Elucidating *Influenza* host-pathogen interactions through data integration and analysis utilizing the BioHealthBase BRC

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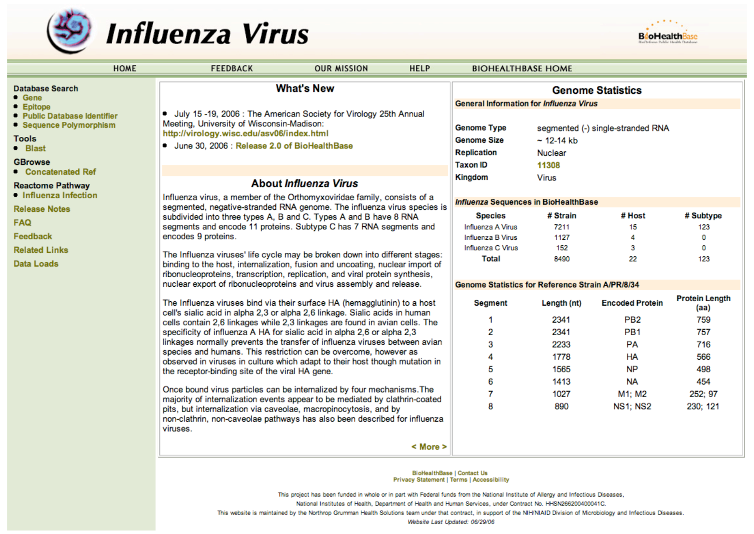
BioHealthBase
BioDefense Public Health Database

BioHealthBase BRC

Introduction

The primary mission of the BioHealthBase Bioinformatics Resource Centers (BRCs) for Biodefense and Emerging/Re-emerging Infectious Diseases is to assist *Influenza virus (A, B, C), Francisella tularensis, Mycobacterium tuberculosis* researchers in their development of vaccines, therapeutics, and diagnostics. The BRCs, contracted through the National Institute of Allergy and Infectious Disease's (NIAID) Division of Microbiology and Infectious Diseases (DMID), will provide both central repositories for a wide variety of scientific data on these pathogenic microorganisms and a platform for software tools that support investigator-driven data analysis. A description of the NIAID BRC program can be found at: http://www.niaid.nih.gov/dmid/genomes/brc/default.htm



What's New

- July 15 -19, 2006 : The American Society for Virology 25th Annual Meeting, University of Wisconsin-Madison: http://virology.wisc.edu/asv06/index.html
- June 30, 2006 : Release 2.0 of BioHealthBase

About *Influenza Virus*

Influenza virus, a member of the Orthomyxoviridae family, consists of a segmented, negative-stranded RNA genome. The influenza virus species is subdivided into three types A, B and C. Types A and B have 8 RNA segments and encode 11 proteins. Subtype C has 7 RNA segments and encodes 9 proteins.

The Influenza viruses' life cycle may be broken down into different stages: binding to the host, internalization, fusion and uncoating, nuclear import of ribonucleoproteins, transcription, replication, and viral protein synthesis, nuclear export of ribonucleoproteins and virus assembly and release.

The Influenza viruses bind via their surface HA (hemagglutinin) to a host cell's sialic acid in alpha 2,3 or alpha 2,6 linkage. Sialic acids in human cells contain 2,6 linkages while 2,3 linkages are found in avian cells. The specificity of influenza A HA for sialic acid in alpha 2,6 or alpha 2,3 linkages normally prevents the transfer of influenza viruses between avian species and humans. This restriction can be overcome, however as observed in viruses in culture which adapt to their host though mutation in the receptor-binding site of the viral HA gene.

Once bound virus particles can be internalized by four mechanisms.The majority of internalization events appear to be mediated by clathrin-coated pits, but internalization via caveolae, macropinocytosis, and by non-clathrin, non-caveolae pathways has also been described for influenza viruses.

< More >

Genome Statistics

General Information for *Influenza Virus*

Genome Type	segmented (-) single-stranded RNA
Genome Size	~ 12-14 kb
Replication	Nuclear
Taxon ID	11308
Kingdom	Virus

Influenza Sequences in BioHealthBase

Species	# Strain	# Host	# Subtype
Influenza A Virus	7211	15	123
Influenza B Virus	1127	4	0
Influenza C Virus	152	3	0
Total	8490	22	123

Genome Statistics for Reference Strain A/PR/8/34

Segment	Length (nt)	Encoded Protein	Protein Length (aa)
1	2341	PB2	759
2	2341	PB1	757
3	2233	PA	716
4	1778	HA	566
5	1565	NP	498
6	1413	NA	454
7	1027	M1; M2	252; 97
8	890	NS1; NS2	230; 121

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This project has been funded in whole or in part with Federal funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, under Contract No. HHSN266200400041C.
This website is maintained by the Northrop Grumman Health Solutions team under that contract, in support of the NIH/NIAID Division of Microbiology and Infectious Diseases.
Website Last Updated: 06/29/06

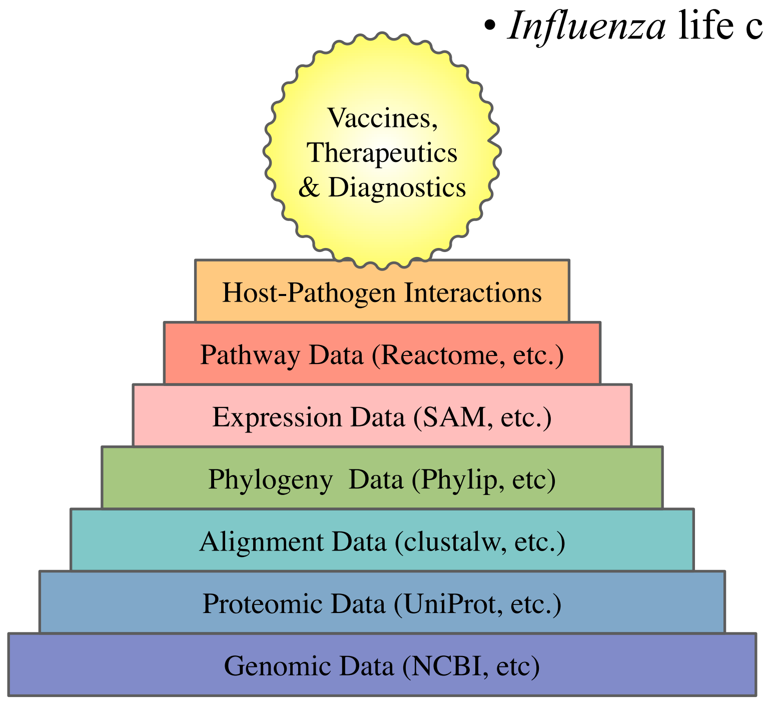
Figure 1. BioHealthBase BRC *Influenza* page.

Current Features

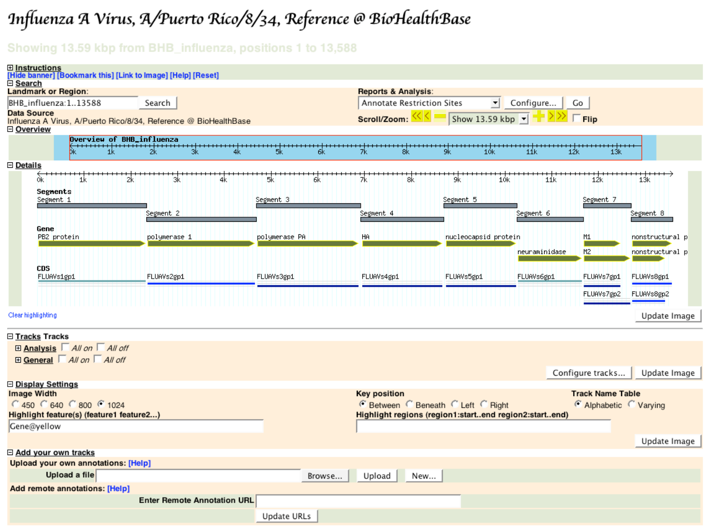
- Integrated data sets from NCBI, UniProt, Pfam, IEDB, etc.
- Web-based data-mining and visualization tools
- Sequence data repository for several strains
- Structural features and functional annotation
- Metabolic and signaling pathway annotation

Influenza Specific Features

- Sequence
  - Creation of consensus sequence based on sequence multi-alignments
- Analysis
  - *Influenza* sequence alignments and polymorphism frequencies
- Epitopes
  - Immune Epitope Database (IEDB) validated epitopes
  - MHC class I predicted epitope using NetCTL
- Visualization
  - Concatenated (8-segment) genome display of A/Puerto Rico/8/34
- Pathways
  - *Influenza* life cycle pathways, host-pathogen interactions in the Reactome database



Vaccines, Therapeutics & Diagnostics
Host-Pathogen Interactions
Pathway Data (Reactome, etc.)
Expression Data (SAM, etc.)
Phylogeny Data (Phylip, etc)
Alignment Data (clustalw, etc.)
Proteomic Data (UniProt, etc.)
Genomic Data (NCBI, etc)



Influenza A Virus, A/Puerto Rico/8/34, Reference @ BioHealthBase

Showing 13.59 kbp from BHB_influenza, positions 1 to 13,588

Landmark or Region: BHB_influenza:1..13588
Data Source: Influenza A Virus, A/Puerto Rico/8/34, Reference @ BioHealthBase
Reports & Analysis: Annotate Restriction Sites
Scroll/Zoom: Show 13.59 kbp ☐ Flip
Analysis ☐ All on ☐ All off
General ☐ All on ☐ All off
Image Width ○ 450 ○ 640 ○ 800 ● 1024
Key position ● Between ○ Beneath ○ Left ○ Right
Track Name Table ● Alphabetic ○ Varying
Highlight feature(s) (feature1 feature2...) Gene@yellow
Highlight regions (region1:start..end region2:start..end)
Upload your own annotations: [Help]
Upload a file
Add remote annotations: [Help]
Enter Remote Annotation URL

Figure 2. Concatenated sequence view of *Influenza*.

Collaborators Welcome

We are currently seeking *Influenza* researchers who would like to work with our team to develop the BioHealthBase application. If you are interested in providing data for analysis, feedback on existing functionality, or recommendations for new functionality, please contact us at feedback@biohealthbase.org.

Reactome Pathways and Host-Pathogen Interactions

Introduction

The Reactome project is a collaboration among Cold Spring Harbor Laboratory, The European Bioinformatics Institute, and The Gene Ontology Consortium to develop a curated resource of core pathways and reactions in human biology. Working in collaboration with *Influenza* experts and Reactome personnel we have added the *Influenza A* virus life cycle foundation to the Reactome project. Additionally, we have added the Toll-like receptor 3 (TLR3) and RIG-I host response pathways to the Reactome project.

Plan:

1. Influenza Life Cycle Foundation
2. Host Response Pathways
3. Detailed Influenza Pathways
4. Connect Host and Pathogen

Current and Future Work

Our initial contributions to the Reactome project include the *Influenza* A virus life cycle stages and their subcomponents. Our immediate priority is to flesh out the *Influenza* pathways that directly relate to NS1, M2 ion channel inhibition and neuraminidase inhibitors. Future work includes efforts to map out host-pathogen interactions between *Influenza* virus and a human host. Our first host pathways, RIG-I and TLR3, have been released in the Reactome release (May 2006) with pathways directly interacting to follow in the future.

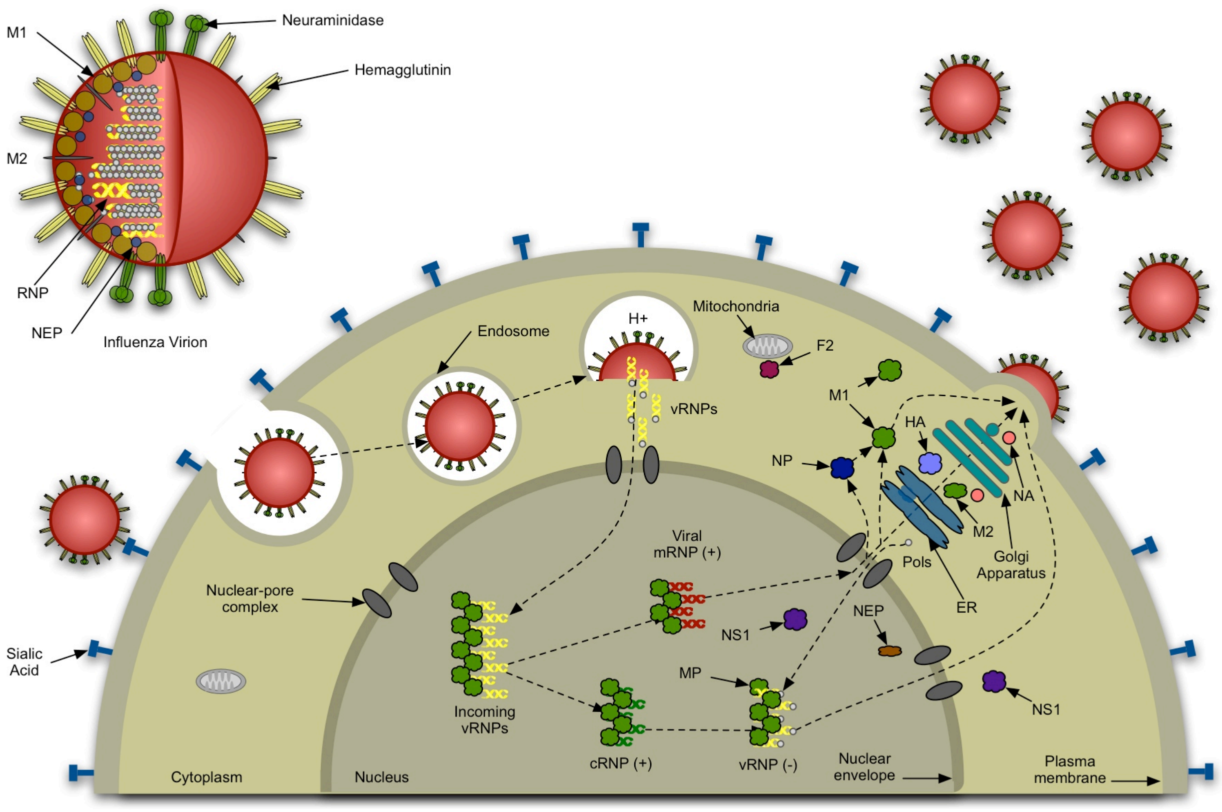
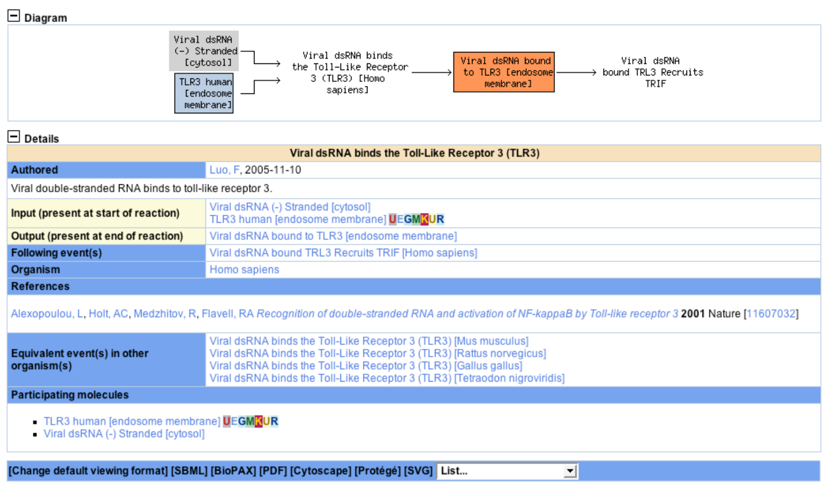


Figure 3. Diagram of the Influenza A virus life cycle.

- Influenza Infection [Homo sapiens]
  - Influenza Life Cycle
    - Binding of the influenza virion to the host cell
    - Entry of Influenza Virion into Host Cell via Endocytosis
      - Clathrin-Mediated Endocytosis of the Influenza Virion
      - Caveolae Endocytosis
      - Non-Clathrin, Non-Caveolae Endocytosis Pathways
      - Macropinocytosis
    - Fusion and Uncoating of the Influenza Virion
      - Fusion of the Influenza Virion to the Host Cell Endosome
      - Uncoating of the Influenza Virion
    - Transport of Ribonucleoproteins into the Host Nucleus
      - Recognition of the Nuclear Localization Signal (NLS) by a Karyopherin Alpha Family Protein
      - Recruitment of Karyopherin Beta to form a Trimeric Complex
      - Docking of trimeric complex at the nuclear pore
      - Transport of the RNP into the host cell nucleus
      - Release of the RNP into the host cell nucleus
    - Influenza Virion RNA Transcription and Replication
      - Viral Messenger RNA Synthesis
      - cRNA Synthesis
      - vRNA Synthesis
    - Assembly and Nuclear Export of Ribonucleoproteins
      - Assembly of Viral RNP Complexes in the Host Cell Nucleus
      - NEP/NS2 Interacts with the Cellular Export Machinery
    - Virus Assembly and Release
      - Assembly of Viral Components at the Budding Site
      - Packaging of Eight RNA Segments
      - Budding
      - Release
  - Host Interactions with Influenza Factors
    - NS1 Mediated Effects on Host Pathways
      - Inhibition of Host mRNA Processing and RNA Silencing
      - Inhibition of Interferon Synthesis
      - Inhibition of PKR
    - Influenza Virus Induced Apoptosis
      - NA activation of TGF-beta
      - PB1-F2 binds to the mitochondrial adenine nucleotide translocator 3 ANT3, inducing apoptosis

Figure 4. *Influenza* A virus Reactome life cycle outline.



Viral dsRNA binds the Toll-Like Receptor 3 (TLR3)	
Authored	Luo, F, 2005-11-10
Viral double-stranded RNA binds to toll-like receptor 3.	
Input (present at start of reaction)	Viral dsRNA (-) Stranded [cytosol] TLR3 human [endosome membrane] UEGMKUR
Output (present at end of reaction)	Viral dsRNA bound to TLR3 [endosome membrane]
Following event(s)	Viral dsRNA bound TRL3 Recruits TRIF [Homo sapiens]
Organism	Homo sapiens
References	
Alexopoulou, L, Holt, AC, Medzhitov, R, Flavell, RA *Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3* **2001** Nature [11607032]	
Equivalent event(s) in other organism(s)	Viral dsRNA binds the Toll-Like Receptor 3 (TLR3) [Mus musculus] Viral dsRNA binds the Toll-Like Receptor 3 (TLR3) [Rattus norvegicus] Viral dsRNA binds the Toll-Like Receptor 3 (TLR3) [Gallus gallus] Viral dsRNA binds the Toll-Like Receptor 3 (TLR3) [Tetraodon nigroviridis]
Participating molecules	

- TLR3 human [endosome membrane] UEGMKUR
- Viral dsRNA (-) Stranded [cytosol]

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Figure 5. Reactome detail view

- Immune System Signaling pathways [Homo sapiens]
  - Innate Immunity Signaling
    - Toll Receptor Cascades
      - Toll Like Receptor 3 (TLR3) Cascade
        - Viral dsRNA binds the Toll-Like Receptor 3 (TLR3)
        - Viral dsRNA bound TRL3 Recruits TRIF
        - Viral dsRNA:TLR3:TRIF Complex Activates RIP1
          - Viral dsRNA:TLR3:TRIF Complex Recruits RIP1
          - Viral dsRNA:TLR3:TRIF Complex Releases Activated RIP1
          - RIP1 phosphorylates IKKs
          - Phospho-IKK Complex phosphorylates IkB within the IkB:NFkB Complex
          - Released NFkB complex is transported to the Nucleus
        - TRAF6 Mediated Induction of the antiviral cytokine IFN-alpha/beta cascade
          - TRAF6 is Recruited to the Viral dsRNA:TLR3:TRIF Complex
          - Formation of the Viral dsRNA:TLR3:TRIF:TRAF6:TAB1:TAB2 Complex
          - Activation and Release of the TRAF6:Phospho-Tak1:Tab1:Phospho-Tab2 Complex
          - Phosphorylation of JNK by the Activated TRAF6:Phospho-TAK1:TAB1:Phospho-Tab2 Complex
          - Phosphorylation of c-Jun by Phospho-JNK
          - TRAF6:Phosho-TAK1:Tab1:PhosphoTAB2 mediated phosphorylation of MAPK1/p38
          - Phosphorylated MAPK1 phosphorylates ATF-2
          - Dimerisation of Activated Protein 1 (AP-1) heterodimer
          - TRAF6:Phosho-TAK1:Tab1:PhosphoTAB2 mediated phosphorylation of the IKK Complex
          - Phospho-IKK Complex phosphorylates IkB within the IkB:NFkB Complex
          - Released NFkB complex is transported to the Nucleus
        - Viral dsRNA:TLR3:TRIF Complex Activates TBK1
          - TBK1 is Recruited to the Viral dsRNA:TLR3:TRIF Complex
          - Viral dsRNA:TLR3:TRIF:TBK1 complex recruits IRF3
          - Phosphorylation and Release of IRF3
          - Dimerization of Phospho-IRF3
          - Dimerized Phospho-IRF3 is Transported To The Nucleus
      - Toll Like Receptor 4 (TLR4) Cascade
    - Complement cascade
    - RIG-I Mediated Induction of the Antiviral Cytokine IFN-alpha/beta Cascades
      - dsRNA binds to RIG-I
      - dsRNA:RIG-I Complex Activates IPS-1
      - IPS-1 stimulated the activation of RIP1
  - Adaptive Immunity Signaling

Figure 6. Host immune system signaling pathways.