

ANSWERING QUESTIONS WITH EXPERIMENTS (AND MODELS)

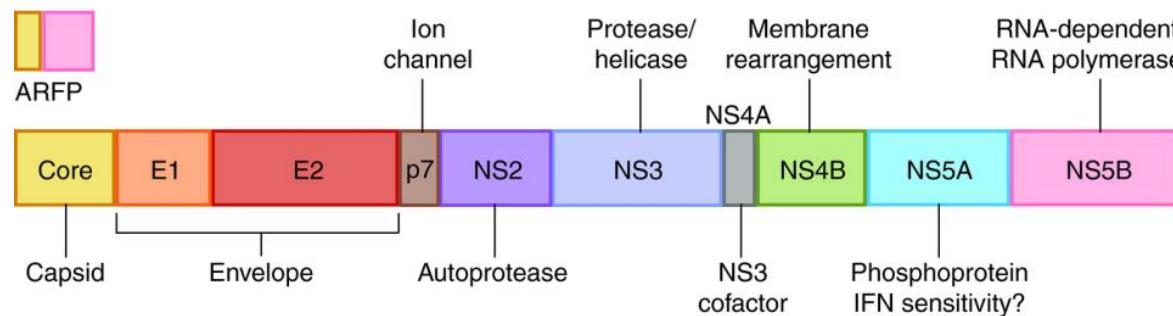
Unit 4
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HEPATITIS C VIRUS

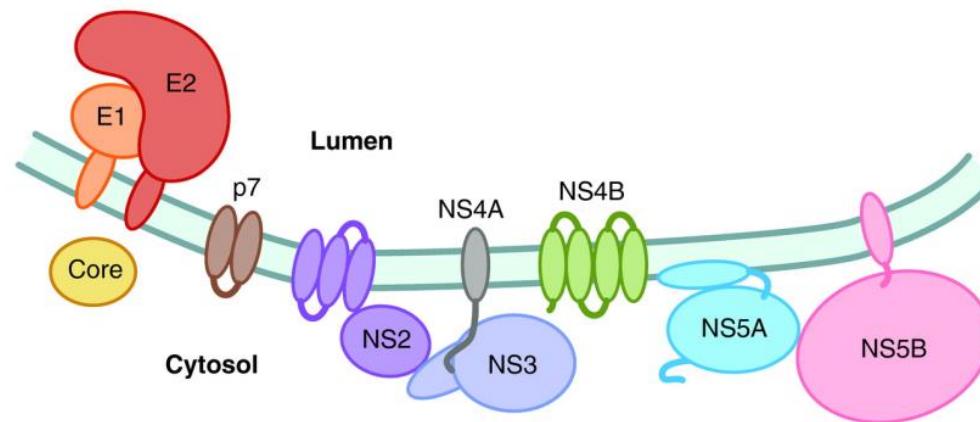
- Enveloped, positive strand RNA virus, *Flaviviridae*
- Isolated in 1989, treatments first emerged in early 1990s
- ~120 million-200 million infections worldwide, number one indication for liver transplant in the U.S.
- 10^{12} viral particles produced/day, $\frac{1}{2}$ life 3 hours in circulation
- Six major genotypes, 3 dominate in the U.S. (1, 2, 3)
 - 30-50% genetic variation among genotypes
 - 1-5% variation among viruses within a single patient
- Replicates via negative-stranded RNA in membranous web in cytoplasm

HCV STRUCTURE

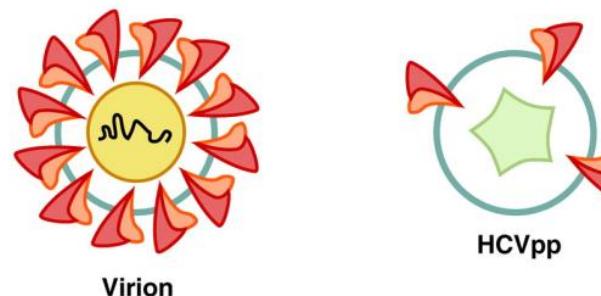
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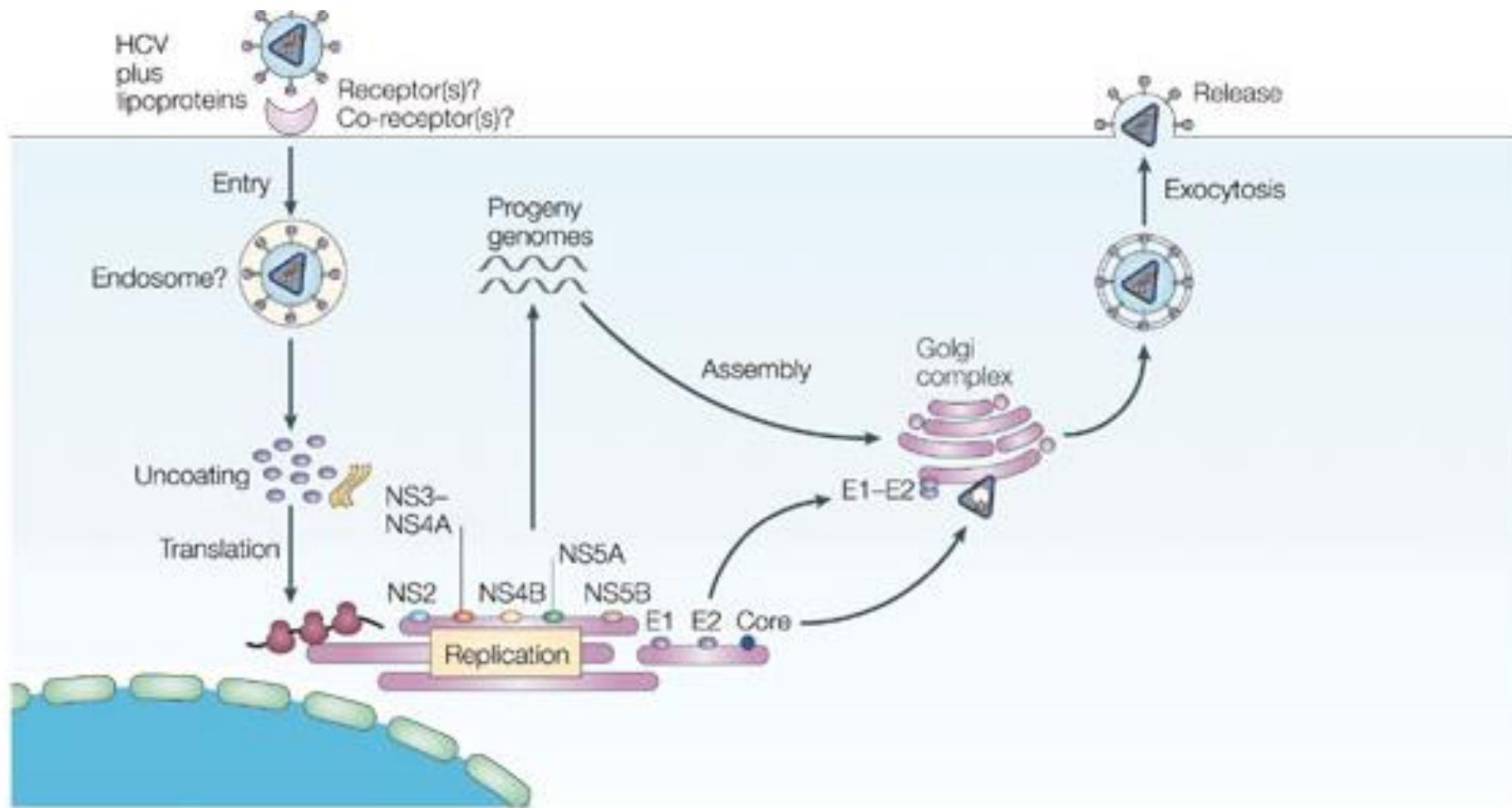


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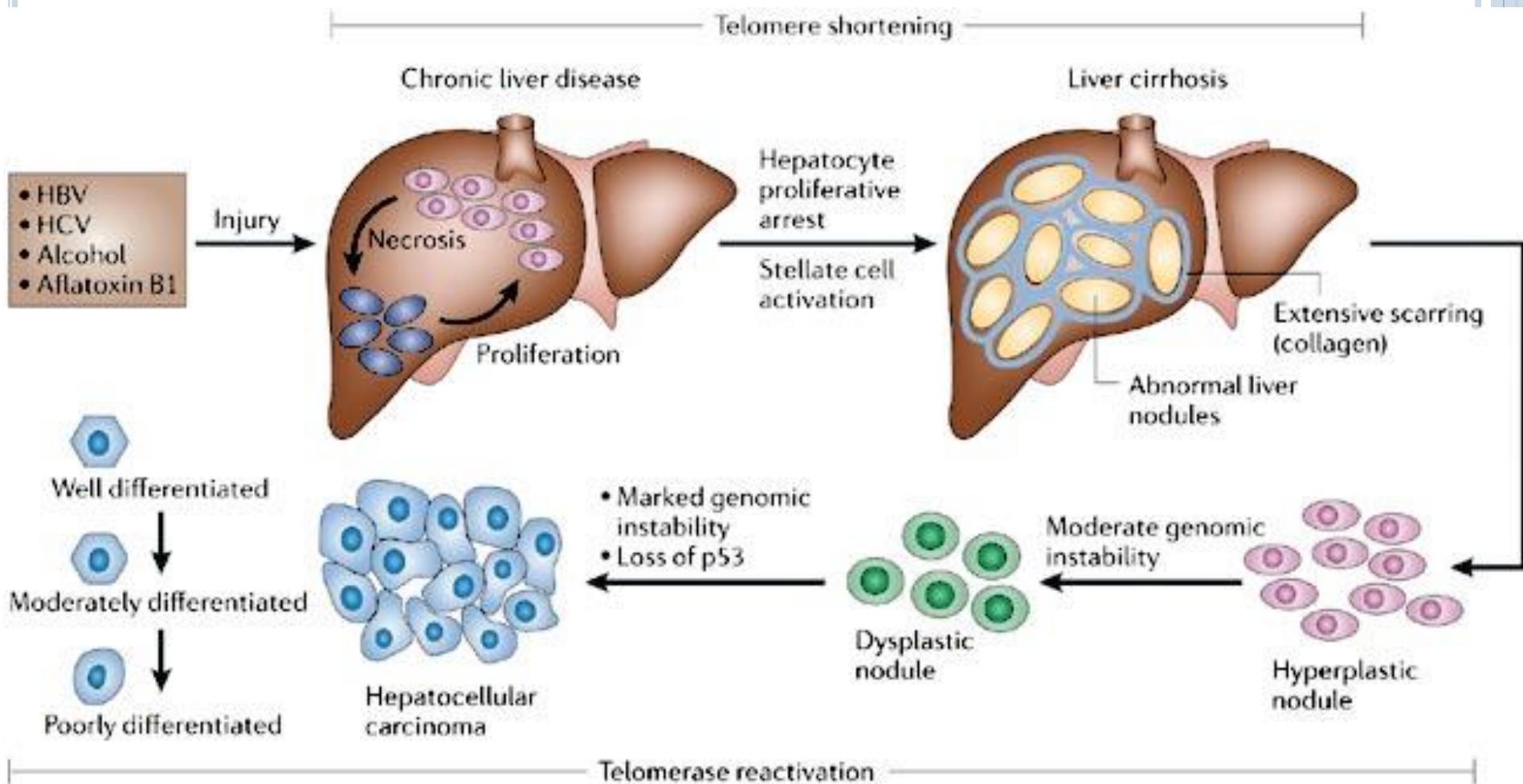
Dustin LB, Rice CM. 2007.
Annu. Rev. Immunol. 25:71–99

HCV LIFE CYCLE



HCV LIFE CYCLE 2

- HCV-associated disease results from viral persistence leading to long term inflammation and cell turnover



SPECIFIC CLEARANCE MECHANISMS FOR PATHOGEN CLASSES (KEEP IN MIND REDUNDANCY)

| Infectious agent | Disease | Humoral immunity | | | | Cell-mediated immunity | |
|------------------|------------------------------------|-----------------------|--------|--------|-----------|---------------------------|--------------------|
| | | IgM | IgG | IgE | IgA | CD4 T cells (macrophages) | CD8 killer T cells |
| Viruses | Variola | Smallpox | | | | | |
| | Varicella zoster | Chickenpox | Yellow | Red | | | |
| | Epstein-Barr virus | Mononucleosis | | Red | | | |
| | Influenza virus | Influenza | | Yellow | | Red | |
| | Mumps virus | Mumps | | Red | | | |
| | Measles virus | Measles | | Red | | | |
| | Polio virus | Poliomyelitis | | Yellow | | | |
| | Human immunodeficiency virus | AIDS | | Yellow | | | |
| Bacteria | <i>Staphylococcus aureus</i> | Boils | Red | Yellow | | | |
| | <i>Streptococcus pyogenes</i> | Tonsilitis | Red | Yellow | | | |
| | <i>Streptococcus pneumoniae</i> | Pneumonia | Red | Yellow | | | |
| | <i>Neisseria gonorrhoeae</i> | Gonorrhea | | Yellow | | | |
| | <i>Neisseria meningitidis</i> | Meningitis | | Red | | | |
| | <i>Corynebacterium diphtheriae</i> | Diphtheria | | Yellow | | | |
| | <i>Clostridium tetani</i> | Tetanus | | Yellow | | | |
| | <i>Treponema pallidum</i> | Syphilis | | Yellow | Transient | | |
| | <i>Borrelia burgdorferi</i> | Lyme disease | | Yellow | Transient | | |
| | <i>Salmonella typhi</i> | Typhoid | | Red | Yellow | | |
| | <i>Vibrio cholerae</i> | Cholera | | Red | Yellow | | |
| | <i>Legionella pneumophila</i> | Legionnaire's disease | | Red | Yellow | | |
| | <i>Rickettsia prowazekii</i> | Typhus | | | | Red | Yellow |
| | <i>Chlamydia trachomatis</i> | Trachoma | | Red | Yellow | Red | Yellow |
| Fungi | <i>Candida albicans</i> | Candidiasis | | Red | Yellow | | |
| | | | | | | Red | Yellow |
| Protozoa | <i>Plasmodium</i> spp. | Malaria | | Red | Yellow | | |
| | <i>Toxoplasma gondii</i> | Toxoplasmosis | | Red | Yellow | | |
| | <i>Trypanosoma</i> spp. | Trypanosomiasis | | Red | Yellow | | |
| | <i>Leishmania</i> spp. | Leishmaniasis | | | | Red | Yellow |
| Worms | Schistosome | Schistosomiasis | | | | Red | Yellow |

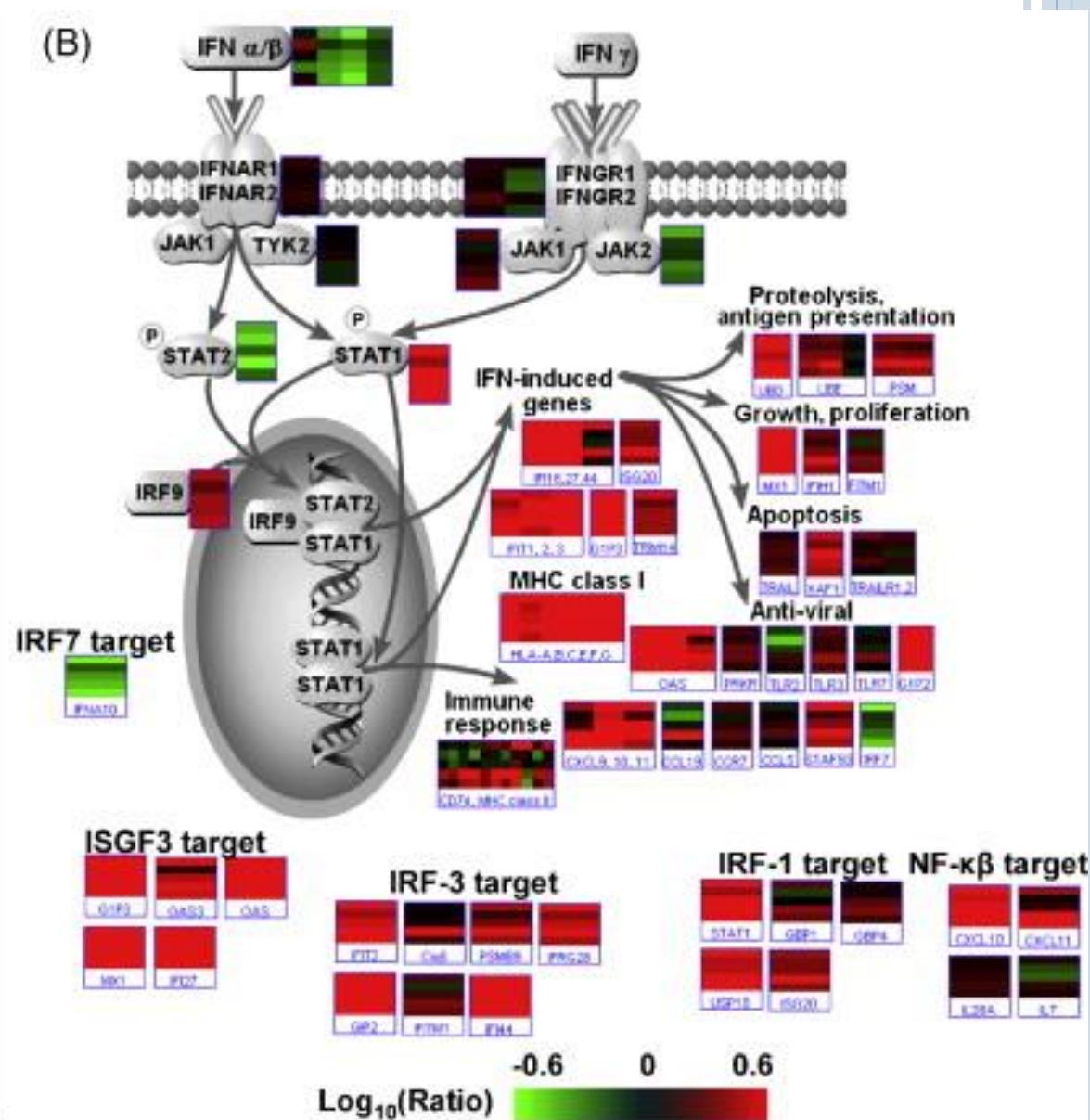
WHAT ARMS OF THE IMMUNE RESPONSE ARE USEFUL AGAINST HCV?

- Innate immunity
 - Antiviral effectors such as IFN that act on host cells, regulating key components of cell biology to limit viral growth and spread
- Antibody-mediated clearance
 - In principle, antibodies should be able to remove virus as it spreads from cell to cell
 - In practice, the correlation of antibody with HCV clearance and outcome is controversial or lacking
 - Patients with high levels of *neutralizing* antibodies nevertheless maintain chronic infection, indicating that neutralizing antibodies are not *sterilizing*
- Cell-mediated clearance
 - Infected cells can be killed before releasing progeny virions
 - Thought to be the primary means of long term control in HCV infection



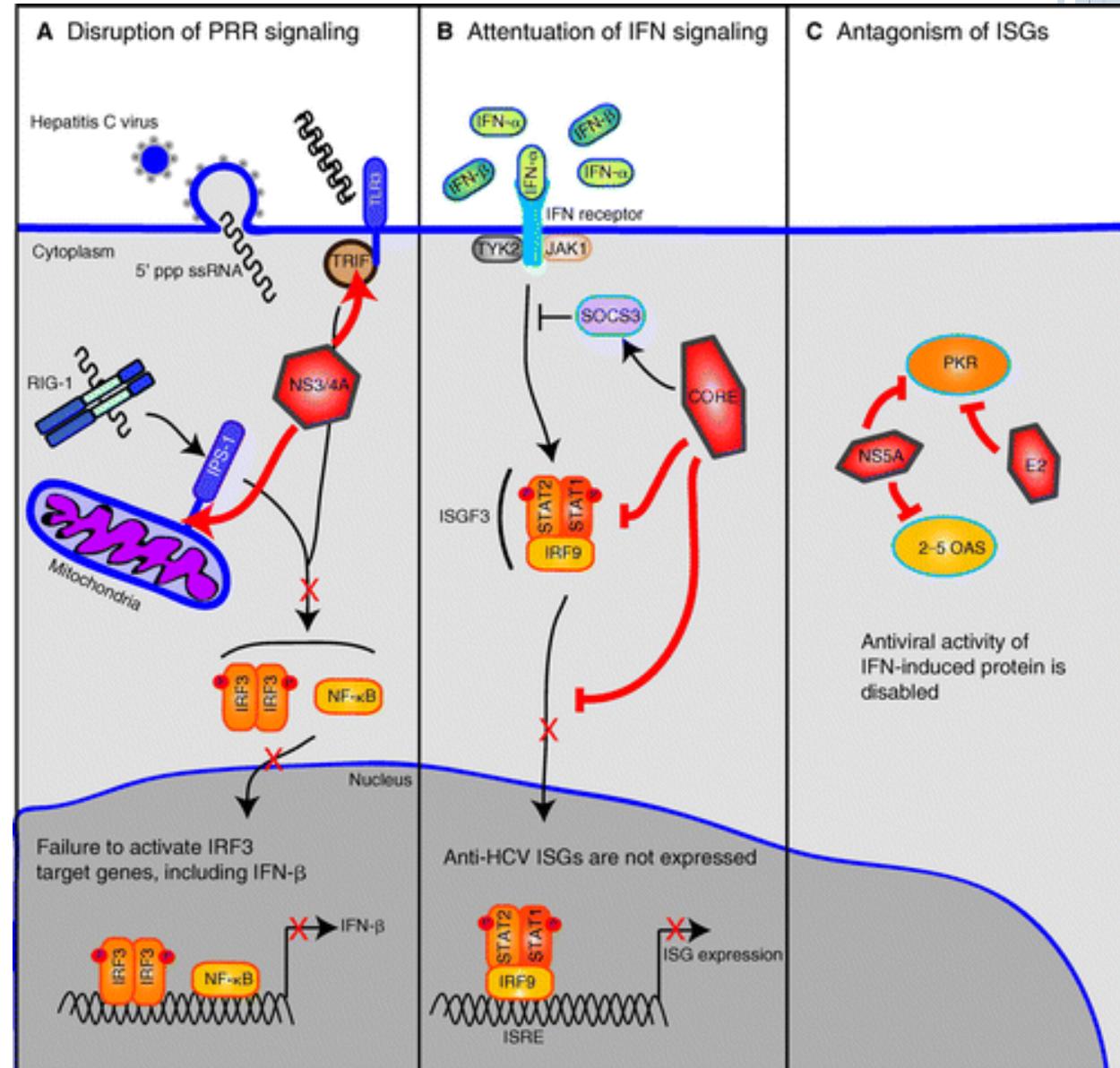
INDUCTION OF INNATE IMMUNITY IN PATIENTS

- IFN-induced genes interfere with viral replication directly:
 - Reducing protein synthesis by inhibiting initiation factors (PKR, ISG56)
 - Targeting of viral RNA (OAS, RNaseL)
- Innate responses can enhance or initiate adaptive responses
 - MHC I expression
 - Chemokine secretion and recruitment of responder cells



INNATE RECOGNITION OF HCV

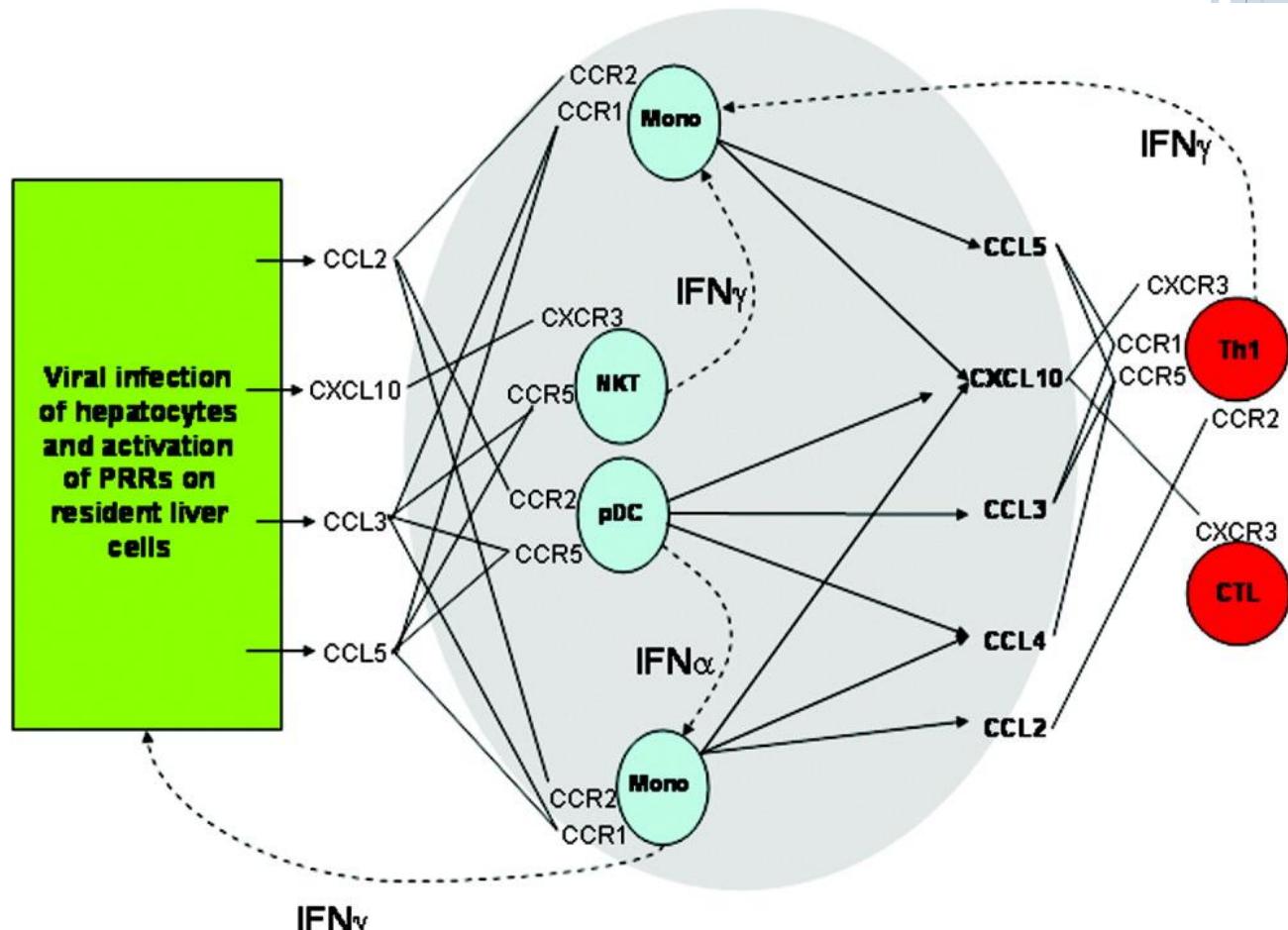
- The generation of dsRNA structures in HCV replication leads to recognition by multiple innate pathways
- HCV subverts these pathways by sequestering or cleaving key components of innate recognition
- The effects are both qualitative and quantitative on the ensuing innate response



Stacy M. Horner, Michael Gale. Journal of Interferon & Cytokine Research. September 2009, 29(9): 489-498

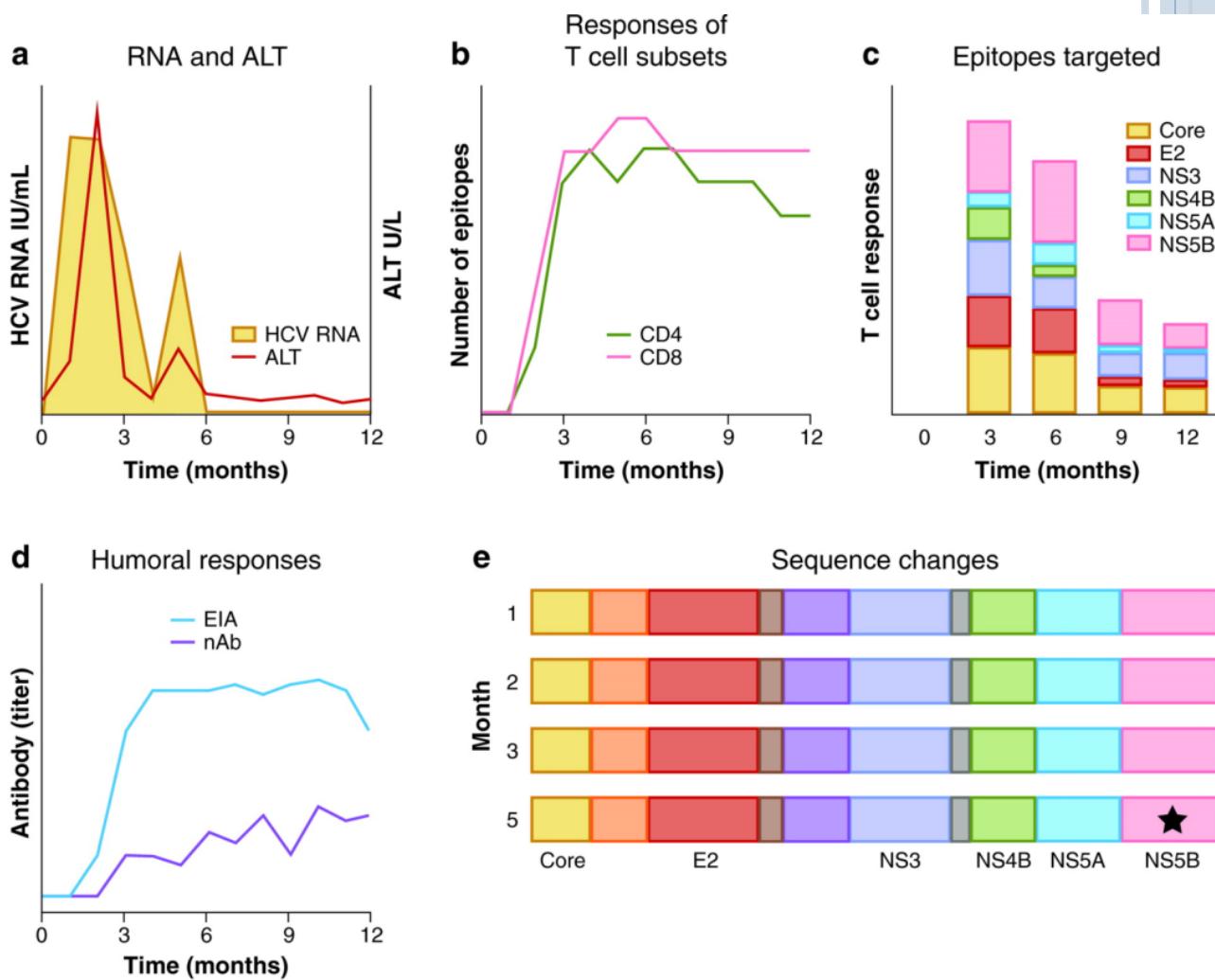
INNATE ACTIVATION OF ADAPTIVE RESPONSES

- The innate response results in the recruitment and “biasing” of key innate and adaptive cell types, including NK cells, NKT cells, antigen-presenting cells (monocytes/macrophages) and ultimately CD4 T cells that will orchestrate the adaptive response



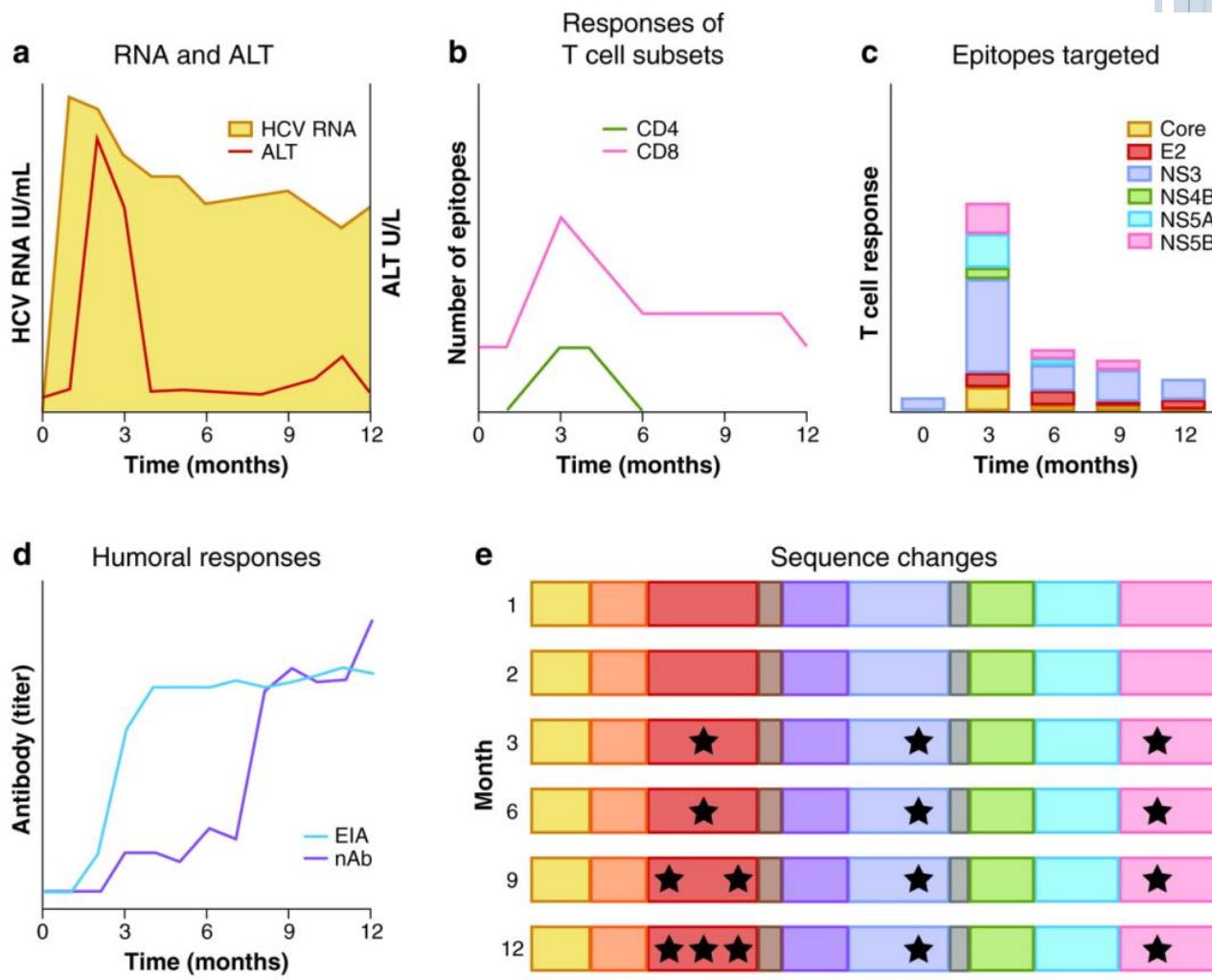
SUCCESSFUL HCV CONTROL (SUSTAINED VIROLOGICAL RESPONSE) IS MEDIATED BY ROBUST ADAPTIVE IMMUNITY

- Broad-based immunological repertoires (targeting multiple epitopes with diverse populations) control acute and prevent the development of chronic infections—particularly CD4 and CD8 cells (the role of antibody is controversial)

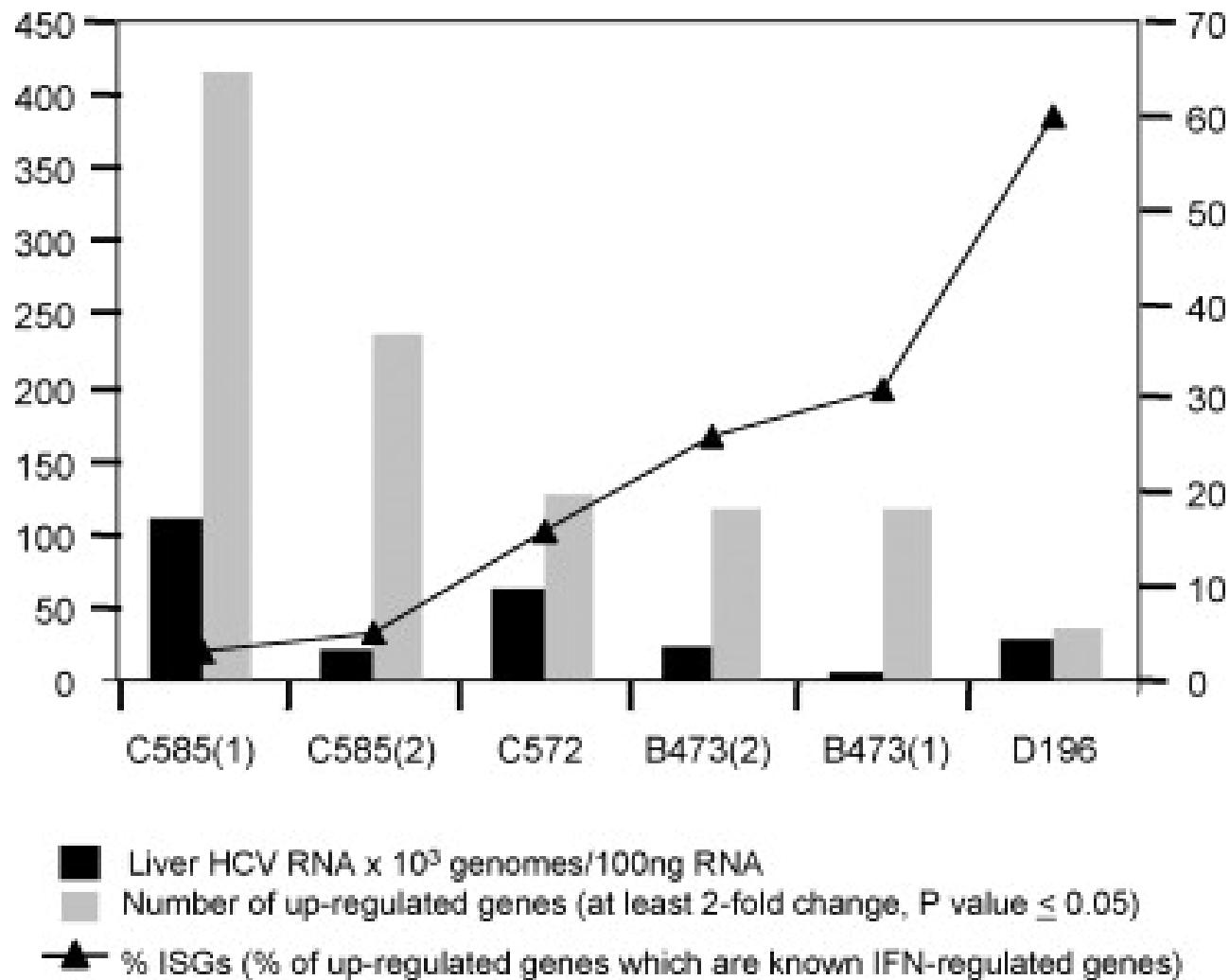


CHRONIC HCV INFECTIONS RESULT FROM POOR T CELL CONTROL, EPITOPE ESCAPE AND LIMITED REPERTOIRES

Limited TCR diversity, restricted epitope targets and dysfunctional T cell regulation result in weak T cell responses that are unable to avoid immunological escape

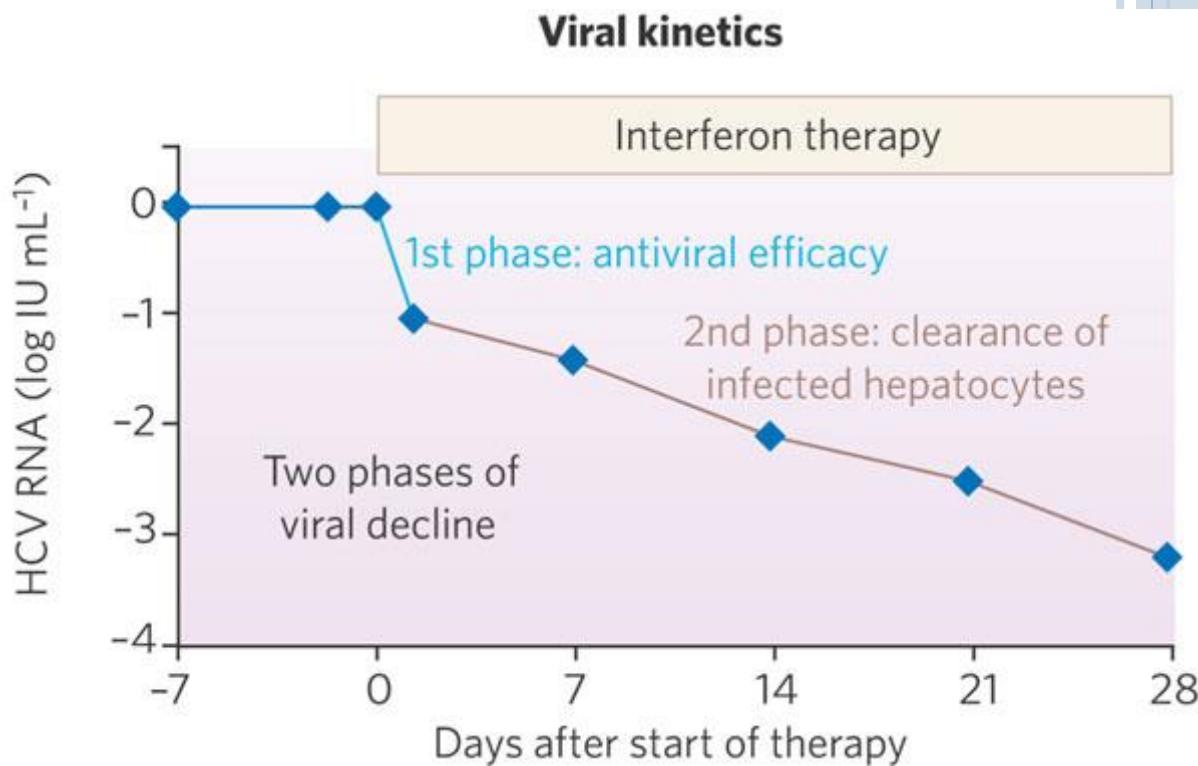


CONTROL OF ACUTE INFECTION CORRELATES WITH INTERFERON-INDUCED GENES



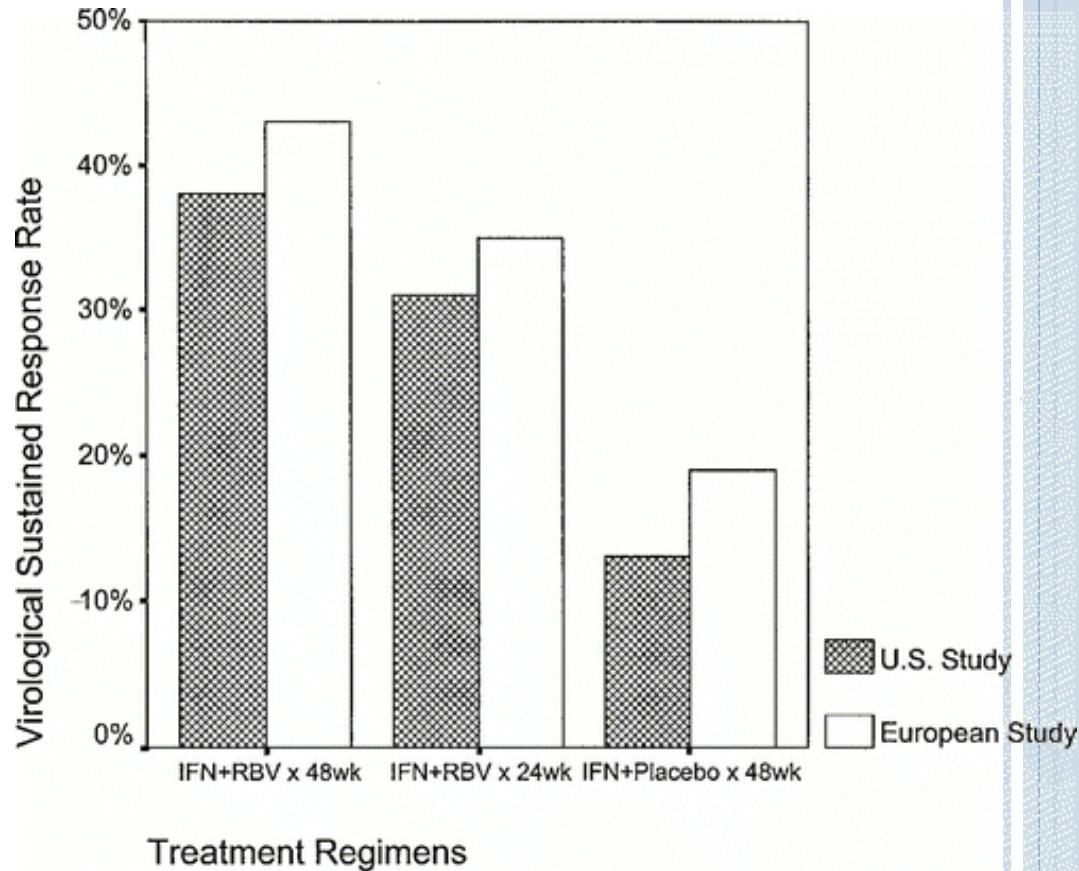
TREATMENT: TYPE I INTERFERON

- First therapy introduced for HCV
- Full mechanism of action unclear—presumably enhances the “normal” interferon response pathways
- Genotype of virus, low baseline levels of HCV RNA and stage of infection are the strongest correlates of efficacy
- Suggestions that immunomodulation may play a role and that high dose-interferon may overcome some of the “regulatory” negative feedback loops active in the infected host
- Overall, the specific mechanism has not been clearly demonstrated biologically



COMBINATION THERAPY IS SIGNIFICANTLY MORE EFFECTIVE

- Interferon alone only yields a 20-25% response rate following a 12-18 month course
- Combination therapy with the “broad based” antiviral ribavirin results in 40% of individuals with SVR (30% genotype 1, 65% genotype 2 or 3)

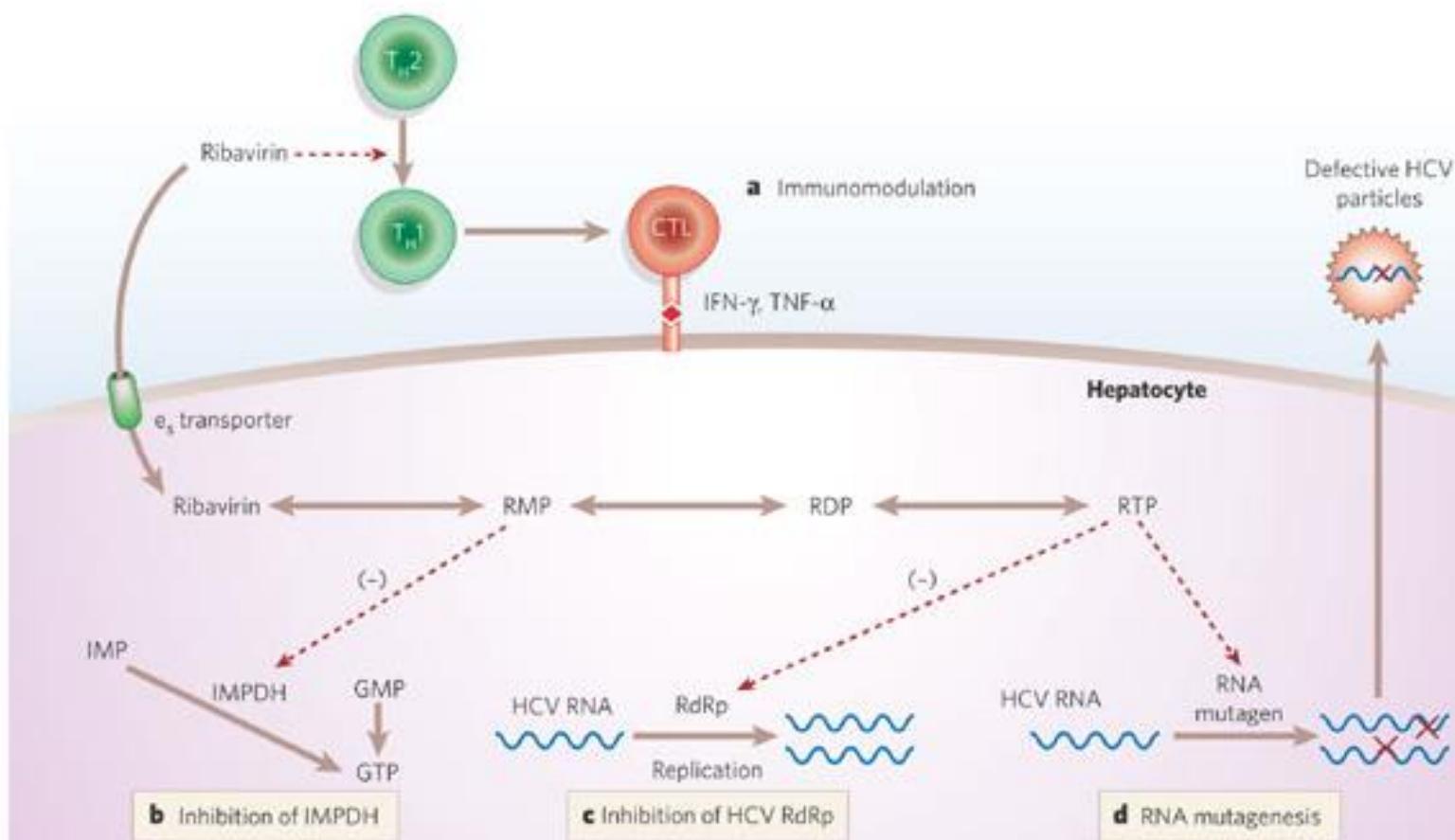


HOW DOES RIBAVIRIN WORK AGAINST HCV?

- Ribavirin was initially designed as a nucleoside analog and developed as an anti-influenza drug, but failed to receive FDA approval or show significant efficacy in humans
- It has been used to treat hemorrhagic fevers, RSV and is again under consideration as combination therapy for influenza
- Proposed Mechanisms:
 - 1) Immunomodulatory properties
 - 2) Inhibition of the inosine monophosphate dehydrogenase (IMPDH)
 - 3) Direct inhibition of the HCV-encoded NS5B RNA polymerase
 - 4) Induction of lethal mutagenesis
 - 5) Modulation of interferon-stimulated gene (ISG) expression



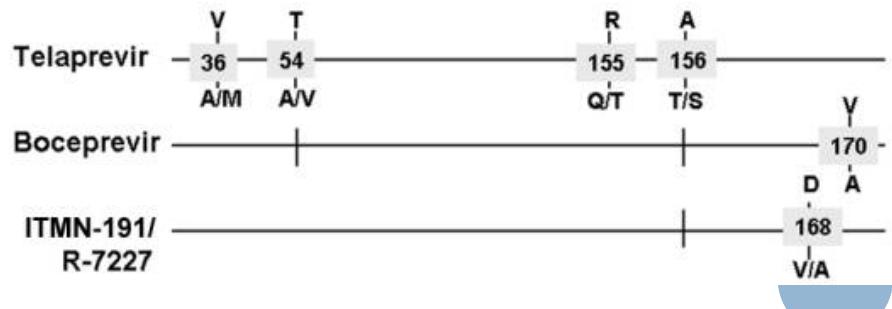
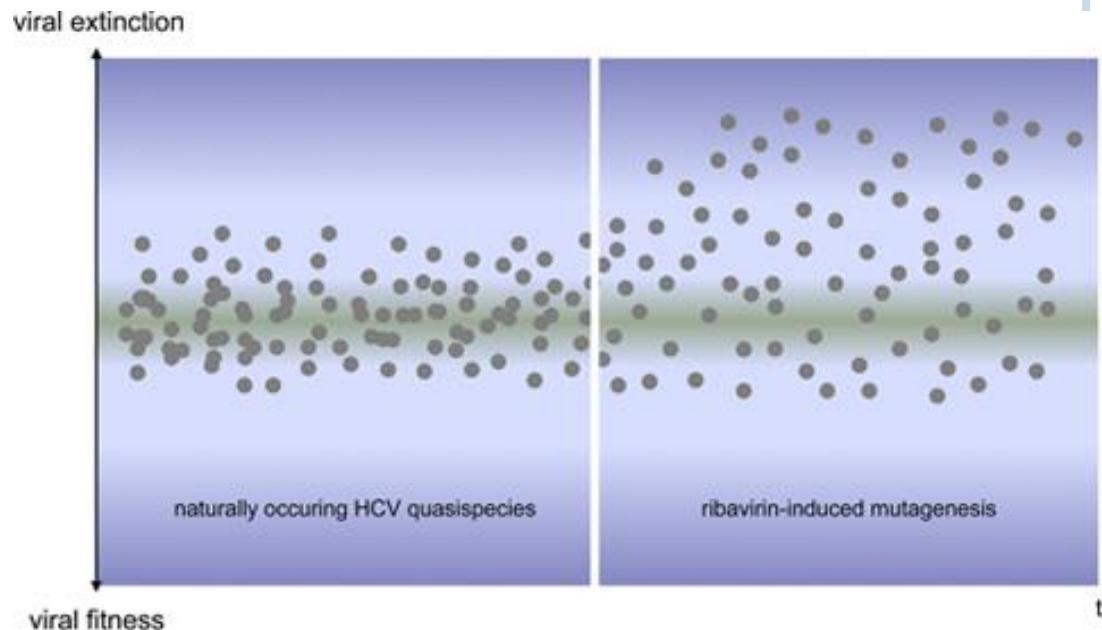
POSSIBLE MECHANISMS FOR RIBAVIRIN MODE OF ACTION



WHAT DATA WOULD HELP RESOLVE RIBAVIRIN'S MECHANISM?

Interferon reduces viral production-- given the proposed mechanisms, how should ribavirin work?

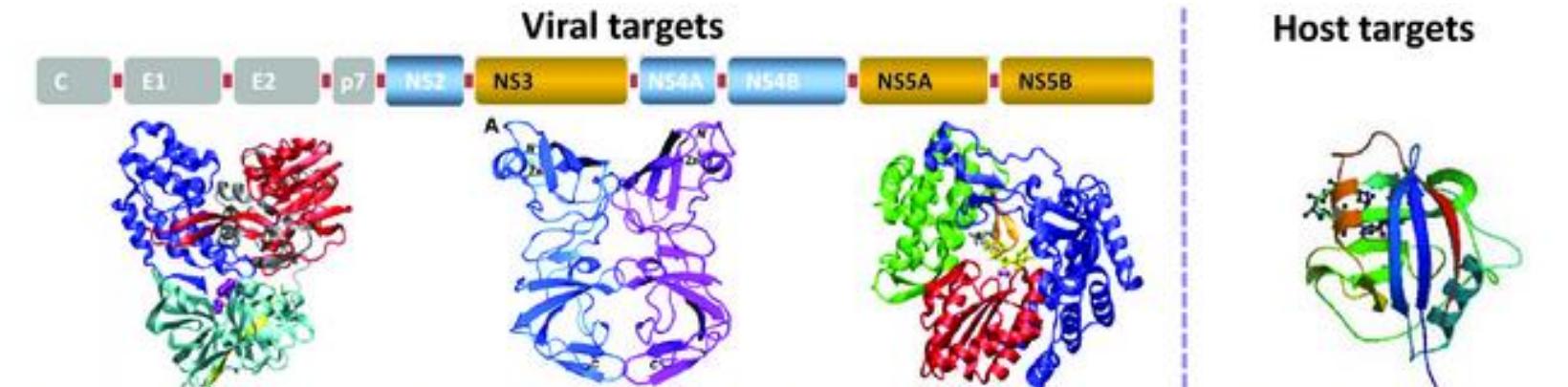
- 1) Immunomodulatory properties—**Should act independently of interferon**
- 2) Inhibition of the inosine monophosphate dehydrogenase (IMPDH)—**Should reduce viral production, be guanosine dependent**
- 3) Direct inhibition of the HCV-encoded NS5B RNA polymerase—**Should reduce viral production, put pressure on polymerase to mutate**
- 4) Induction of lethal mutagenesis—**Viral production maintained, infected cell number maintained (clearance by decay), new cells infected at a lower rate**
- 5) Modulation of interferon-stimulated gene (ISG) expression—**Direct antiviral effects like interferon, should shift ISG expression from negative feedback pathways and be synergistic with poor interferon responders.**



DETERMINING AN ANTIVRAL TREATMENT'S MODE OF ACTION

- Biological *in vitro* experiments with HCV have been difficult to perform as a result of the limited nature of developed culture systems
- Alternative drugs that perform a single “ribavirin function” do not recapitulate ribavirin efficacy, suggesting that multiple pathways may be acting together
- Biological mechanisms can often seem plausible, but can be difficult to prove conclusively that they play an important role (particularly when the drug is “reverse engineered” to the pathogen)
- Mathematical modeling from real infection data provides a compelling argument for the viral life cycle stage(s) that might be affected

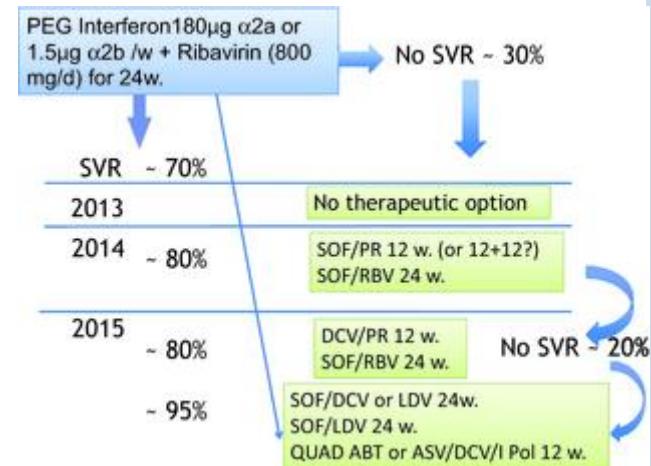
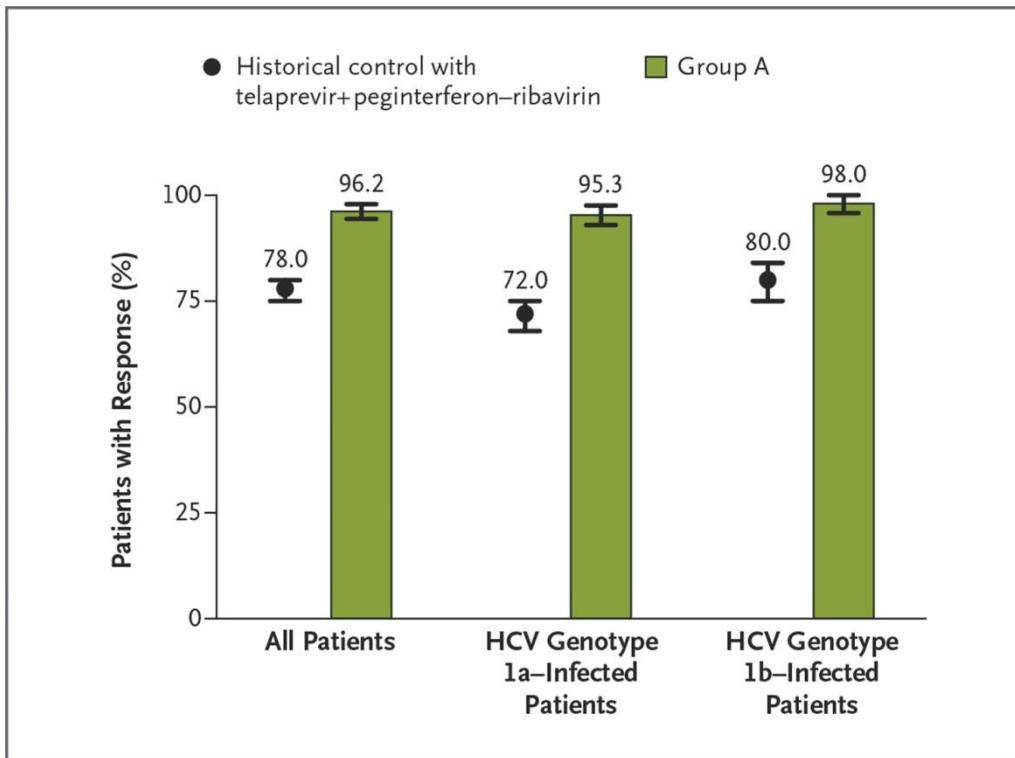
NEW DRUG TREATMENTS FOR HCV



The diagram illustrates the HCV genome structure and its viral targets. At the top, the genome is shown as a series of colored boxes: C (grey), E1 (light blue), E2 (medium blue), p7 (green), NS2 (dark blue), NS3 (yellow), NS4A (light blue), NS4B (blue), NS5A (yellow), and NS5B (yellow). Below the genome, four 3D ribbon models represent the targets: 1) NS3 protease (red and green), 2) NS4B (purple), 3) NS5A (green and blue), and 4) NS5B polymerase (red and blue).

| Viral targets | | | | Host targets |
|--|--|---|---|--------------|
| NS3 | NS5A | NS5B | Cyclophilin A | |
| The NS3/4A serine protease | Multifunctional phosphoprotein, component of the HCV-RNA replication complex | RNA-dependent RNA polymerase | Host protein interacting with NS5A and the NS5B | |
| Boceprevir Telaprevir ABT-450/r, ACH-1625 Asunaprevir, TMC-435 (Simeprevir), BI-201335 Danoprevir/r, GS-9451 MK-5172 | Daclatasvir GS-5885 ABT-267 PPI-668 MK | <u>Nucleos(t)ide analogue</u> GS-7977 (Sofosbuvir), Mericitabine, IDX-184 <u>Non-nucleoside analogue</u> BI-207127, ABT-333 ABT-072, BMS-791325 Tegobuvir, Setrobuvir VX-222, Filibuvir | Alisporivir | SCY-635 |

Rates of Sustained Virologic Response among All Patients and According to HCV Genotype in the Historical Control Group and in Group A.



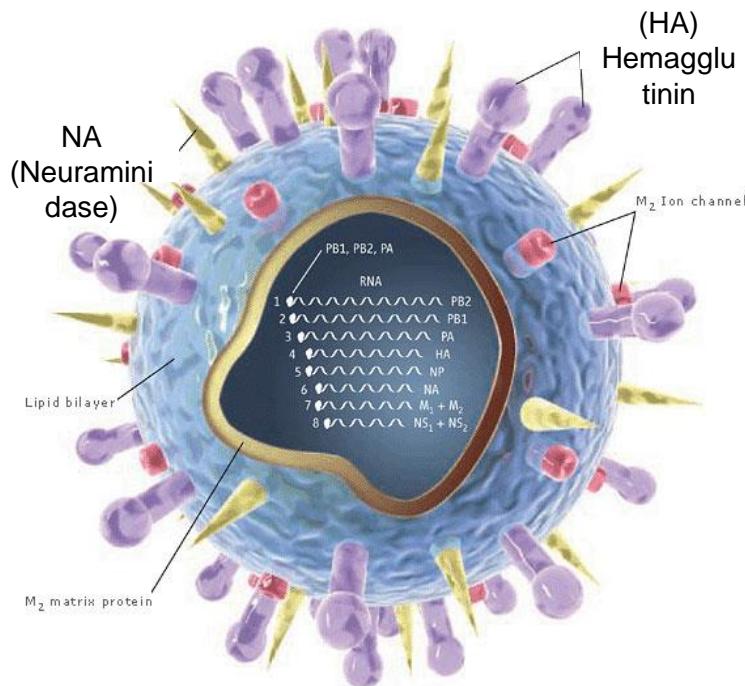
FELD JJ ET AL. N ENGL J MED 2014;370:1594-1603.



The NEW ENGLAND
JOURNAL of MEDICINE

INFLUENZA A VIRUS

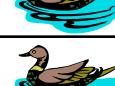
- Negative sense, segmented RNA virus
- *Orthomyxoviridae*
- Eight genes, 11 proteins (three alternate reading frames)
- Two non-structural proteins (NS₁ and PB₁-F₂)
- Surface proteins HA and NA determine serotype



Modified from: Kaiser. *Science* 2006, 312:380-382.

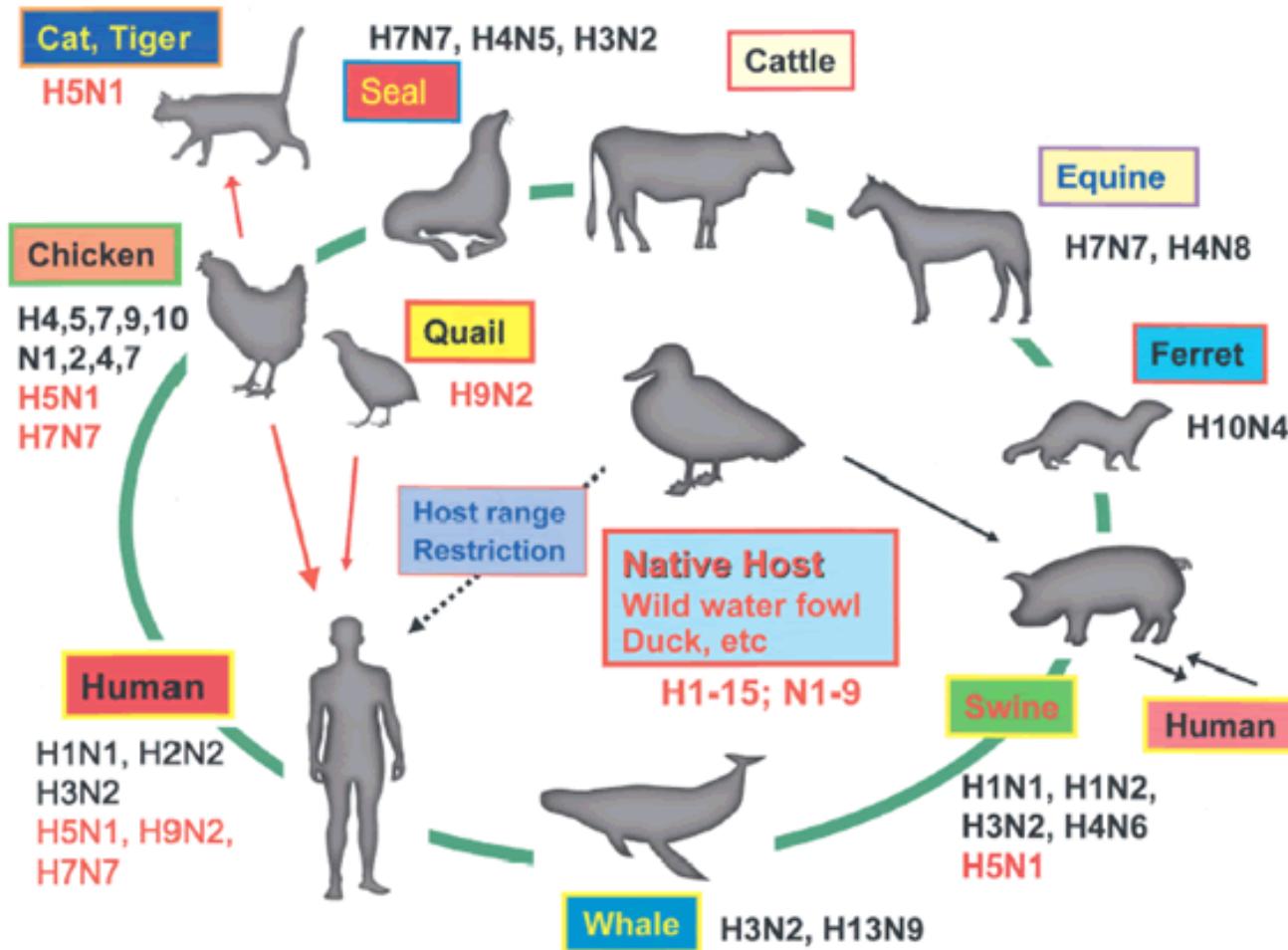
Influenza A HA and NA Subtypes

| | | | |
|-----|---|---|---|
| H1 |  |  |  |
| H2 |  |  |  |
| H3 |  |  |  |
| H4 | | | Other Animals |
| H5 |  |  |  |
| H6 |  |  | |
| H7 |  |  | Other Animals |
| H8 | |  | |
| H9 |  |  |  |
| H10 | |  | |
| H11 | |  | |
| H12 | |  | |
| H13 | |  | |
| H14 | |  | |
| H15 | |  | |
| H16 | | | |

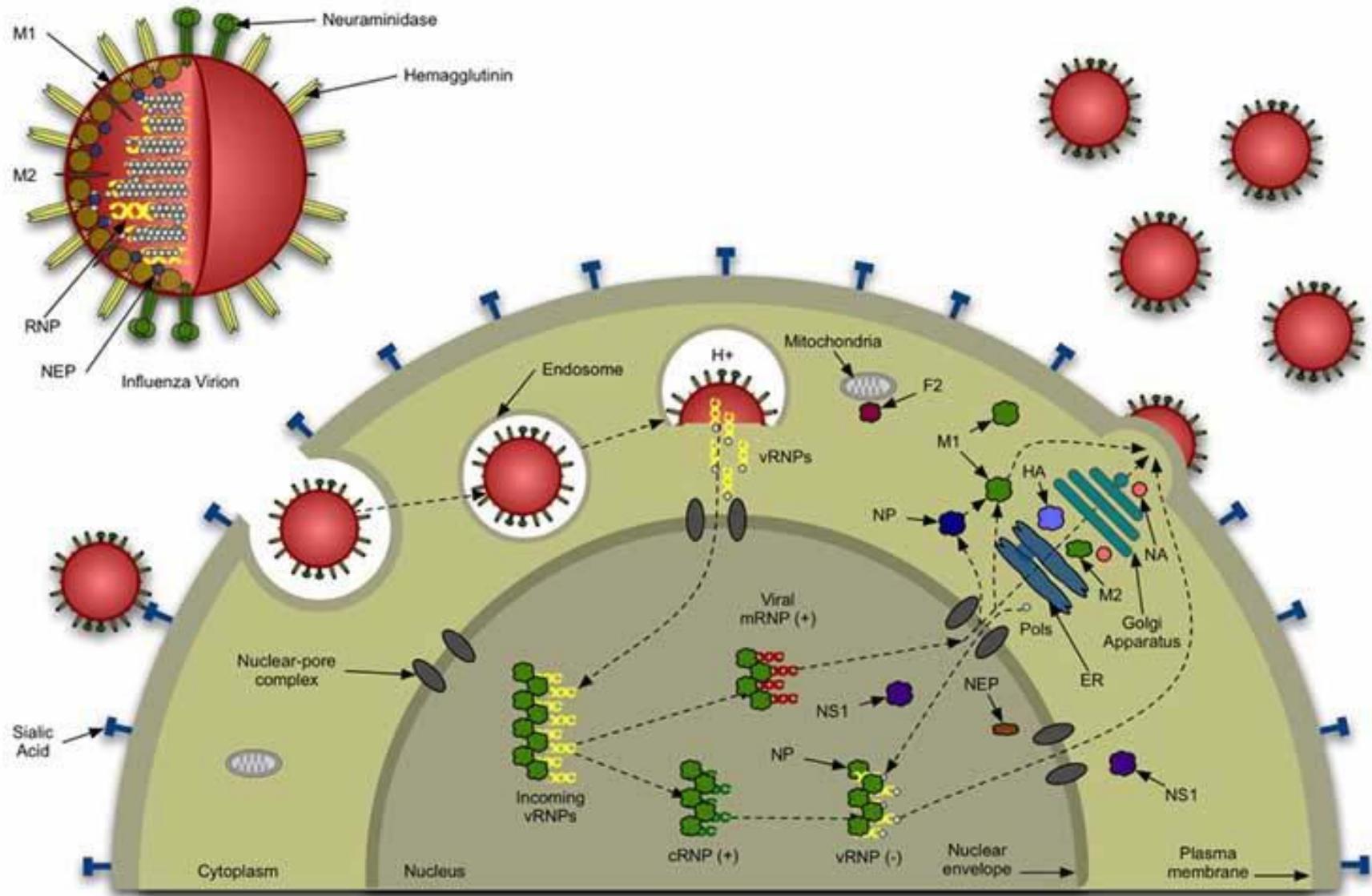
| | | | |
|----|---|---|---|
| N1 |  |  |  |
| N2 |  |  |  |
| N3 | |  |  |
| N4 | |  | |
| N5 | |  | |
| N6 | |  | |
| N7 | |  | Other Animals |
| N8 | |  | Other Animals |
| N9 | |  | |



DIVERSE HOST TROPISM ALLOWS RESTRICTION AND RECOMBINATION

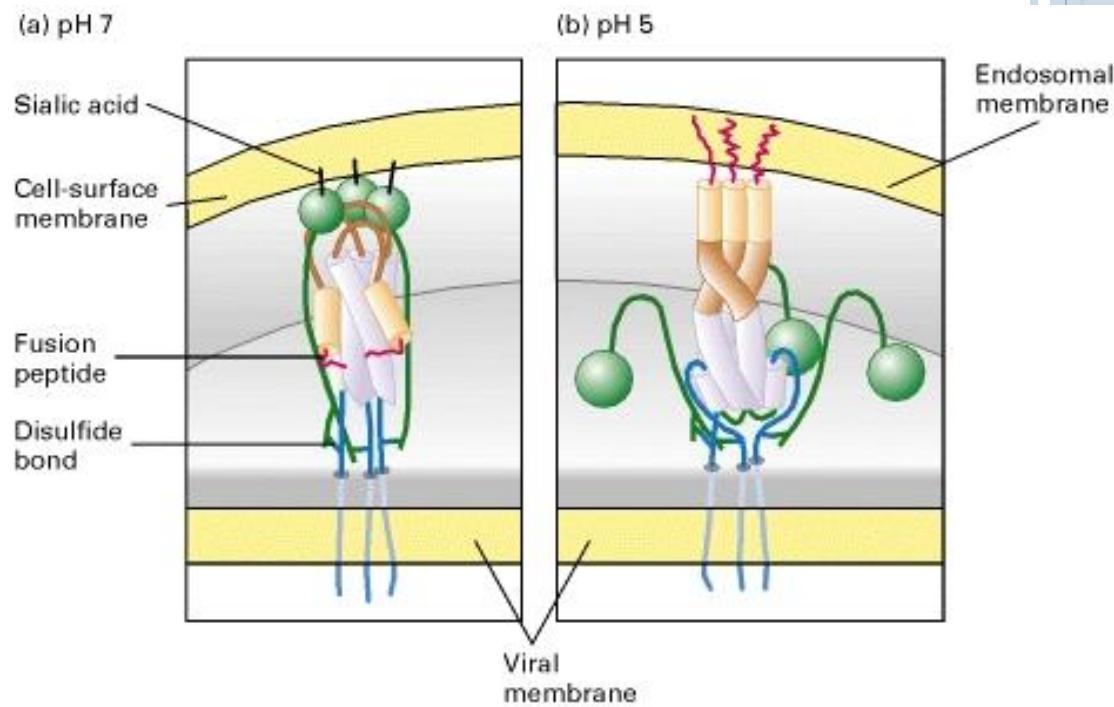


INFLUENZA LIFE CYCLE



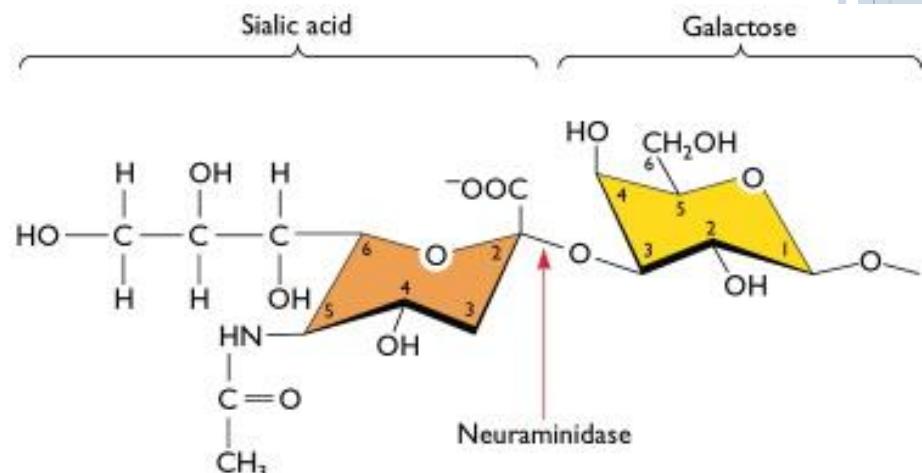
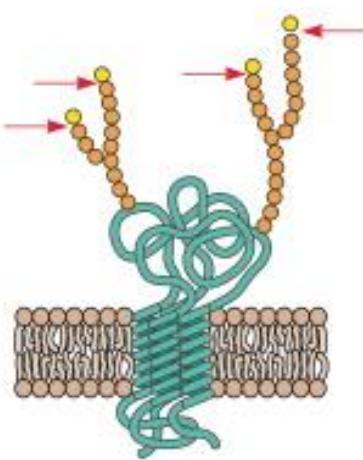
HA IS REQUIRED FOR CELL ENTRY

- HA binding to sialic acid on the surface of cells mediates initial attachment
- Virus is endocytosed, where the endosome is acidified
- This triggers a conformational change in the virus, resulting in membrane fusion
- For HA to be active, it needs to be cleaved by a protease into two pieces—this protease is generally restricted to the respiratory epithelium



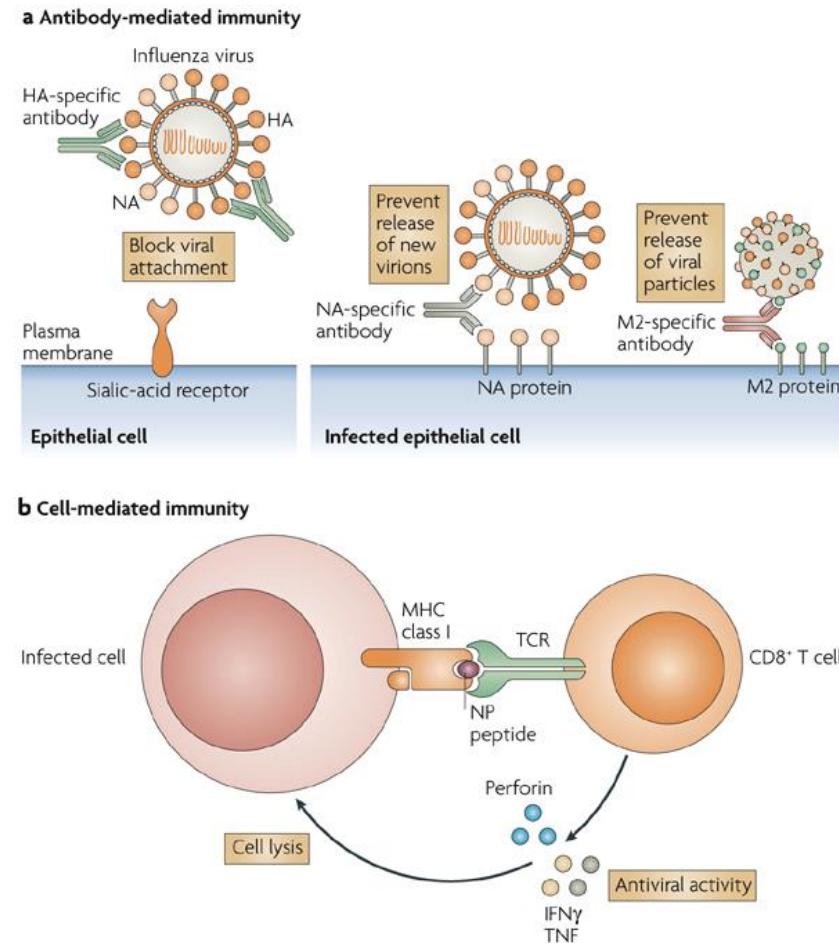
NEURAMINIDASE ACTS TO CLEAVE THE SIALIC ACID RECEPTORS FROM THE CELL SURFACE

- IAV must balance the binding and entry activity of HA with the sialic acid cleavage activity of NA so that virus efficiently enters and buds from the cell surface—thus HA and NA are often “matched” for activity



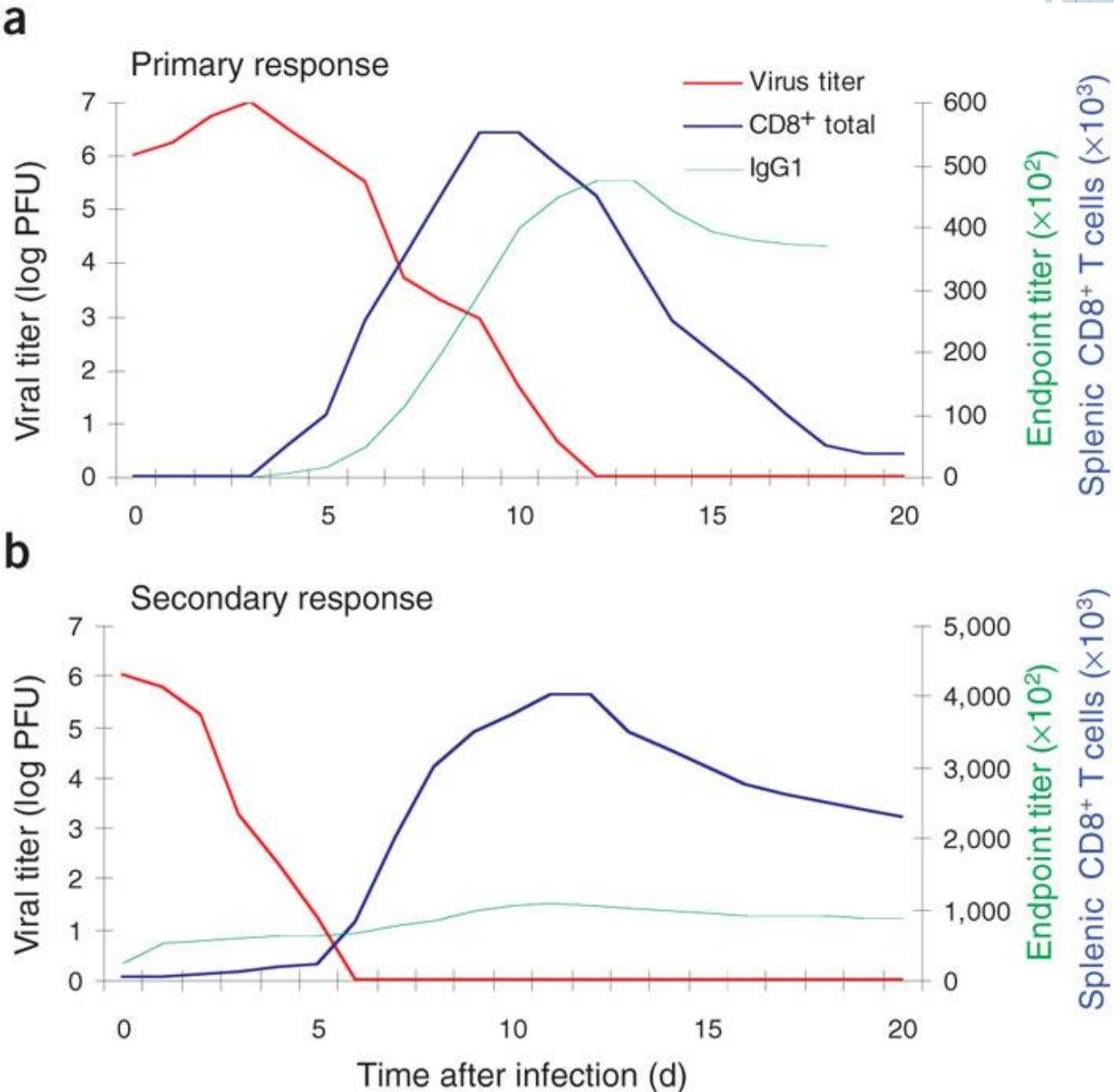
IMMUNE MECHANISMS OF PROTECTION

- Antibody mediated immunity exerts the most pressure on the virus, leading to seasonal antigenic drift and pandemic strains of antigenic shift
- Internal proteins are relatively conserved allowing heterologous cellular protection
- Mutation of dominant CD8 epitopes over time suggests that CTLs provide immunological pressure

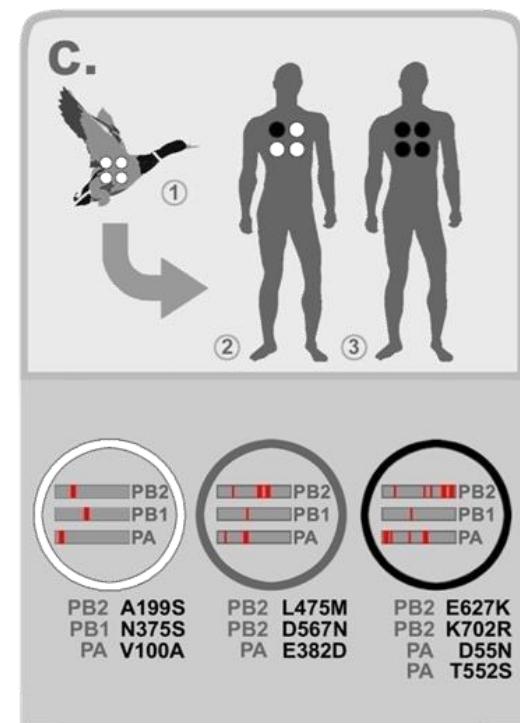
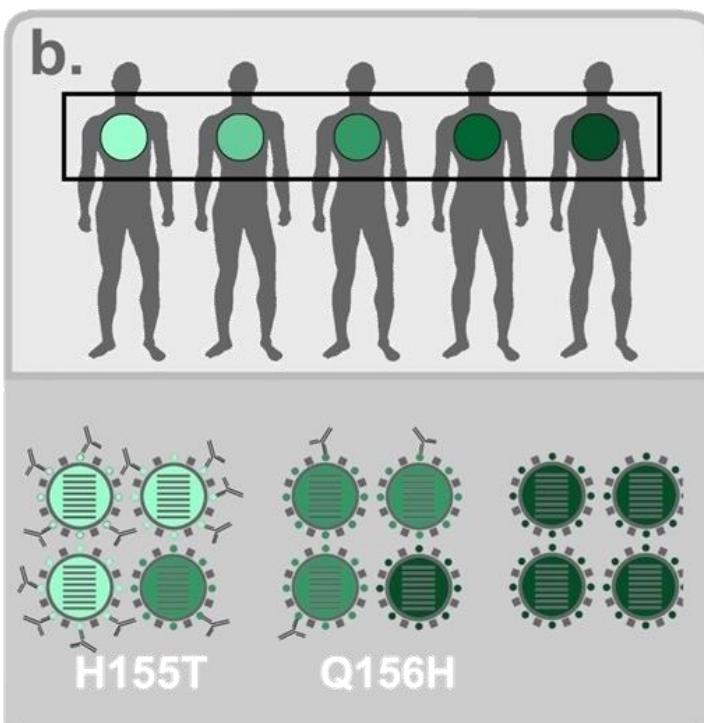
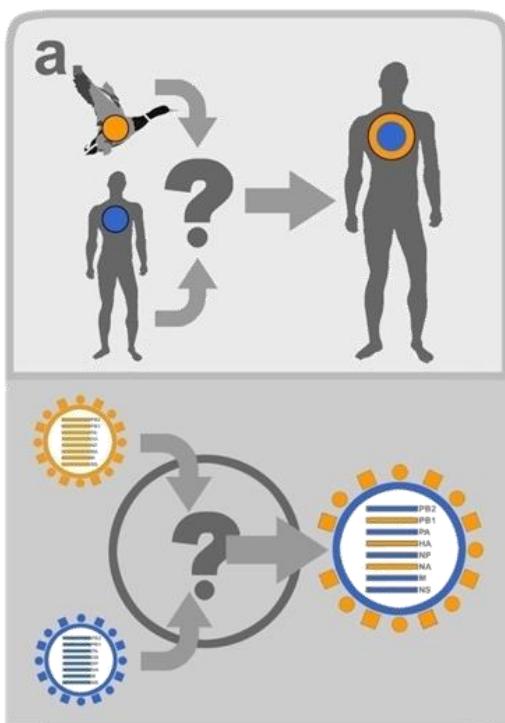


IMMUNE COURSE OF INFLUENZA INFECTION

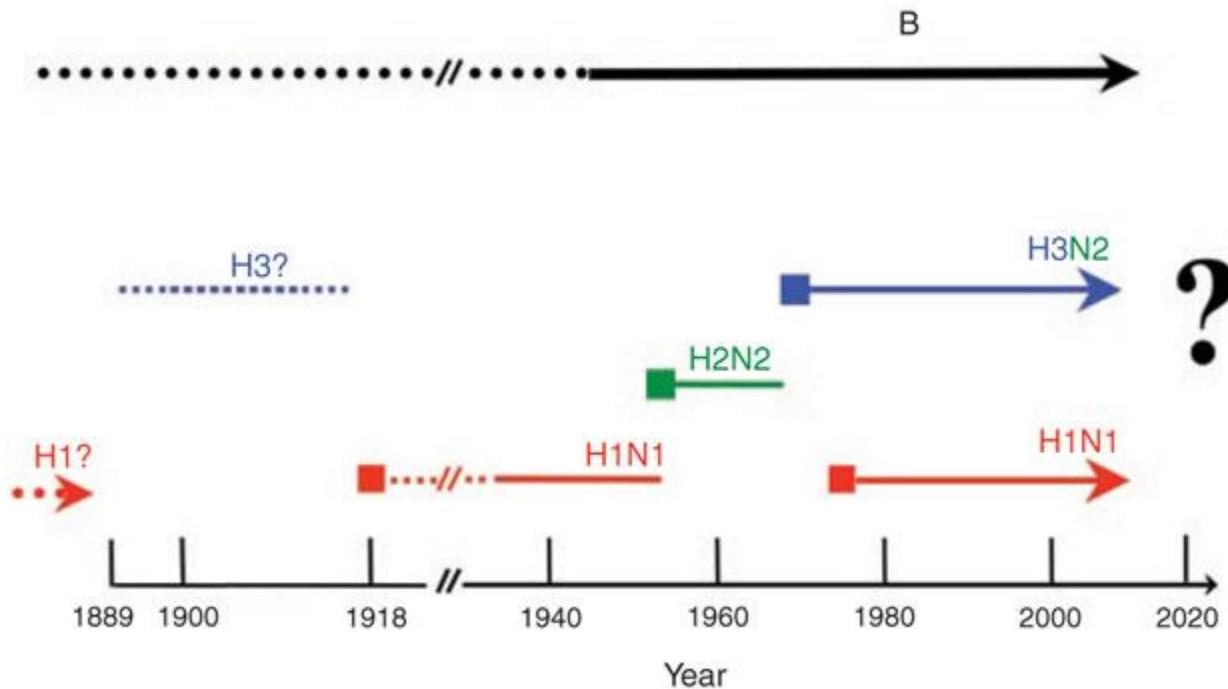
- Influenza is initially controlled by antibody and CD8+ T cells
- Secondary infection with heterologous virus is cleared with CD8+ T cell activity much more rapidly
- Homologous infection can be prevented by antibody (sterilizing immunity)



INFLUENZA EVOLUTION

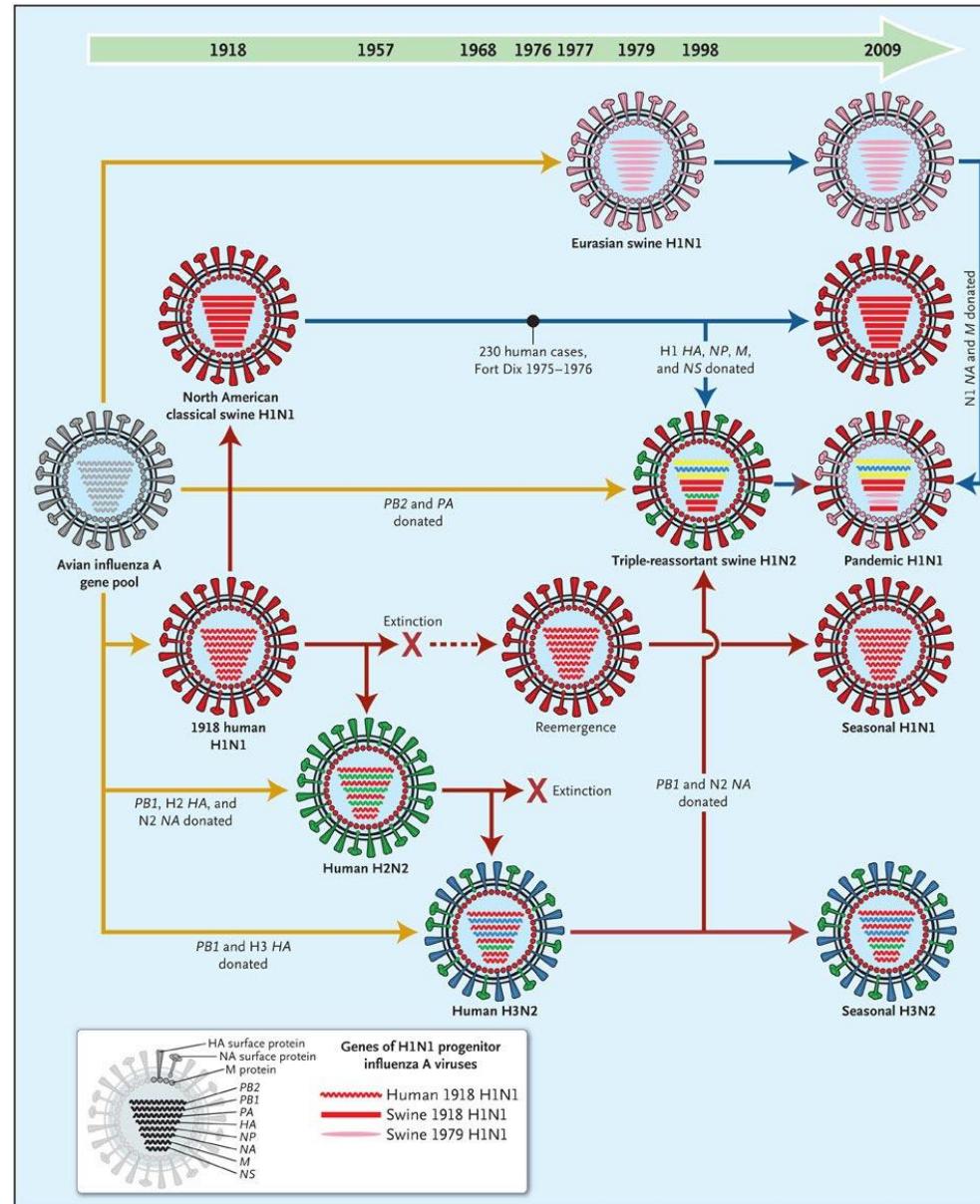


HUMAN INFLUENZA PANDEMICS



EVOLUTION OF HUMAN INFLUENZA FROM 1918

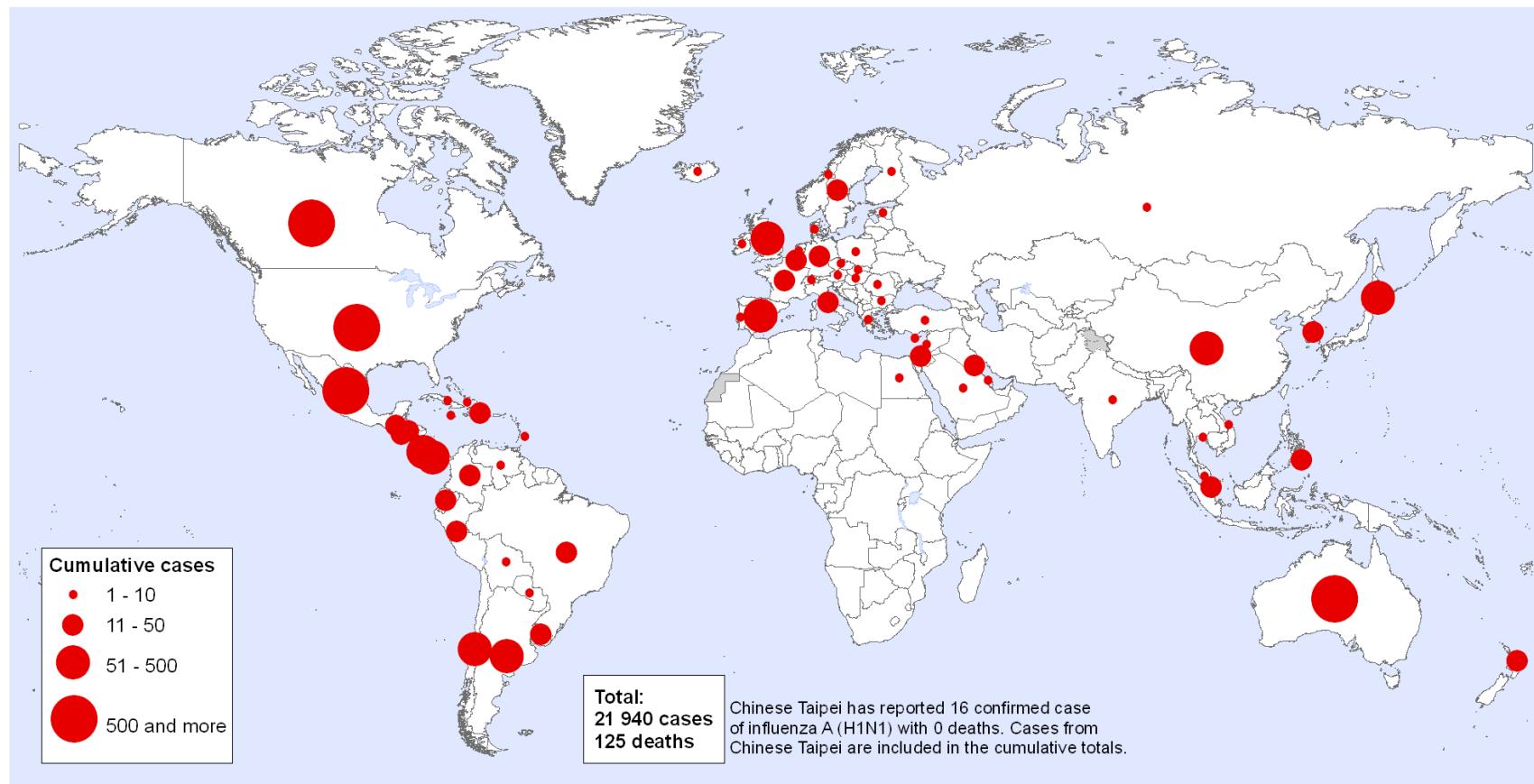
- All current human influenza is majority-derived from the 1918 pandemic
- Distinct reservoirs have allowed evolution to occur with varying pressures, providing diverse sources for new gene introductions into the human pool



SWINE-ORIGIN H1N1 INCIDENCE

New Influenza A (H1N1),
Number of laboratory confirmed cases as reported to WHO

Status as of 05 June 2009
06:00 GMT



The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

Map produced: 05 June 2009 08:10 GMT

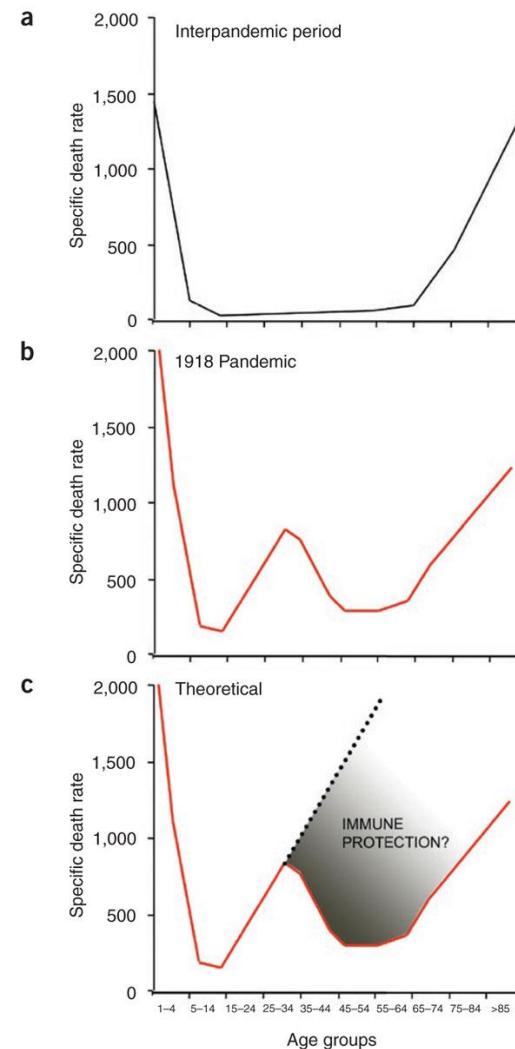
Data Source: World Health Organization
Map Production: Public Health Information and Geographic Information Systems (GIS)
World Health Organization



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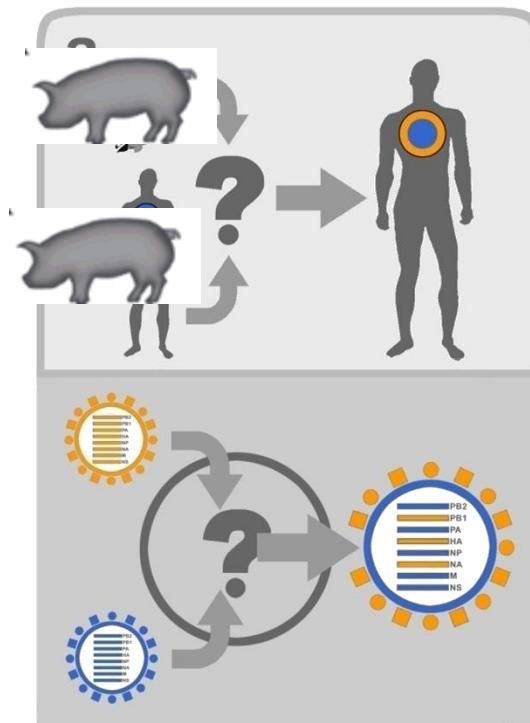
1918 (AND POSSIBLY SwORH1N1) MORTALITY CURVES SUGGEST PREVIOUS EXPOSURE

- The “U” shaped curve of regular influenza infection demonstrates the highest mortality among children (naïve) and the elderly (immunocomprised)
- The 1918 pandemic had a “W” shaped curve, with a spike in deaths among young adults—immunopathology or prior protection for ~40 year olds?



PREDICTIONS OF THE 2009/H1N1 PANDEMIC

- The 2009 H1N1 pandemic emerged as a particularly novel threat: an antigenic shift event between two swine viruses, without the “human” virus component expected to be required
- The initial rapid spread bred fears of an equally high incidence of severe morbidity and mortality (~90,000 deaths in the US, ~1.8 million hospitalizations)



PRE-EXISTING CROSS-REACTIVE IMMUNITY TO 2009/H1N1

ORIGINAL ARTICLE

Cross-Reactive Antibody Responses to the 2009 Pandemic H1N1 Influenza Virus

Kathy Hancock, Ph.D., Vic Veguilla, M.P.H., Xiuhua Lu, M.D., Weimin Zhong, Ph.D., Ebeneé N. Butler, M.P.H., Hong Sun, M.D., Feng Liu, M.D., Ph.D., Libo Dong, M.D., Ph.D., Joshua R. DeVos, M.P.H., Paul M. Gargiullo, Ph.D., T. Lynnette Brammer, M.P.H., Nancy J. Cox, Ph.D., Terrence M. Turphey, Ph.D., and Jacqueline M. Katz, Ph.D.

Table 1. Cross-Reactive Microneutralization Antibody Response against Pandemic Influenza A (H1N1) Virus in Pediatric and Adult Recipients of Seasonal Trivalent Inactivated Influenza Vaccines.*

| Type of Vaccine, Influenza Season, and Influenza Virus Used in Assay | Age Group | No. of Subjects | Increase in Antibody Titer by a Factor of ≥ 4 | Geometric Mean Titer† | | Microneutralization Titer of ≥ 40 for Children or ≥ 160 for Adults‡ | | | | |
|--|---------------|--------------------|--|-----------------------------------|----------------------------------|---|---------------------------|--|--|--|
| | | | | Before Vaccination (95% CI) | After Vaccination (95% CI) | Before Vaccination | After Vaccination % | | | |
| Children | | | | | | | | | | |
| Trivalent inactivated influenza vaccine | | | | | | | | | | |
| 2005–2007 | 6 mo to 9 yr | 33 | | | | | | | | |
| Seasonal H1N1 | | | 67 | 26 (16–40) | 267 (171–418) | 45 | 94 | | | |
| Pandemic H1N1 | | | 0 | 5 (5–6) | 6 (5–6) | 0 | 0 | | | |
| 2007–2008 | 5 yr to 9 yr | 13 | | | | | | | | |
| Seasonal H1N1 | | | 85 | 42 (22–80) | 575 (303–1093) | 54 | 100 | | | |
| Pandemic H1N1 | | | 0 | 10 (7–15) | 12 (8–17) | 8 | 15 | | | |
| 2008–2009 | 6 mo to 23 mo | 9 | | | | | | | | |
| Seasonal H1N1 | | | 100 | 5 (4–7) | 285 (202–402) | 0 | 100 | | | |
| Pandemic H1N1§ | | | 0 | 5 | 5 | 0 | 0 | | | |
| Trivalent inactivated influenza vaccine with adjuvant | | | | | | | | | | |
| 2008–2009 | 6 mo to 59 mo | 45¶ | | | | | | | | |
| Seasonal H1N1 | | | 96 | 12 (8–18) | 193 (134–280) | 24 | 100 | | | |
| Pandemic H1N1 | | | 2 | 6 (5–7) | 8 (7–9) | 0 | 4 | | | |

TABLE CONTINUED

| | | | (%) | (%) | (%) | (%) | (%) |
|---|----------------|------|-----|----------------|------------------|-----|-----|
| Adults | | | | | | | |
| Trivalent inactivated influenza vaccine | | | | | | | |
| 2007–2008 | 18 yr to 64 yr | 148 | | | | | |
| | Seasonal H1N1 | | 75 | 48 (40–58) | 598 (497–720) | 29 | 93 |
| | Pandemic H1N1 | | 22 | 25 (21–31) | 54 (44–65) | 7 | 25 |
| 2008–2009 | 18 yr to 40 yr | 83 | | | | | |
| | Seasonal H1N1 | | 78 | 29 (22–38) | 546 (418–713) | 20 | 88 |
| | Pandemic H1N1 | | 12 | 11 (9–14) | 21 (16–26) | 6 | 7 |
| Older adults | | | | | | | |
| Trivalent inactivated influenza vaccine | | | | | | | |
| 2007–2008 | ≥60 yr | 63 | | | | | |
| | Seasonal H1N1 | | 54 | 31 (22–42) | 143 (105–194) | 14 | 54 |
| | Pandemic H1N1 | | 5 | 92 (71–121) | 97 (74–127) | 33 | 43 |
| 2008–2009 | ≥60 yr | | | | | | |
| | Seasonal H1N1 | 49** | 18 | 22 (17–28) | 51 (39–66) | 6 | 14 |
| | Pandemic H1N1 | 50** | 0 | 47 (36–61) | 51 (39–65) | 8 | 8 |

EARLY PANDEMIC H1N1: APRIL – JULY 2009

Table 2. Estimates of pandemic (H1N1) 2009-related cases and rates of illness and hospitalization by age distribution of confirmed case-patients, United States, April–July 2009

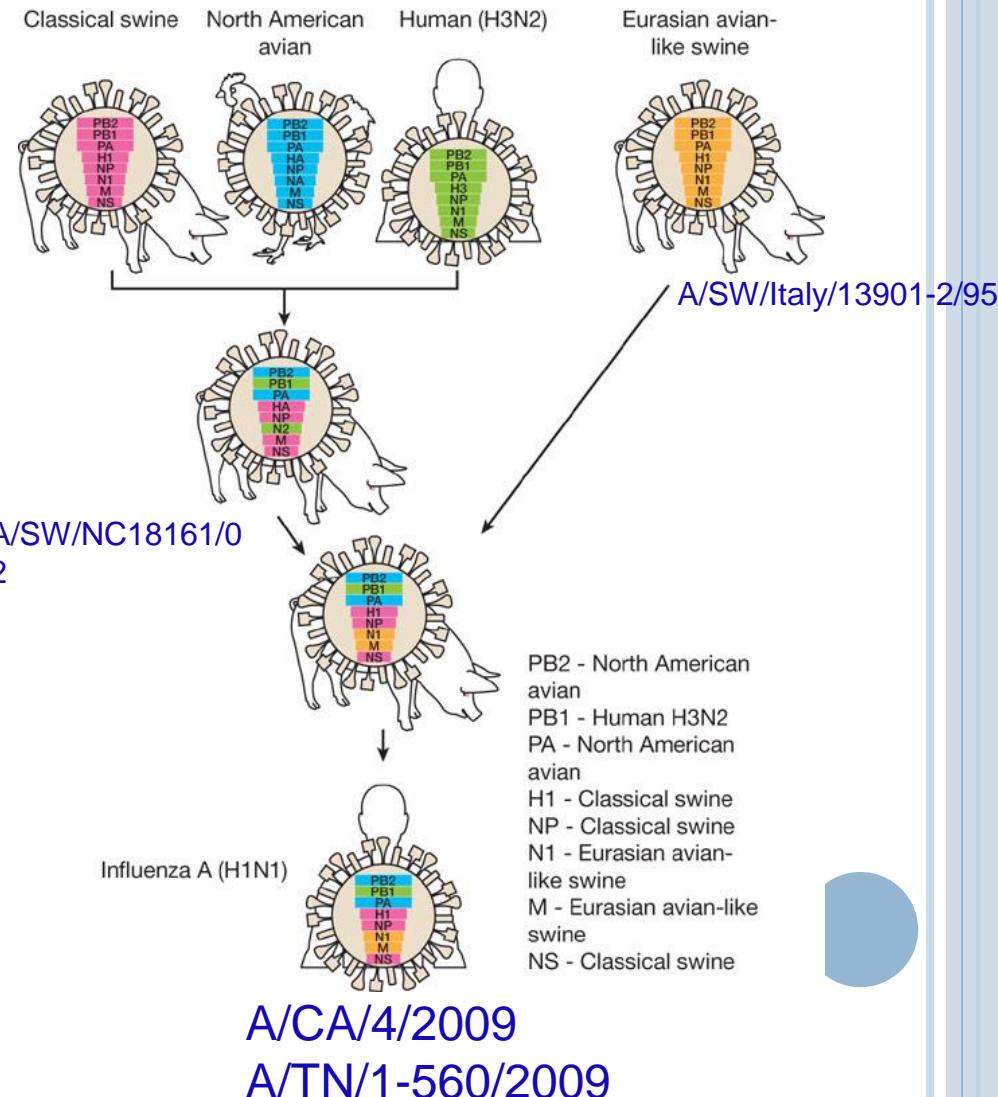
| Parameter | Estimated no. case-patients | | Estimated rate/100,000* | |
|--|-----------------------------|---------------------|-------------------------|-------------|
| | Median | 90% range | Median | 90% range |
| Total no. case-patients by age group, y† | 3,052,768 | 1,831,115–5,720,928 | 997 | 598–1,868 |
| 0–4 | 397,033 | 238,149–744,045 | 1,870 | 1,122–3,505 |
| 5–24 | 1,820,284 | 1,091,845–3,411,237 | 2,196 | 1,317–4,115 |
| 25–49 | 612,862 | 367,608–1,148,511 | 577 | 346–1,081 |
| 50–64 | 180,297 | 108,146–337,879 | 319 | 192–599 |
| ≥65 | 42,292 | 25,368–79,256 | 107 | 64–201 |
| No. hospitalized case-patients by age group, y | 13,764 | 9,278–21,305 | 4.5 | 3.0–7.0 |
| 0–4 | 2,768 | 1,866–4,285 | 13.0 | 8.8–20.2 |
| 5–24 | 4,991 | 3,364–7,725 | 6.0 | 4.1–9.3 |
| 25–49 | 3,440 | 2,319–5,324 | 3.2 | 2.2–5.0 |
| 50–64 | 1,912 | 1,289–2,959 | 3.4 | 2.3–5.2 |
| ≥65 | 654 | 441–1,012 | 1.7 | 1.1–2.6 |
| Multiplier | | | | |
| Hospitalized | 2.7 | 1.7–4.5 | – | – |
| Nonhospitalized | 79 | 47–148 | – | – |
| Through May 12 | 33 | 23–49 | – | – |
| After May 12 | 84 | 50–163 | – | – |

*United States Population Estimates, 2009.

†Age distributions from line list and aggregate reports of laboratory-confirmed cases and hospitalizations to the Centers for Disease Control and Prevention through July 23, 2009.

2009 PANDEMIC H1N1

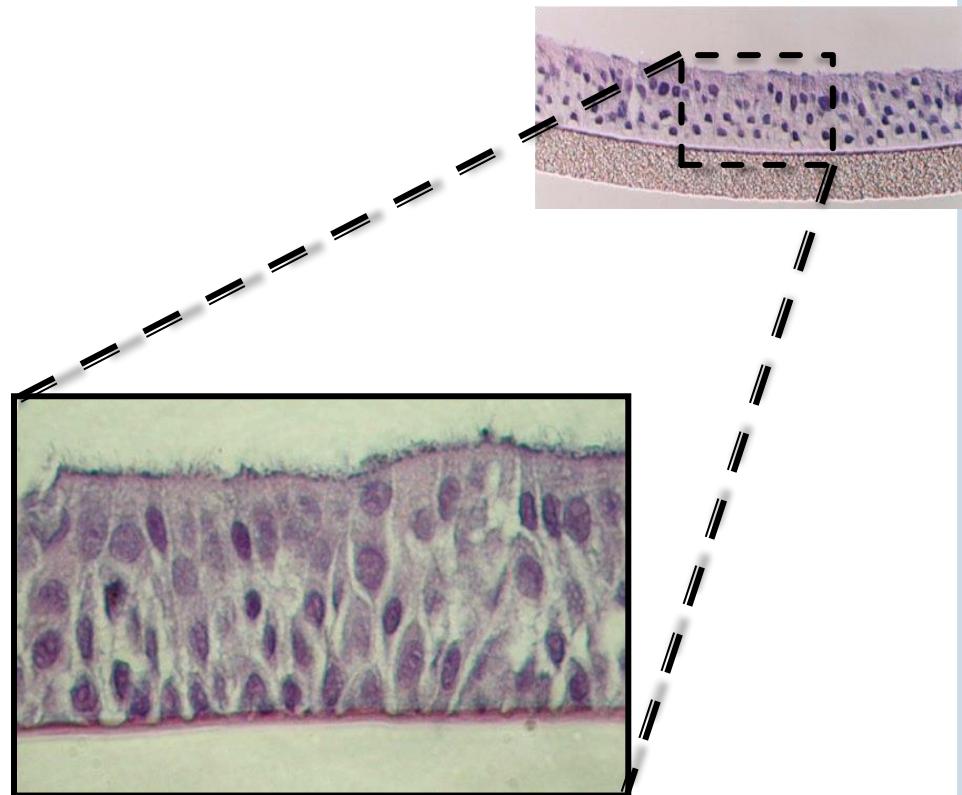
- 2009/H1N1 resulted from the recombination of two viruses (American and Eurasian Swine)
- The American Swine virus was itself a recombinant of three viruses that established itself in 1998
- These viruses are genetically distant from the human seasonal H1N1 (reference strain A/Brisbane/59/07)



H1N1 SWINE FLU STUDIES: RESPONSE IN HUMAN CELLS

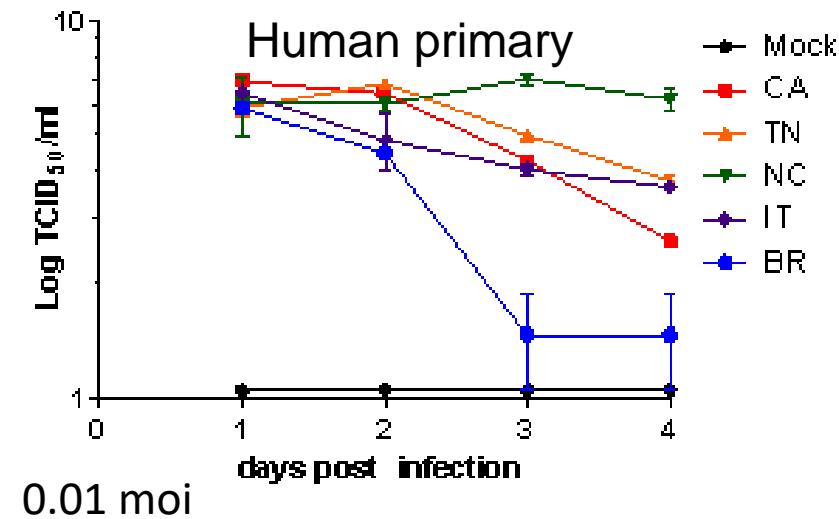
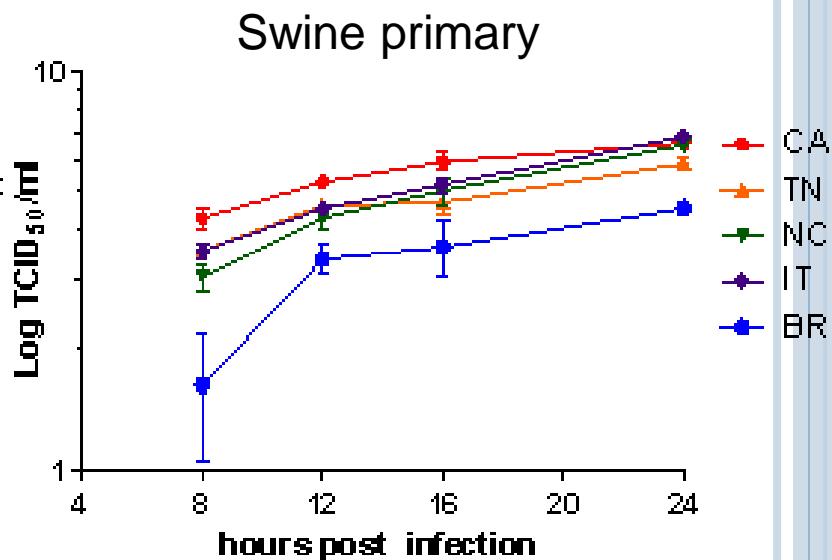
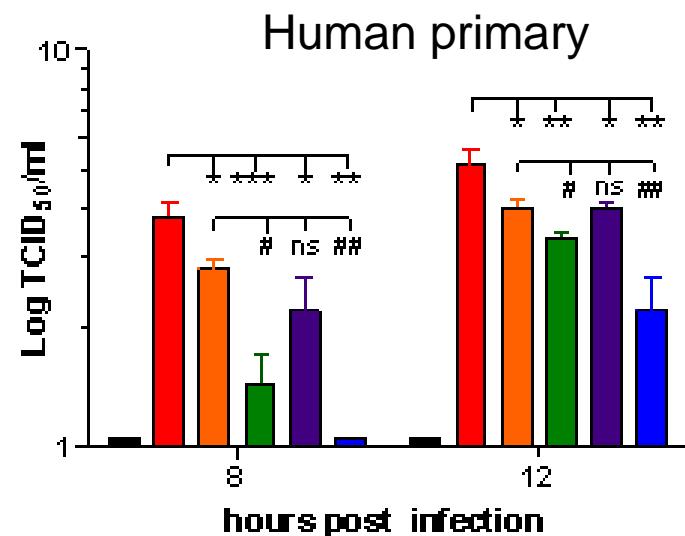
Measures:

- Infectivity and growth of virus (TCID₅₀, immunofluorescence)
- Secretion of inflammatory mediators from apical and basolateral surfaces (multiplexed immunoassay)
- Transcriptional response over the first 24 hours (Exon arrays, fluidigm analysis)
- Confirm results by “swapped viruses” made by reverse genetics



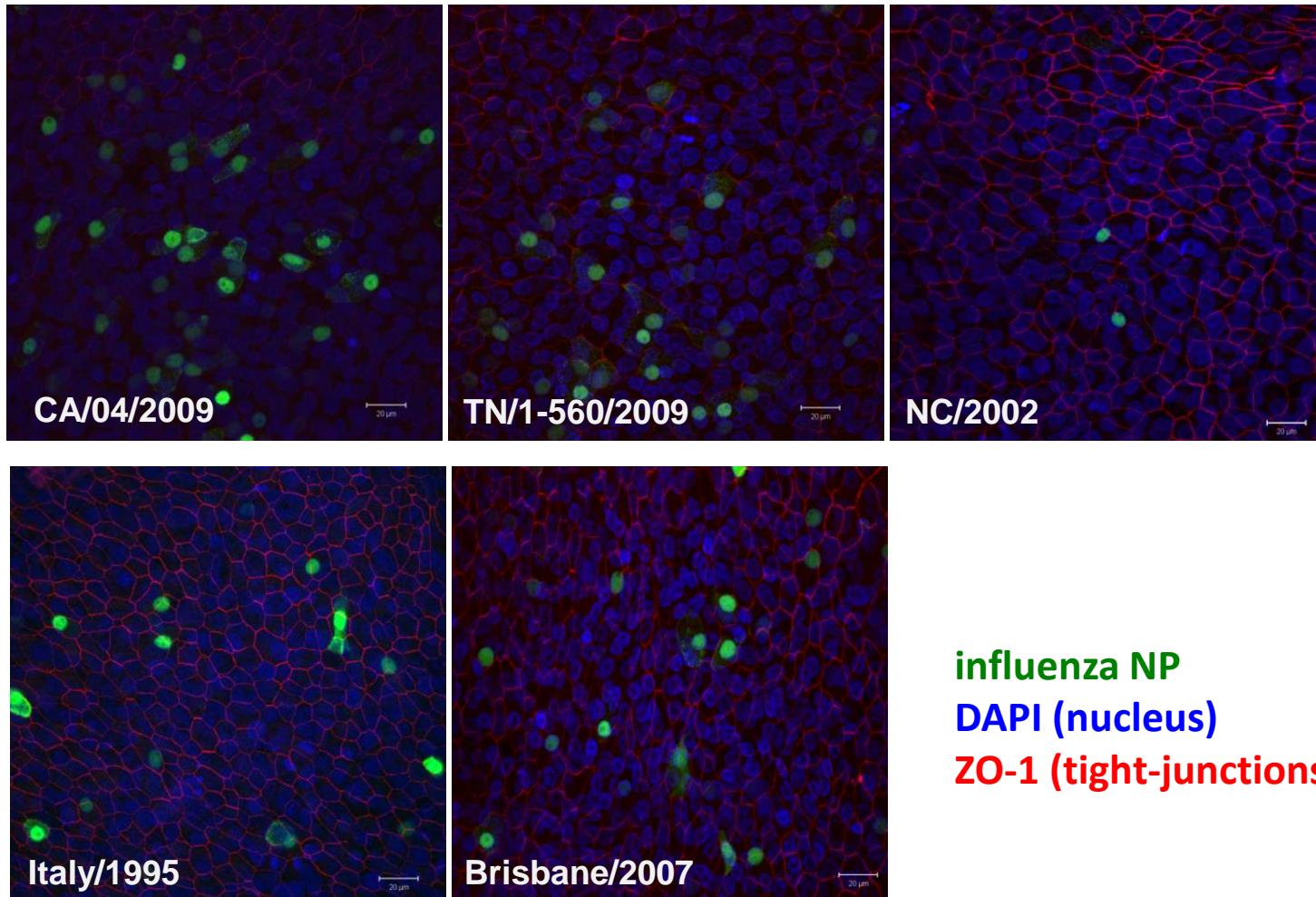
EpiAirway™,
MatTek

VIRAL GROWTH KINETICS IN HAE CELLS



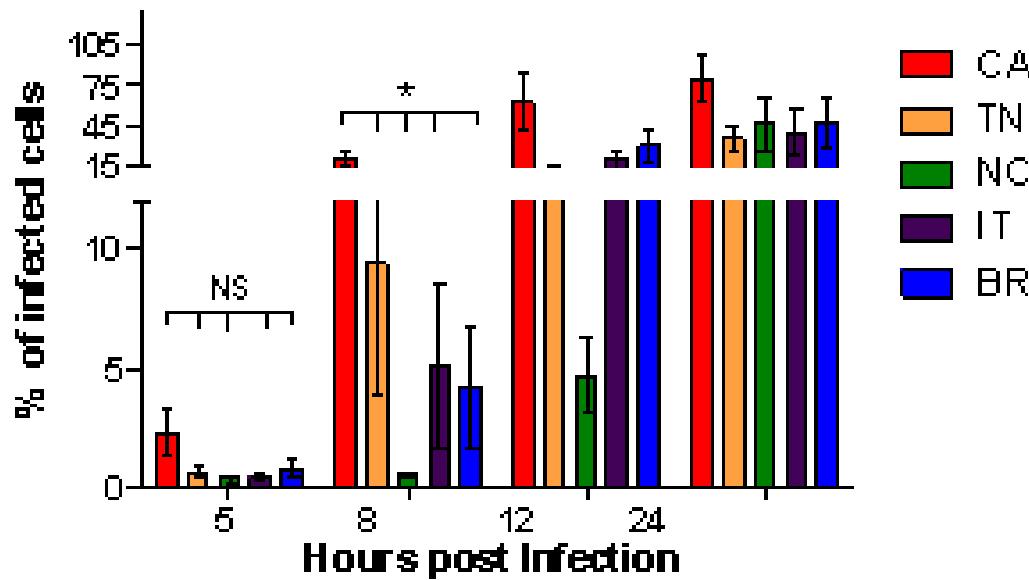
All continued
shedding from
healthy
monolayers for
>3 weeks

Influenza NP detection in 3D HAE cultures viral growth kinetics in HAE cells



8 hr post infection- 0.01 moi

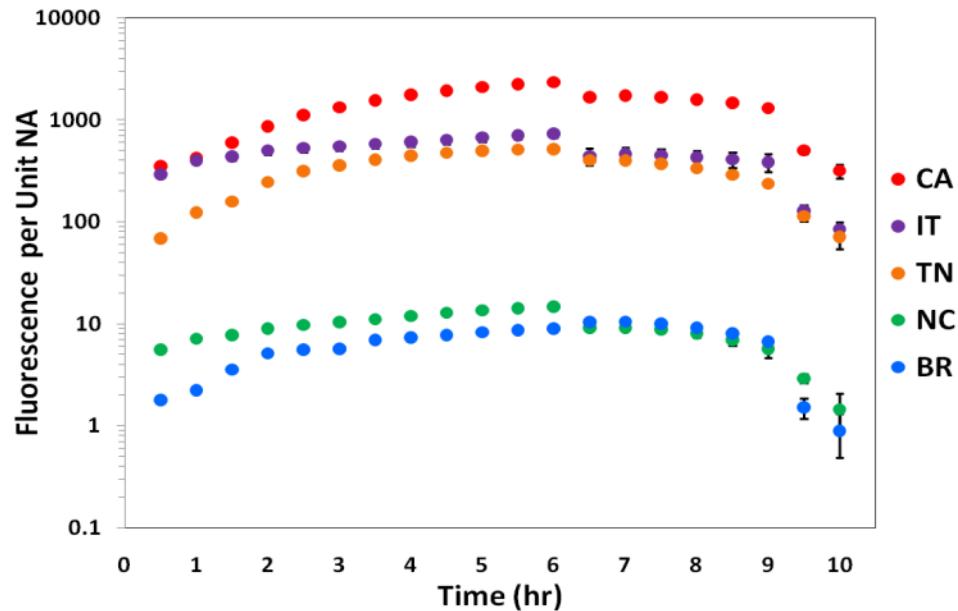
MORE RAPID COLONIZATION OF CULTURE BY PANDEMIC AND ESW VIRUS



By 12 hours, pandemic strains and Italy have infected
~50%-75% of the culture

HIGHER NA ACTIVITY IN PANDEMIC AND ESw

- NA activity measured as ability to convert sialic acid containing substrate
- Results normalized to functional viral titer, so NA activity/infectious virion
- Higher NA activity may relate to ability of virus to spread efficiently



GROWTH SUMMARY

- The pandemic virus acquired a rapid growth phenotype in human cells similar to the Esw virus
- This phenotype associates with both the NA and M of Esw virus
- The Esw virus transmits more efficiently in ferrets
- Titer and infected cell number can be de-coupled across infections/individuals



ODE MODEL OF INFLUENZA INFECTION—ANDREAS HANDEL, UGA

$$\frac{dU}{dt} = \lambda D - \frac{b}{1 + s_1 X} UV \quad \text{uninfected cells}$$

$$\frac{dE}{dt} = \frac{b}{1 + s_1 X} UV - \frac{g}{1 + s_3 X} E \quad \text{latent infected cells}$$

$$\frac{dI}{dt} = \frac{g}{1 + s_3 X} E - dI \quad \text{productively infected cells}$$

$$\frac{dD}{dt} = dI - \lambda D \quad \text{dead cells}$$

$$\frac{dV}{dt} = \frac{p}{1 + s_2 X} I - cV - \gamma \frac{b}{1 + s_1 X} VU \quad \text{free virus}$$

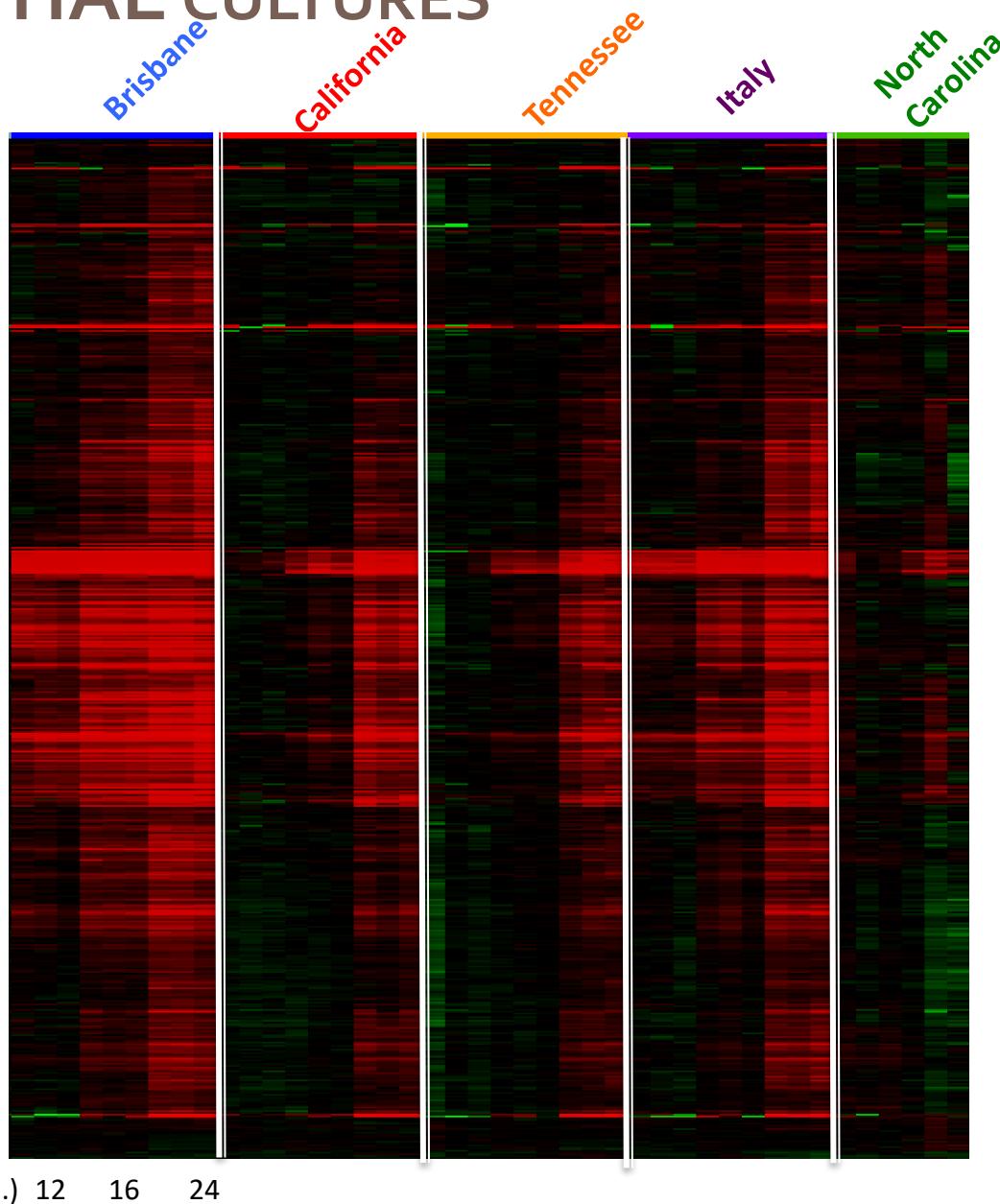
Why wasn't the Esw virus a pandemic?



TRANSCRIPTOME ANALYSIS OF PANDEMIC VIRUS

INFECTED HAE CULTURES

mRNA expression in
hAE cultures
infected at
MOI=0.01

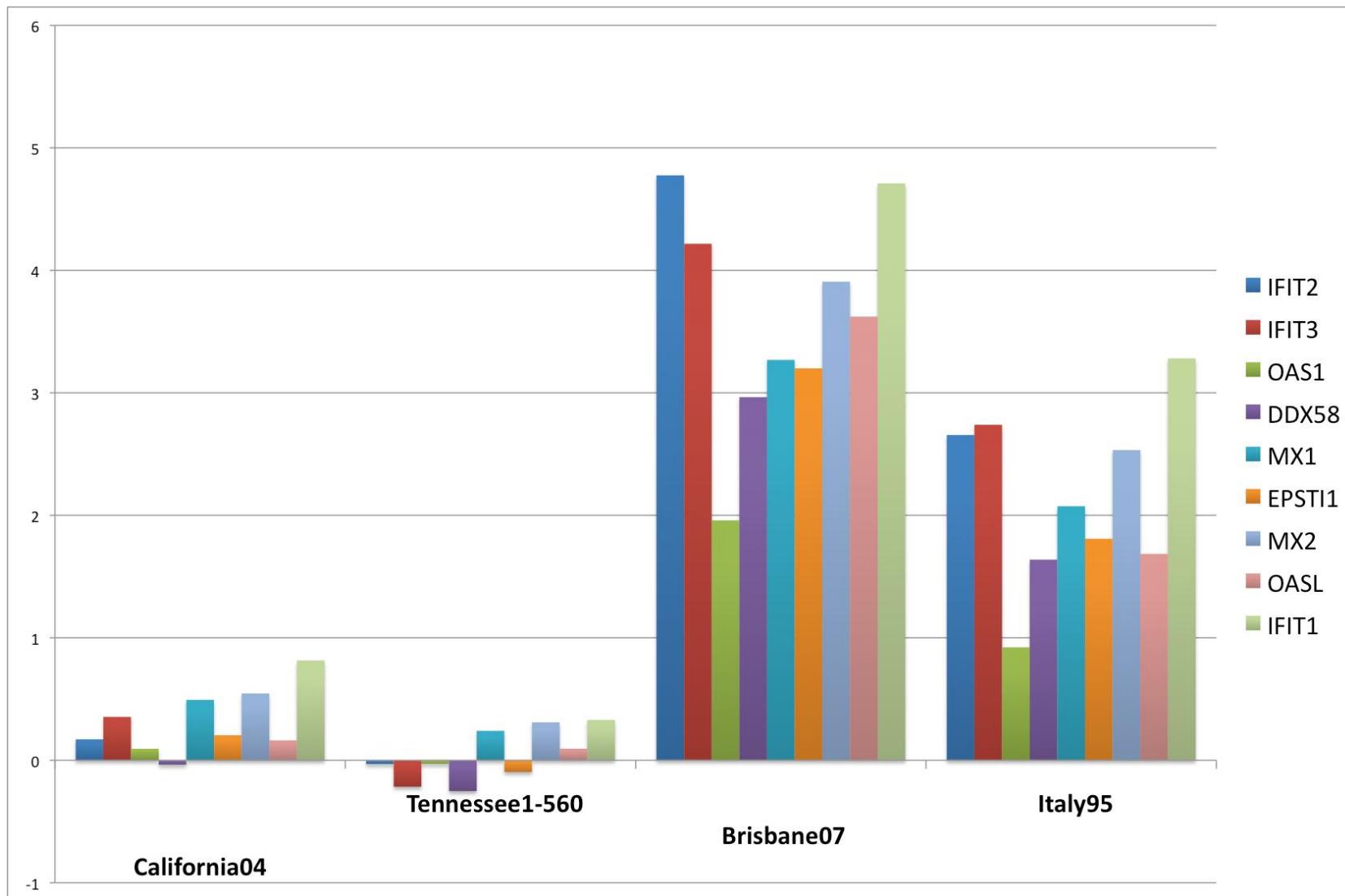


BIC applied to k-means clustering:
2 clusters
271 upregulated in all
24 downregulated or differential

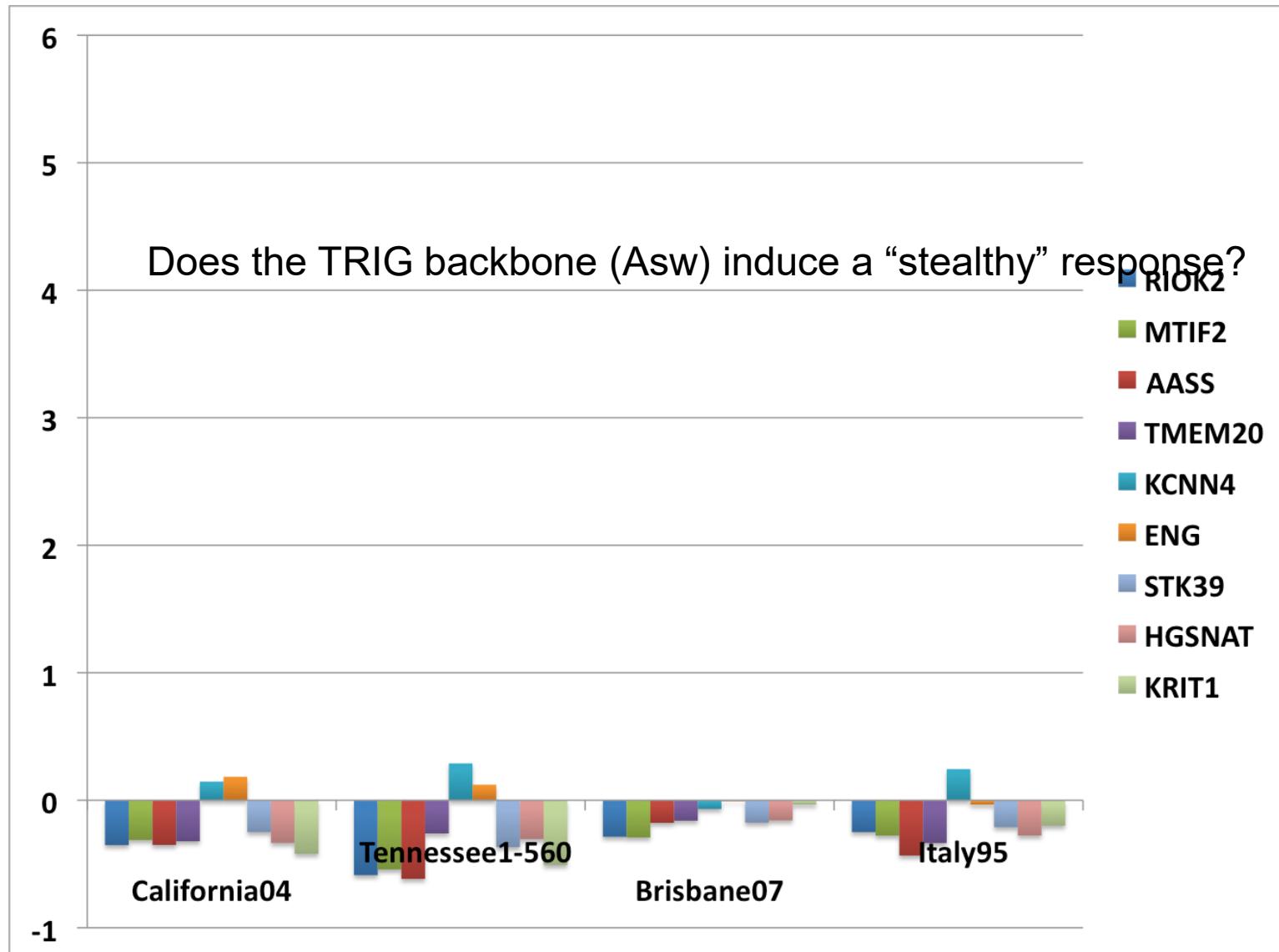


Time (hours p.i.) 12 16 24

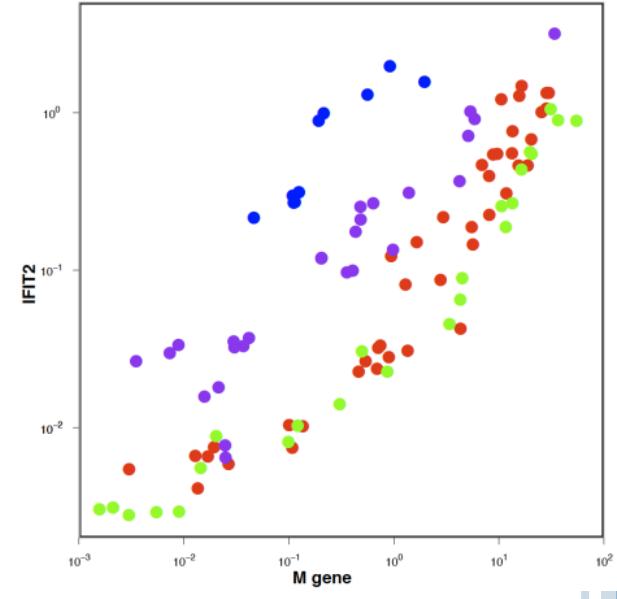
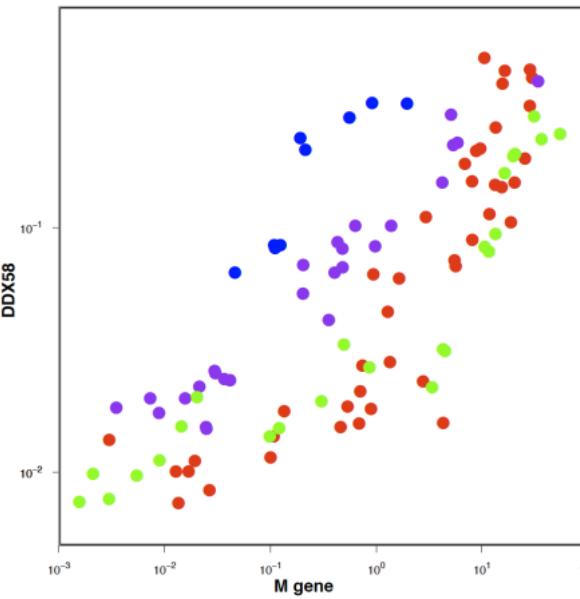
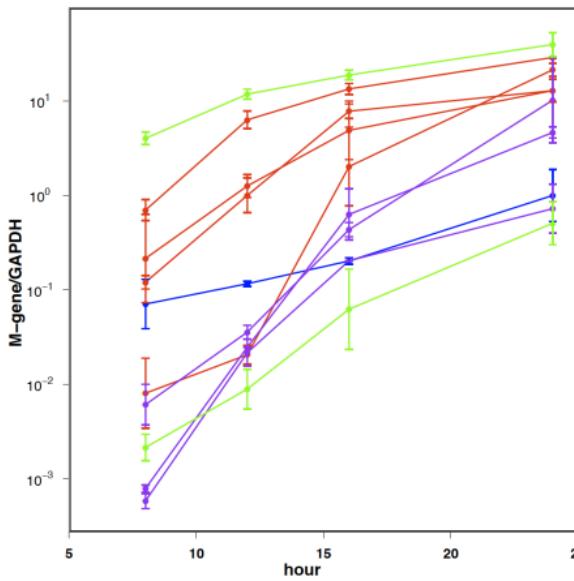
TOP 9 MOST SIGNIFICANT DIFFERENTIALLY EXPRESSED GENES 12 HOURS POST-INFECTION WITH A/BRISBANE/59/2007(H1N1)



TOP 9 MOST SIGNIFICANT DIFFERENTIALLY EXPRESSED GENES AT 12 HOURS POST-INFECTION WITH A/CALIFORNIA/04/2009(H1N1)



HOST RESPONSE AS A FUNCTION OF VIRUS



Brisbane California Italy

North
Carolina

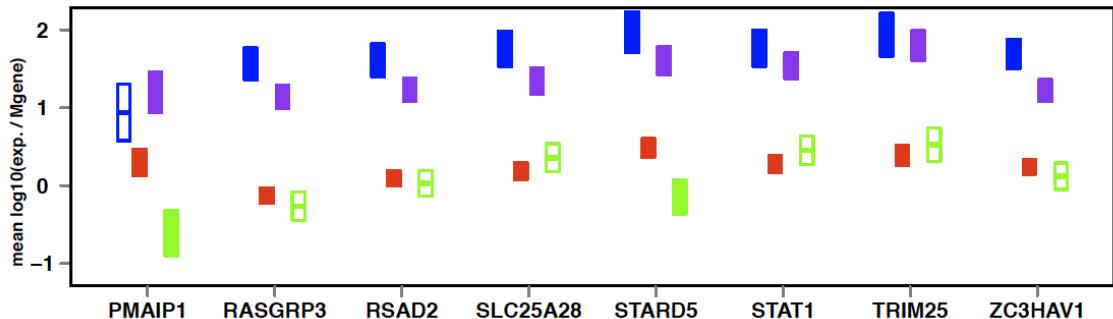
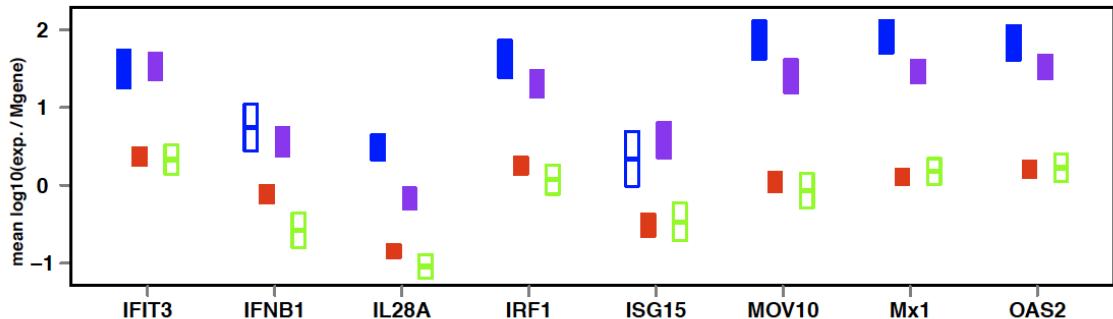
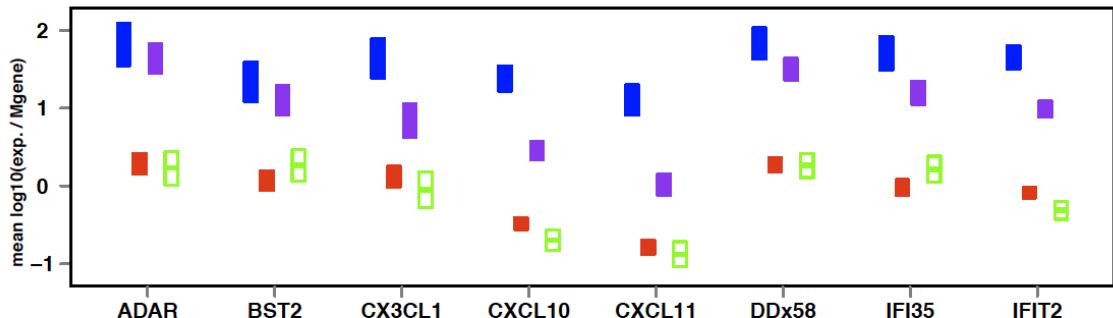
Fluidigm Real Time PCR from
primary human cell infections
(2 donors)



HOST RESPONSE AS A FUNCTION OF VIRUS II

Brisbane
California
Italy
North Carolina

$$\frac{\text{expression} - \text{expression}_{\text{mock}}}{\max(\text{expression})} \quad M_{\text{gene}}$$

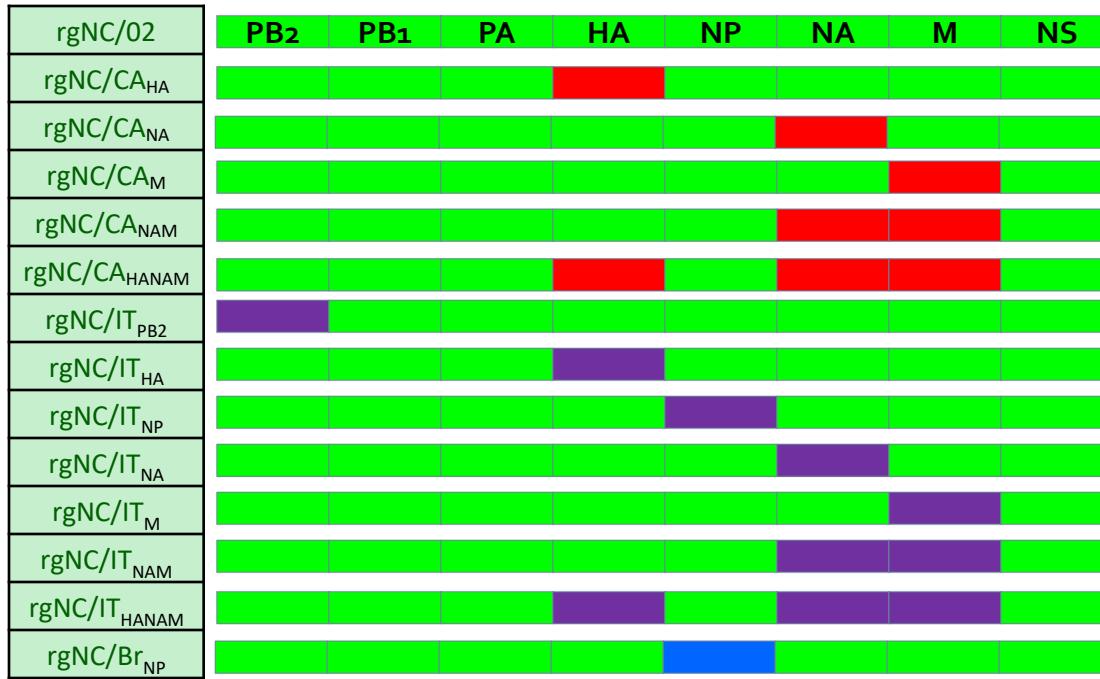


SWAPS

What's the mechanistic basis of the stealthy (or noisy) phenotype?

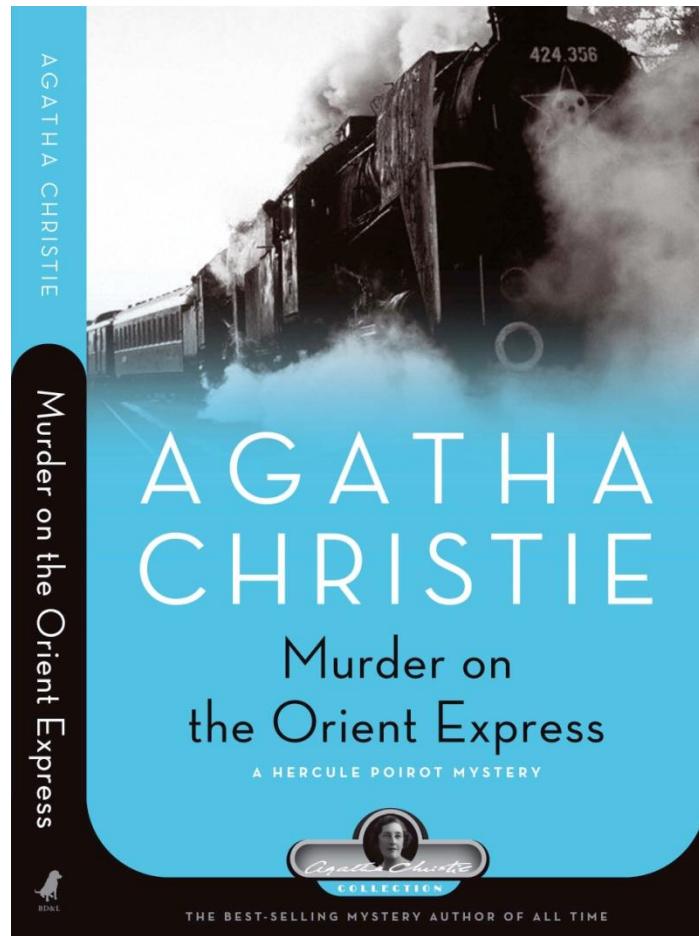
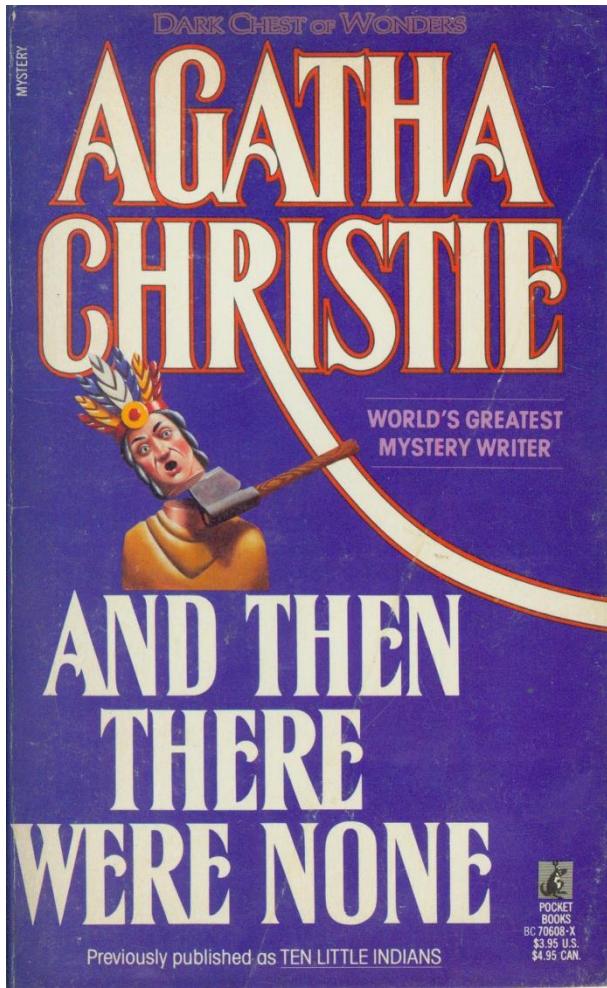


CA
IT
BR
N
C

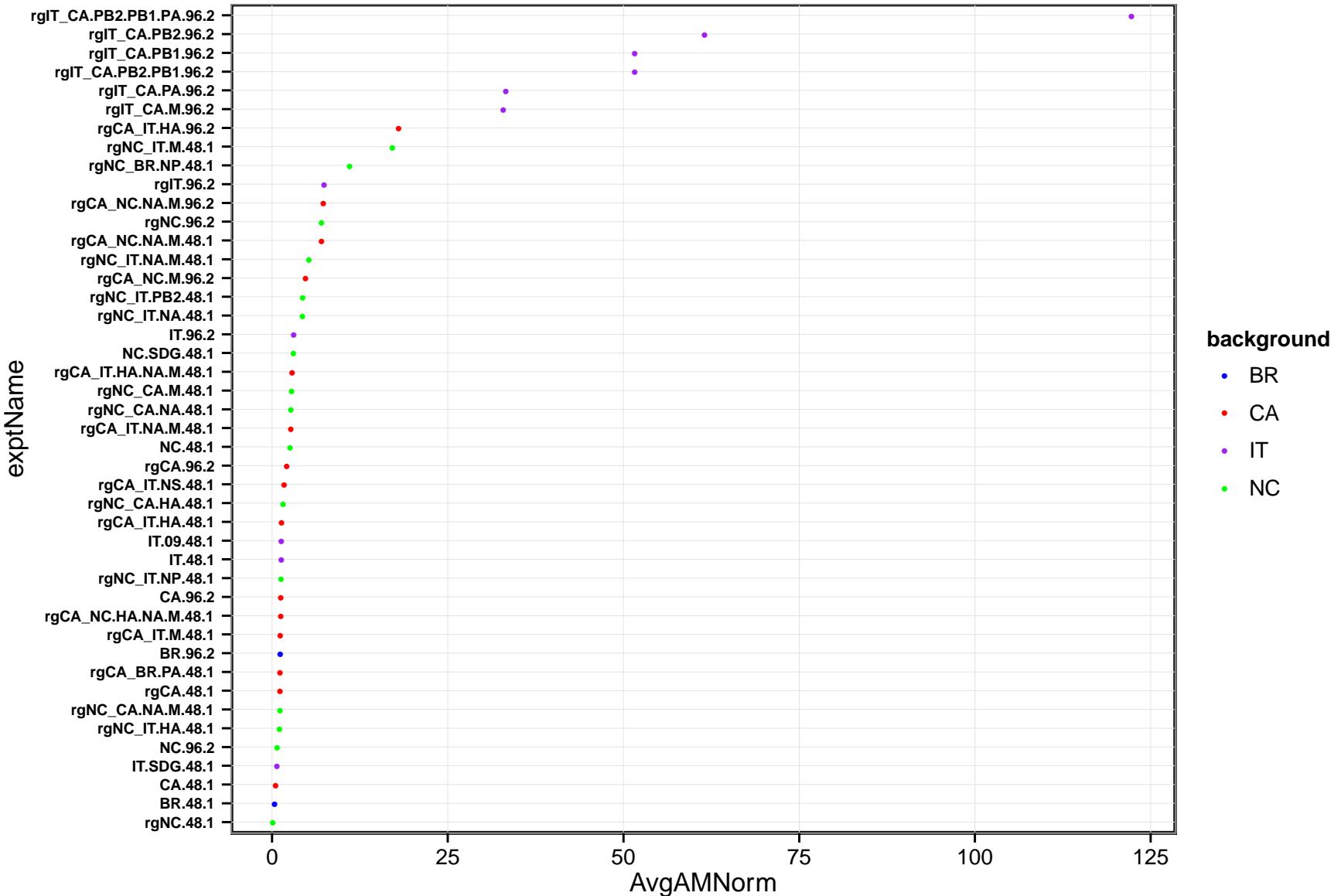


CA
IT
BR
N
C

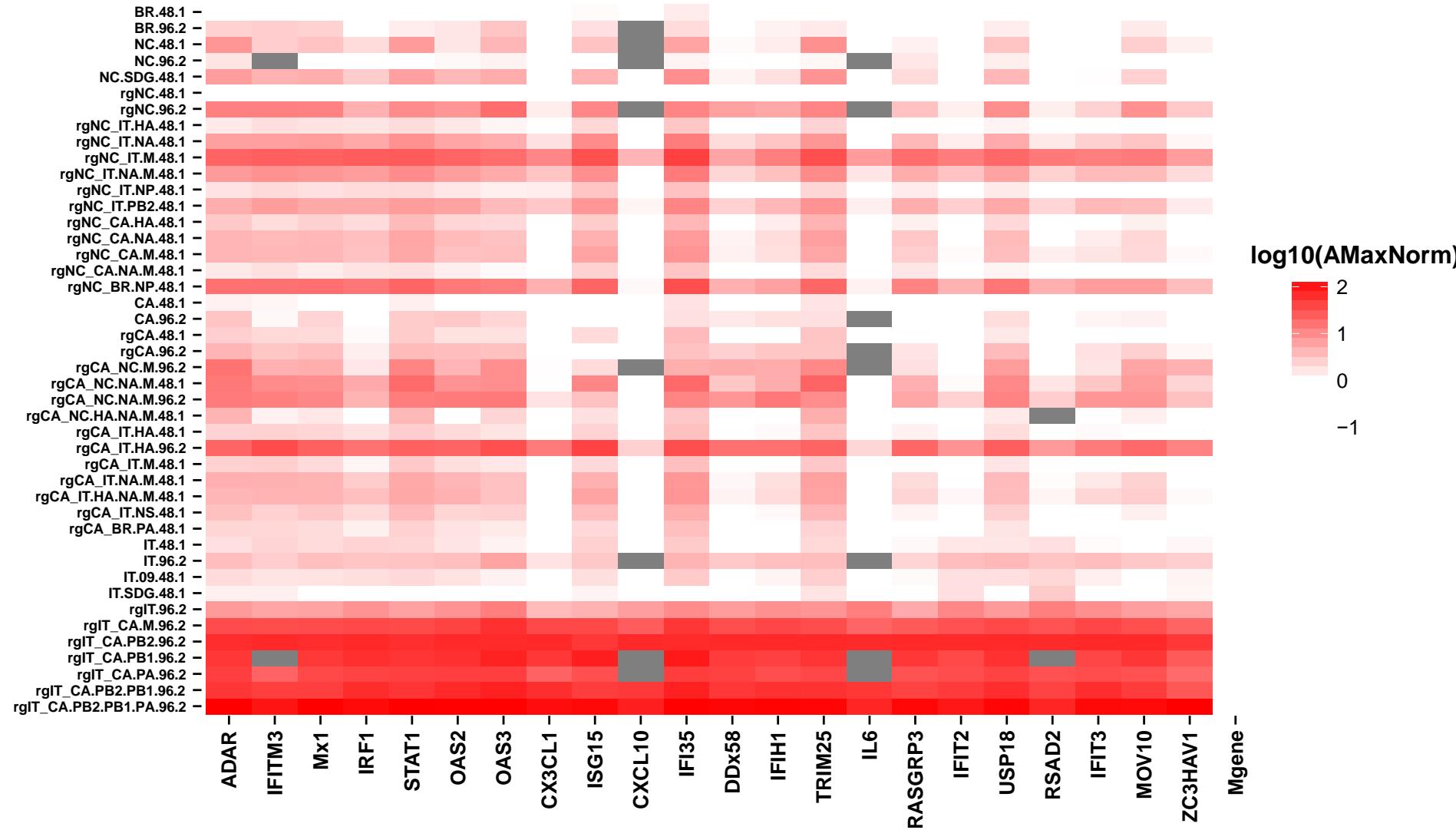




Average amplitude across all genes normalized to M-gene



Amplitude (“A”) normalized to M-gene



THE PANDEMIC STRAIN IS EFFICIENT AND STEALTHY

- Rapid + stealthy growth = Pandemic

[Morbidity and Mortality Weekly Report](#)

Limited Human-to-Human Transmission of Novel Influenza A (H3N2) Virus — Iowa, November 2011

- The set of genes induced by diverse viruses is largely equivalent in the first 24 hours— “the flu program”
- The pandemic strategy is distinct from the well-adapted human seasonal virus
- Kinetic differences in the first ~18 hours of infection are critical to the quality and quantity of the later response
- The stealthy phenotype is mediated by contributions of the P-gene complex, with potential roles for NP and NS

ODE MODEL OF INFLUENZA INFECTION

$$\frac{dU}{dt} = \lambda D - \frac{b}{1 + s_1 X} UV \quad \text{uninfected cells}$$

$$\frac{dE}{dt} = \frac{b}{1 + s_1 X} UV - \frac{g}{1 + s_3 X} E \quad \text{latent infected cells}$$

$$\frac{dI}{dt} = \frac{g}{1 + s_3 X} E - dI \quad \text{productively infected cells}$$

$$\frac{dD}{dt} = dI - \lambda D \quad \text{dead cells}$$

$$\frac{dV}{dt} = \frac{p}{1 + s_2 X} I - cV - \gamma \frac{b}{1 + s_1 X} VU \quad \text{free virus}$$

$$\frac{dX}{dt} = wI - \delta X \quad \text{innate immune response (IFN)}$$

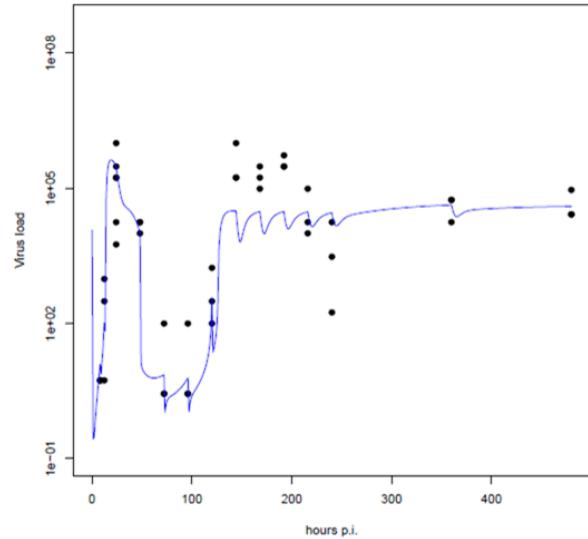
AICC VALUES OF 8 DIFFERENT MODELS

- 1. No IR and no cell-regrowth
- 2. No IR, with cell-regrowth
- 3. With IR reducing virus production, no cell-regrowth
- 4. With IR reducing infection rate, no cell-regrowth
- 5. With IR prolonging latency, no cell-regrowth
- 6. With IR reducing virus production, with cell-regrowth
- 7. With IR reducing infection rate, with cell-regrowth
- 8. With IR prolonging latency, with cell-regrowth

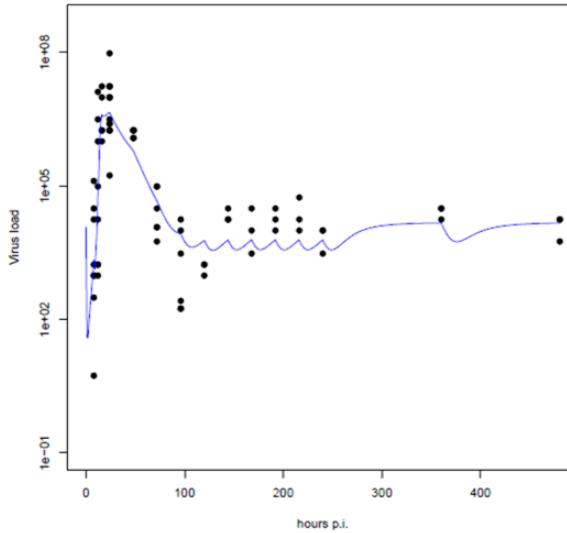
| Model | BB | CA | IT | NC |
|-------|-------|-------|-------|------|
| 1 | 54.5 | 54.7 | 33.1 | 28.2 |
| 2 | 48.8 | -22.6 | 0.8 | 28.5 |
| 3 | 52.8 | 24.8 | 17.0 | 30.3 |
| 4 | 59.9 | 33.2 | 38.3 | 33.6 |
| 5 | 53.2 | 32.1 | 24.6 | 31.7 |
| 6 | -11.6 | -17.6 | -11.1 | 33.2 |
| 7 | 54.5 | -17.7 | 6.1 | 29.3 |
| 8 | 56.1 | -17.3 | 6.2 | 34.3 |

FITS FOR MODEL 6—IR REDUCES VIRUS PRODUCTION AND CELLS REGROW

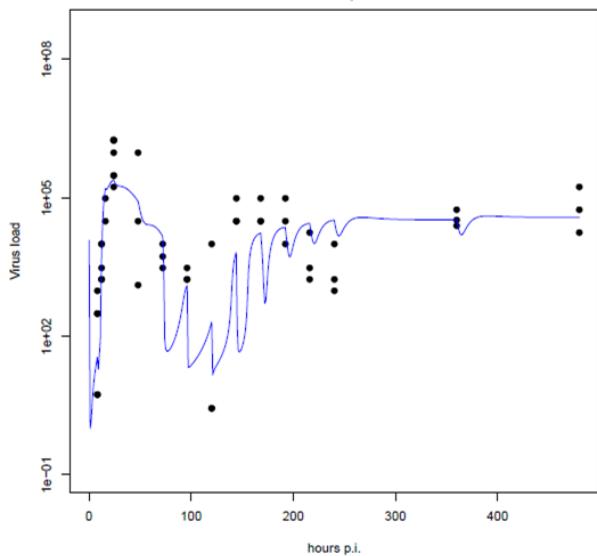
BR



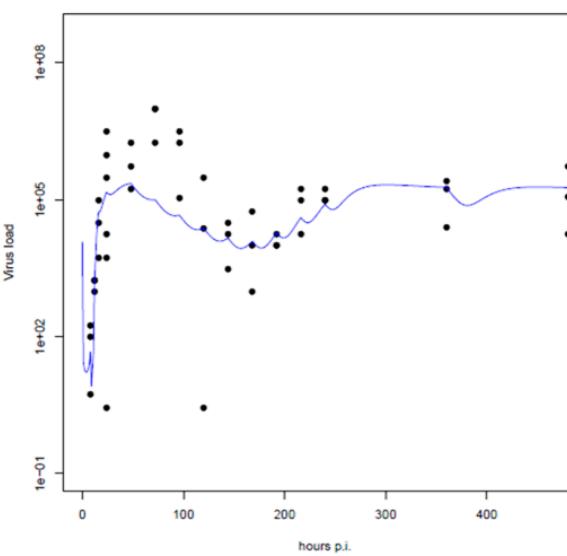
CA



IT

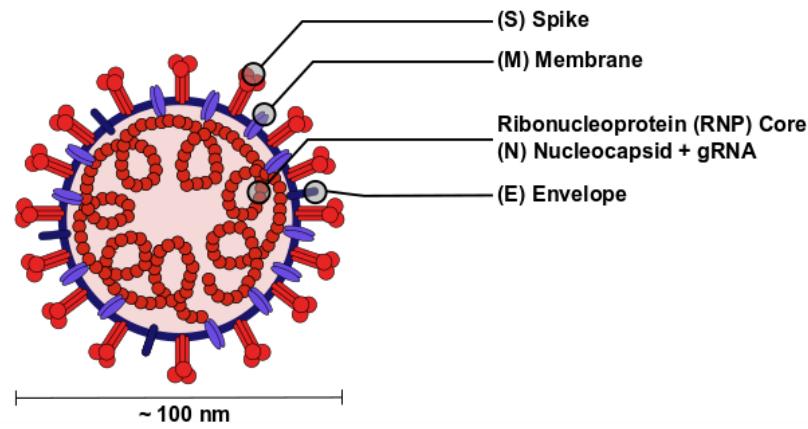


NC



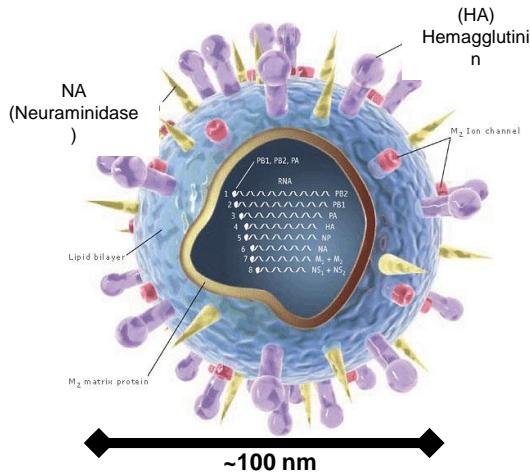
SARS-CoV-2 vs. INFLUENZA VIRUS

The Coronavirus Virion



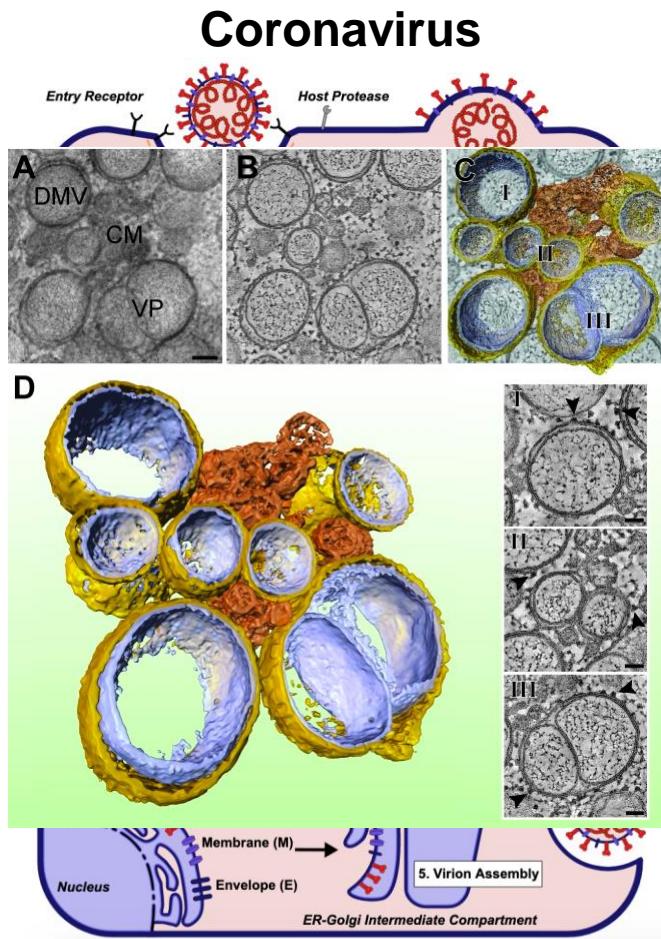
(+) ssRNA genome ~28-32 Kb
29 proteins

The Influenza Virus Virion

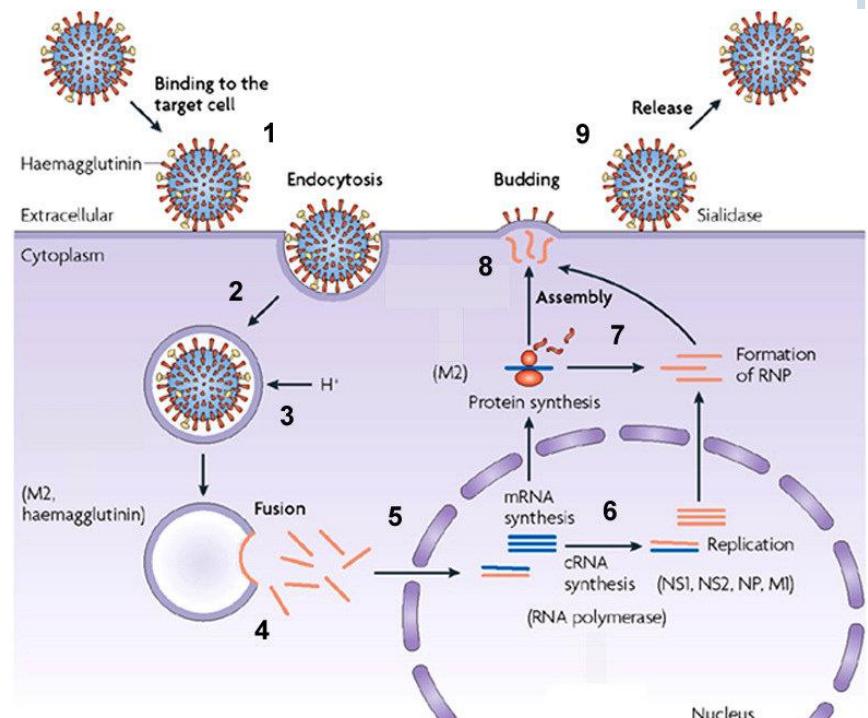


(-) segmented ssRNA genome ~28-32 Kb
~14 Kb, 10-14 proteins

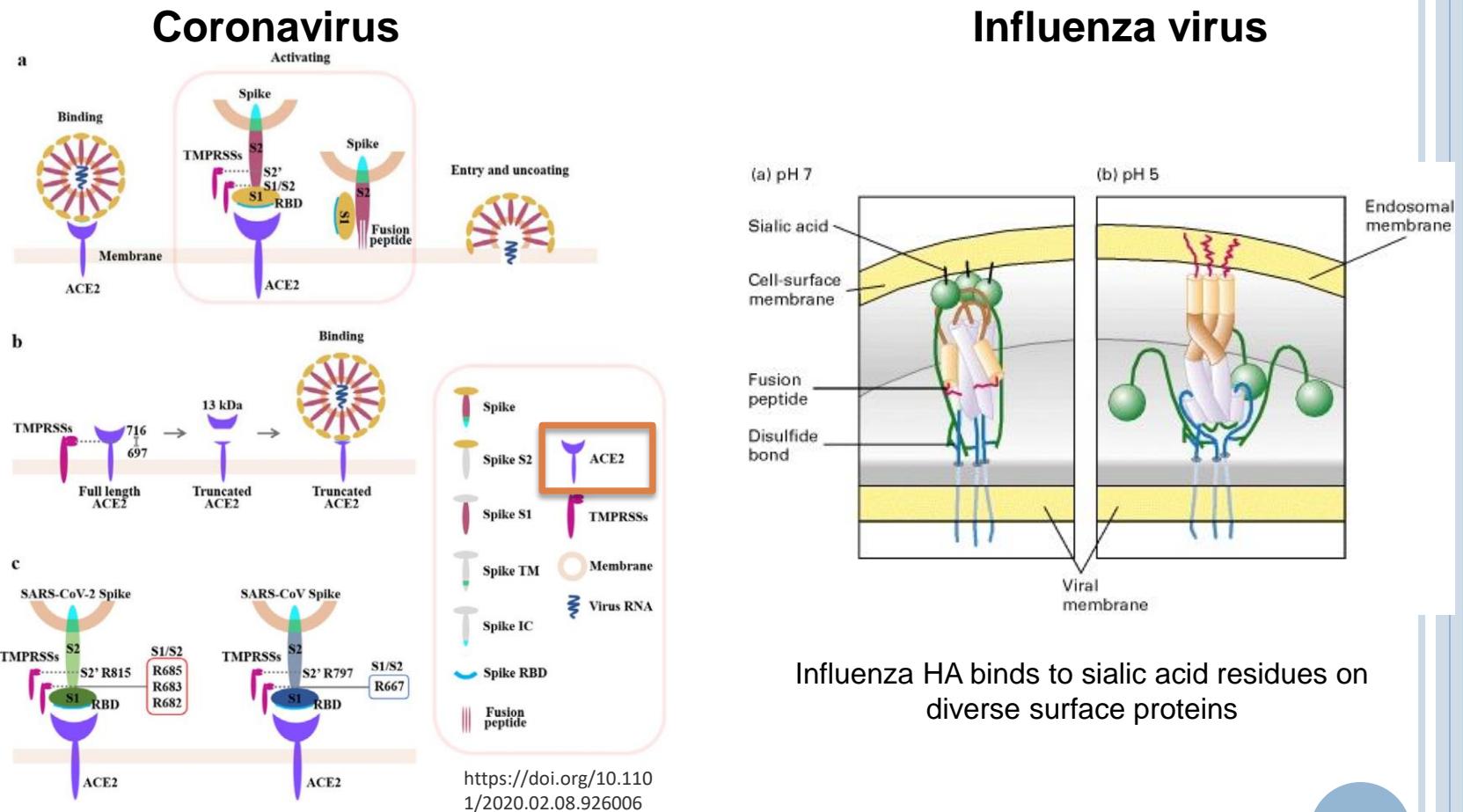
Coronavirus and influenza virus replication cycles



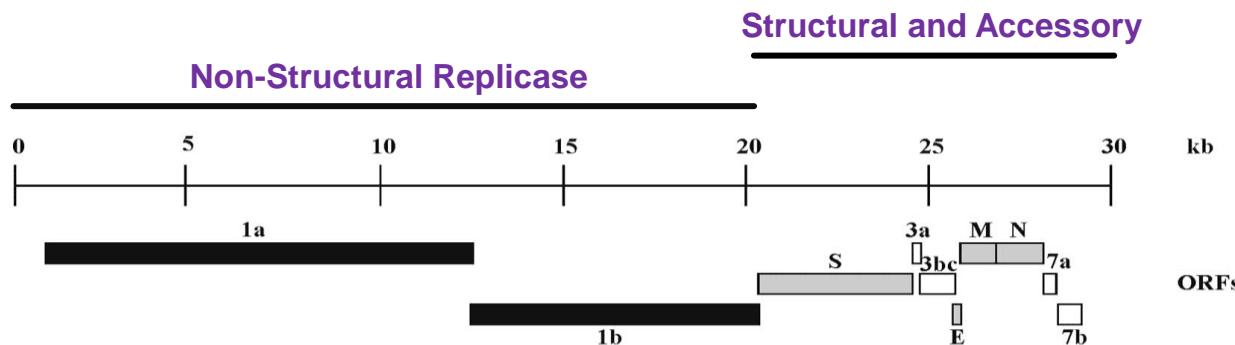
Influenza virus



DISTINCT RECEPTOR BINDING FEATURES OF SARS VS. INFLUENZA VIRUSES



Coronavirus Genome Encodes Several IFN Antagonists



1. Non-Structural Proteins (nsp1-16)

Conserved across CoVs

Various, required functions

IFN antagonists: nsp1, PLP2

(nsp3)

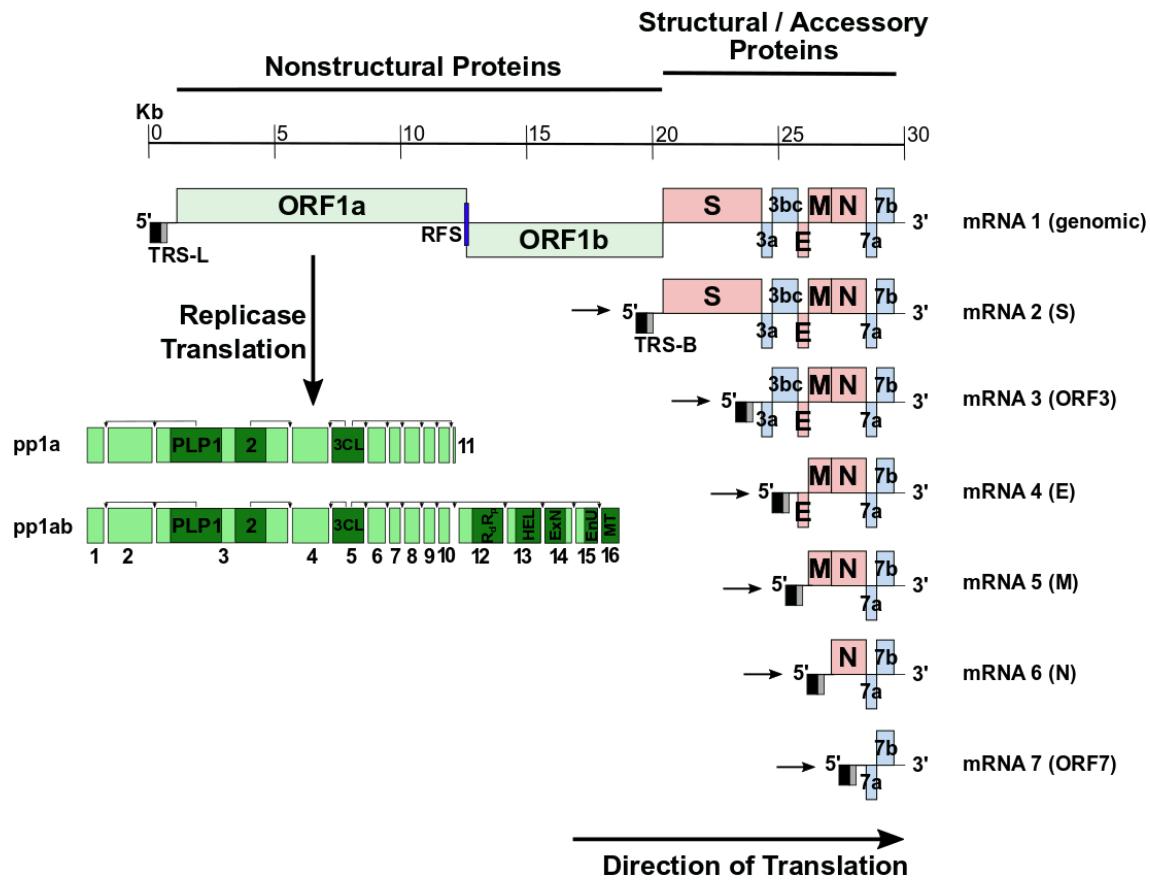
2. Accessory Proteins

Unique to subfamilies and species

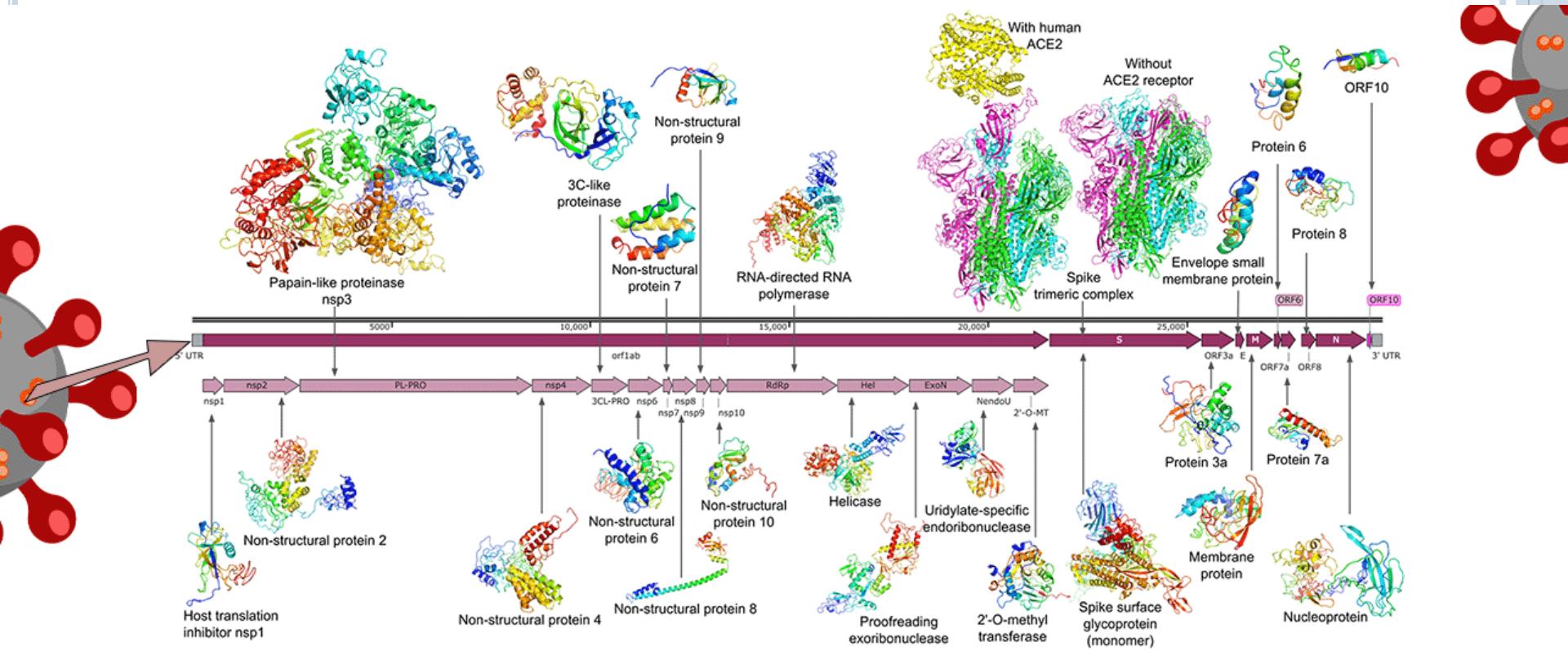
Function dispensable for replication

Encode virulence factors

Coronavirus Genome Structure and Duplication



LARGE SARS-CoV-2 PROTEOME CONTAINS MANY IMMUNOMODULATORY NON-STRUCTURAL PROTEINS



SARS-CoV-2 vs. INFLUENZA VIRUS SUMMARY

SARS-CoV-2

- RNA virus (+ sense)
- Single segment
- Large genome
- Multiple immune antagonists
- Specific receptor (ACE2)

Influenza virus

- RNA virus (- sense)
- 8 segments
- Much smaller genome (than CoV)
- Single immune antagonist (ds RNA sequestration)
- Non-specific receptor

