine-tuned anti-viral transcription (Amit et al., 2009). In a similar approach Zaslavsky *et al* identified a temporal cascade of transcriptional factors regulating antiviral transcriptional response to Newcastle disease virus in DCs (dendritic cells) (Zaslavsky et al., 2010). Transcriptional profiling of non-coding host RNAs during virus infection has revealed their regulatory roles in innate immunity. In case of influenza infection, specific changes in levels of miRNAs and other small non-coding RNAs have been reported to regulate the early innate immune response and cell death induction (Li et al., 2010; Peng et al., 2011).

An additional layer of complexity is added at single cell level, where in a homogenous population, individual cells may exhibit different phenotypes based on genetic and proteomic status as well as in stochastic events. Indeed, Shalek *et al* discovered a bimodal variation in messenger RNA abundance and splicing patterns by performing single cell RNAseq based profiling of DCs in response to LPS. They also identified a module of 137 highly variable genes which regulated antiviral signaling through STAT2 and IRF7 (Shalek et al., 2013). In terms of metabolic profiling, a limited number of Lipidomics studies have

been conducted to understand lipid biomarkers associated with viral pathogenicity and anti-viral immune responses (Diamond et al., 2010; Morita et al., 2013; Tam et al., 2013). In an important study, Luber *et al*/compared the lipid profile of CD8+ and CD4+ DCs in response to influenza virus infection and discovered differential expression of PRRs and sensitivity of viral infection (Luber et al., 2010). This study underscored the molecular basis of variable response of different immune effector cells to viral infections. The IFN signaling culminates with expression of hundreds of ISGs, which have varied role in anti-viral response (de Veer et al., 2001). Overexpression of selected set of genes to discover novel anti-viral molecules has been a fruitful approach. Charlie Rice's group has conducted screens of over 400 ISGs, using a panel of reporter viruses and discovered several anti-viral effectors, many of them having broad spectrum effect such as cGAS (Schoggins et al., 2014; Schoggins et al., 2011). Tripartite motif (TRIM) family of proteins has been reported to perform protein modifications and have antimicrobial activity. In an overexpression accompanied with knockdown screen of 75 TRIM family proteins, several member proteins were found to regulate IFN signaling in response to viral infections (Versteeg et al., 2013). Using a cDNA overexpression approach additional regulators of antiviral signaling have been discovered, such as STING in DNA sensing and MafB in IRF3 regulation (Ishikawa and Barber, 2008; Kim and Seed, 2010). Finally, genetic polymorphisms associated with innate immunity genes in humans can yield a lot of information about regulation of innate immune response and viral disease pathogenesis. Genome Wide association (GWA) studies, Exome sequencing, global profiling of single nucleotide polymorphisms (SNPs) and gene copy number variations (CNV) can be used to identify genes responsible for susceptibility to enhanced viral disease or predisposition to autoimmune disorders. Jean-Laurent Casanova's group has successfully used this approach to identify the role of ISG15 in regulation of innate immune signaling and to associate IRF7 and TLR3 deficiencies with severe influenza pathogenesis and herpes encephalitis, respectively, in humans (Ciancanelli et al., 2015; Zhang et al., 2015; Guo et al., 2011). Integrative analysis of published genome-wide datasets at large scale using uniform statistical methods can help visualize unique and diverse molecular patterns in antiviral immunity. On those lines, recently Gorenshiteyn et al performed a tour de force comprehensive computational analysis of 38,088 diverse genome scale immunological experimental datasets. This allowed them to create an extensive molecular interaction network capable of predicting antiviral responses (Gorenshiteyn et al., 2015). Along similar lines, in context of influenza A viruses, we have performed a meta-analysis of genome scale RNAi datasets and integrated it with global protein interaction data to uncover a functional biochemical landscape for influenza-host interactions, which allowed us to predict several novel antiviral host factors (unpublished). These data and tools are accessible at <http://www.metascape.org/IAV>.

Systems Studies on Influenza A viruses

Influenza viruses have negative sense segmented RNA genome, which can acquire mutations rapidly through genetic shift and drift. This allows the virus to change its host specificity, virulence and escape the restriction posed by drugs or vaccines (Medina and Garcia-Sastre, 2011). Factors contributing to emergence of novel influenza strains are poorly understood and new strains often cause intermittent pandemics at global scale, case in point the 2009 H1N1 pandemic. The seasonal influenza strains usually have low pathogenicity; however the

1918 H1N1, H5N1 ‘Bird Flu’ and recently emerged H7N9 viruses have shown severe pathogenesis and increased mortality in humans. Orthogonal integration of transcriptional profiling, proteomic and RNAi data to generate detailed virus-host interaction network has been done with many viral pathogens including Influenza A viruses (Konig et al., 2008; Watanabe et al., 2010). These studies are contributing to emergence of ‘Systems Virology’ as a discipline in its own (Law et al., 2013); along the way these studies are also yielding knowledge about anti-viral immune responses (Baas et al., 2006; Shapira et al., 2009). For instance Brass *et al* conducted a genome wide RNAi screen to identify host dependency factors for IAV, and in the process they also discovered IFITM proteins as broad spectrum anti-viral effectors (Brass et al., 2009). Apart from revealing antiviral signaling components and effectors, system level studies have also pointed to a major role of early innate immune response in the viral pathogenesis. In the case of Influenza A virus infections, transcriptional and proteomic profiling of infected animal models has shown co-operative action among IAV genome segments and host innate immunity and apoptosis genes in severe pathogenesis (Brown et al., 2010; Cheung et al., 2012; Kash et al., 2006; Kroeker et al., 2012). In another such study, Brandes et al applied transcriptional and chemokine profiling during IAV infection to differentiate the contribution of virus vs host in IAV associated pathogenesis and discovered an innate immune chemokine loop as important contributor (Brandes et al., 2013). Michael G. Katze's group has done extensive application of systems methods to understand influenza biology, specially the factors regulating viral pathogenesis (Korth et al., 2013). We, in collaboration with other groups have undertaken the ‘Fluomics’ initiative to achieve systems level understanding of influenza biology (<http://www.fluomics.org/>). A much desired application of the system based knowledge of antiviral immunity to viruses, is the development of more effective vaccines. This goal is compounded by additional variables such as age, immune status of the individuals, vaccine formulation and administration regimen (Hagan et al., 2015). Scientists have started analyzing the innate and adaptive immune responses in humans post vaccination in comprehensive manner in order to discover biomarkers of efficient vaccine response to influenza and other viruses (Andersen-Nissen et al., 2012; Nakaya et al., 2011; Querec et al., 2009; Tsang et al., 2014). These studies are contributing to the emergence of ‘Systems Vaccinology’ as a new research discipline (Nakaya et al., 2012).

Conclusion and perspective

As High throughput methods of data acquisition evolve, their precision and accuracy will increase and related costs will come down. This will make systems approaches to biological problems more popular and common practice. In order to make novel discoveries and avoid rediscovering known facts, it is crucial to integrate biologically diverse data sets. More powerful and universal algorithms are desired for this purpose. As increasing number of research groups generate omics datasets, data management is a crucial issue to avoid getting lost in translation. It's important that omics datasets are made available to the research community through easy to access databases where different studies can be compared in useful ways. Systems level understanding of antiviral innate immunity holds the key to the design of more effective and broad spectrum antivirals and vaccines. With advances in pharmacogenomics, system level understanding of innate immunity in context of individual

patient genetics could be translated into personalized medicine for treating viral infections and inflammatory disorders. However, to realize this goal, active collaborations are warranted for knowledge and resource sharing between clinical and systems biology researchers. In conclusion, systems biology approaches to understand functioning of antiviral immunity is poised to change paradigms, introduce new concepts in virus-host interactions and usher the anti-viral modalities into the era of ‘Systems medicine’.

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Highlights

- Cellular anti-viral innate immune response is highly dynamic and complex.
- Systems biology approach is well suited to study anti-viral innate immunity.
- Systems studies are explaining Influenza pathogenesis and vaccine efficacy.

Figure 1. Scheme of antiviral innate immune signaling in mammalian cells

Upon infection viral ligands (ss/ds RNA/DNA, RNA/DNA hybrids, glycoproteins) are detected by host viral sensors (PRRs, TLRs, ALRs, NLRs) which signal through STING and MAVS to activate IRFs and NFκB, which in turn move in to nucleus, bind to IFN promoter and drive IFN expression. Primary IFNs are secreted out of the cell where they bind to IFNAR and initiate JAK-STAT pathway. This involves formation of ISGF3 with STATs and IRF9, which translocate into the nucleus and bind to ISRE elements to drive expression of ISGs. Among ISGs are antiviral effectors, IFN signaling enhancers (IFNs, IRFs, STATs, PRRs) and inhibitors (SOCS, USP18). PRRs can also lead to formation of inflammasome complex which activates caspase 1 and initiates cell death response upon viral infection.

Figure 2. Systems biology approach to study biological systems

Systems biology studies involve parallel measurement of varied properties of the biological system using high throughput methods. The acquired data is then analyzed using computational tools to predict key regulators of the biological process being studied. Next, a mathematical model is generated to predict interaction between key regulators under different conditions and how it will affect the phenotype. This model is tested by selected perturbations and repetitive cycle of experimentation, until a robust model for the system is generated

Table 1**Systems Biology and Innate immunity Resources**

Resource (reference)	Web address
Innate Immunity Resources	
InnateDB (Breuer et al., 2013)	www.innatedb.com/
Interferome (Rusinova et al., 2013)	www.interferome.org/
The Immunological Genome Project (Heng et al., 2008)	www.immgen.org/
The Immunology Database and Analysis Portal (Bhattacharya et al., 2014)	www.immport.org/
Reference Database of Immune Cells (RefDIC) (Hijikata et al., 2007)	http://refdic.rcai.riken.jp/welcome.cgi
Immune Response In Silico (IRIS) (Abbas et al., 2005)	http://research-public.gene.com/share/clark.iris.2004/iris/iris.html
Innate Immunity in Heart, Lung and Blood Disease	www.regepi.bwh.harvard.edu/IIPGA2/
Collection of immune-related functional relationship networks (Gorenshteyn et al., 2015)	http://immunet.princeton.edu/
Omics Databases/ repositories	
Gene Expression Omnibus	www.ncbi.nlm.nih.gov/geo/
Array Express Archive	www.ebi.ac.uk/arrayexpress/
PRIDE Archive - proteomics data repository (Martens et al., 2005)	www.ebi.ac.uk/pride/archive/
Virus Pathogen Resource (ViPR)	www.viprbrc.org/
Proteomics research resource for integrative biology	www.panomics.pnnl.gov/data/
International hapMap project (International HapMap, 2003)	www.hapmap.org/
The Human protein atlas (Uhlen et al., 2010)	www.proteinatlas.org/
Human Proteome map (Kim et al., 2014)	www.humanproteomemap.org/
LipidMAPS (Fahy et al., 2007)	www.lipidmaps.org/
The Alliance for Cellular Signaling (Natarajan et al., 2006)	www.afcs.org/
Omics data analysis tools	
DAVID (Huang da et al., 2009)	http://david.abcc.ncifcrf.gov/
Cytoscape (Shannon et al., 2003)	www.cytoscape.org
STRING (Jensen et al., 2009)	www.string-db.org
Cell Region-Based Rendering And Layout: CEREBRAL (Barsky et al., 2007)	www.pathogenomics.ca/cerebral/
Network Analysis Tools (NEAT) (Brohee et al., 2008)	http://rsat01.biologie.ens.fr/rsa-tools/NeAT_home.html
VisANT (Hu et al., 2013)	http://visant.bu.edu/
Osprey (Breitkreutz et al., 2003)	http://biodata.mshri.on.ca/osprey/servlet/Index
Ingenuity Pathway Analysis (IPA)	www.ingenuity.com
Biobase	www.biobase-international.com
Other useful resources	
Pathguide (Bader et al., 2006)	www.pathguide.org
Systems Biology Markup Language (Hucka et al., 2003)	www.sbml.org
Influenza Research database	www.fludb.org/
Systems Influenza Program	www.systemsinfluenza.org
Influenza 'Big Data' bank and analysis resource	www.metascope.org/IAV/