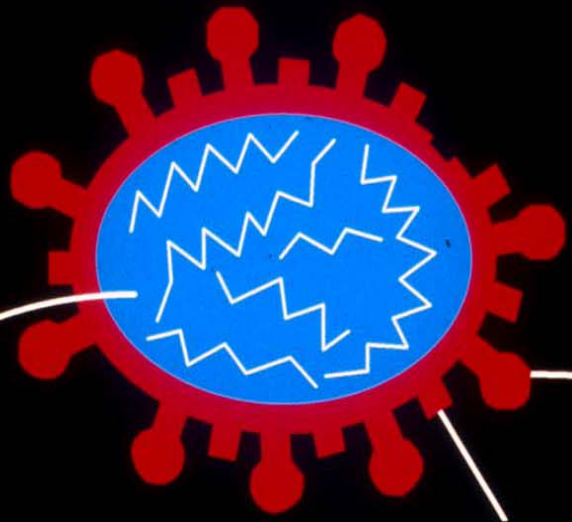
Influenza Virus Nomenclature



Neuraminidase

Type of Nuclear Protein

Hemagglutinin

A/USSR/90/77 (H1N1)

Virus Type

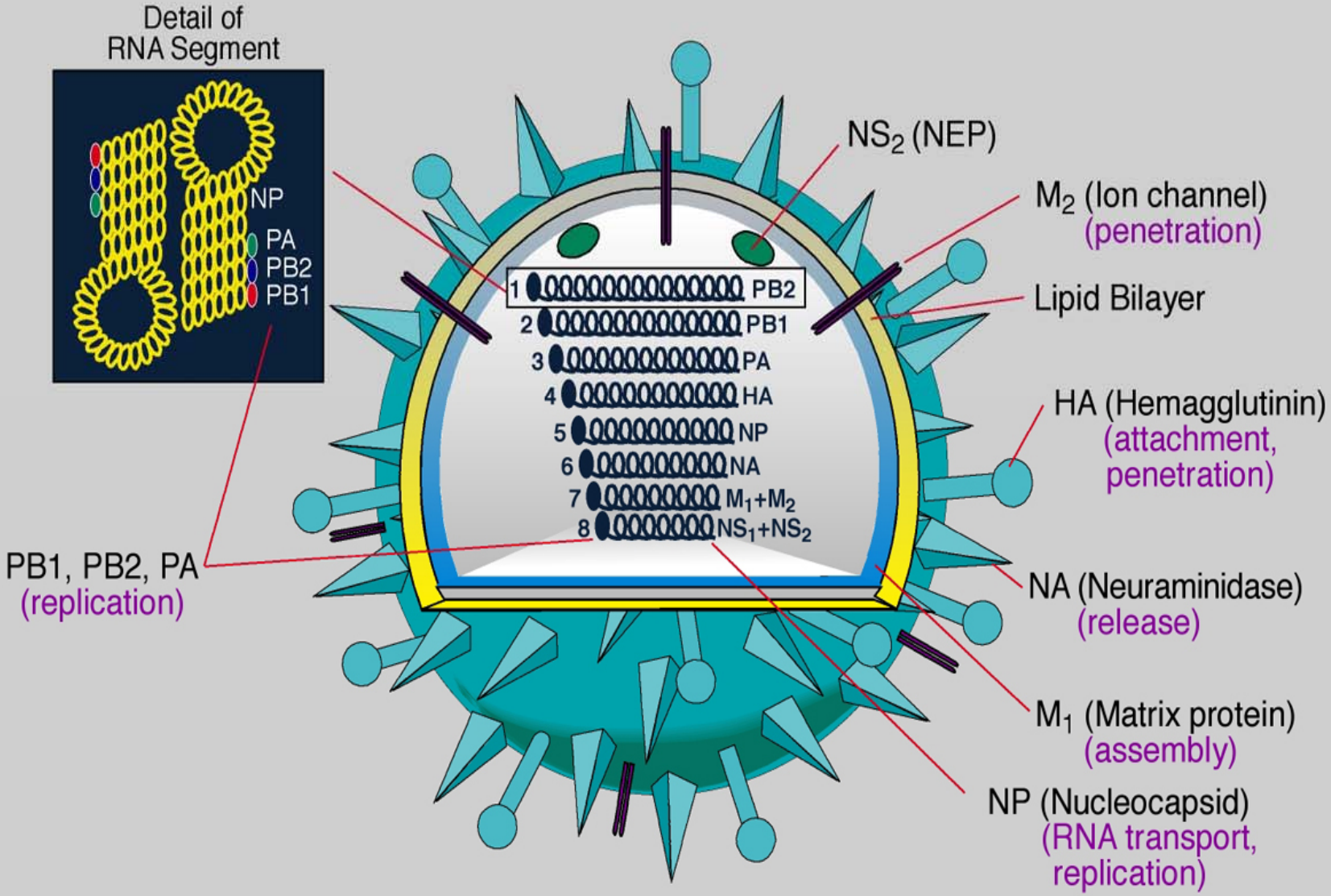
Geographic Origin

Strain Number

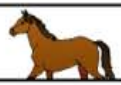
Year of Isolation

Virus Subtype

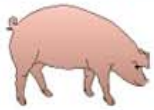
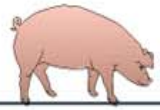
Proteins and RNA's of Influenza A Virus



Where Do These Non-Human Viruses Come From?

H1				
H2				
H3				
H4				
H5				
H6				
H7				
H8				
H9				
H10				
H11				
H12				
H13				
H14				
H15				

Distribution of Neuraminidases in Nature

N1	  
N2	  
N3	
N4	
N5	
N6	
N7	  
N8	  
N9	

Influenza A viruses in humans this century

H1N1 → H2N2 → H3N2 → H1N1

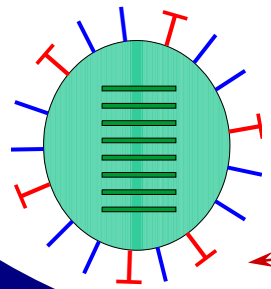
Spanish Influenza — Asian Influenza — Hong Kong Influenza

1918 — 1957 — 1968 — 1977

?

Influenza A reservoir

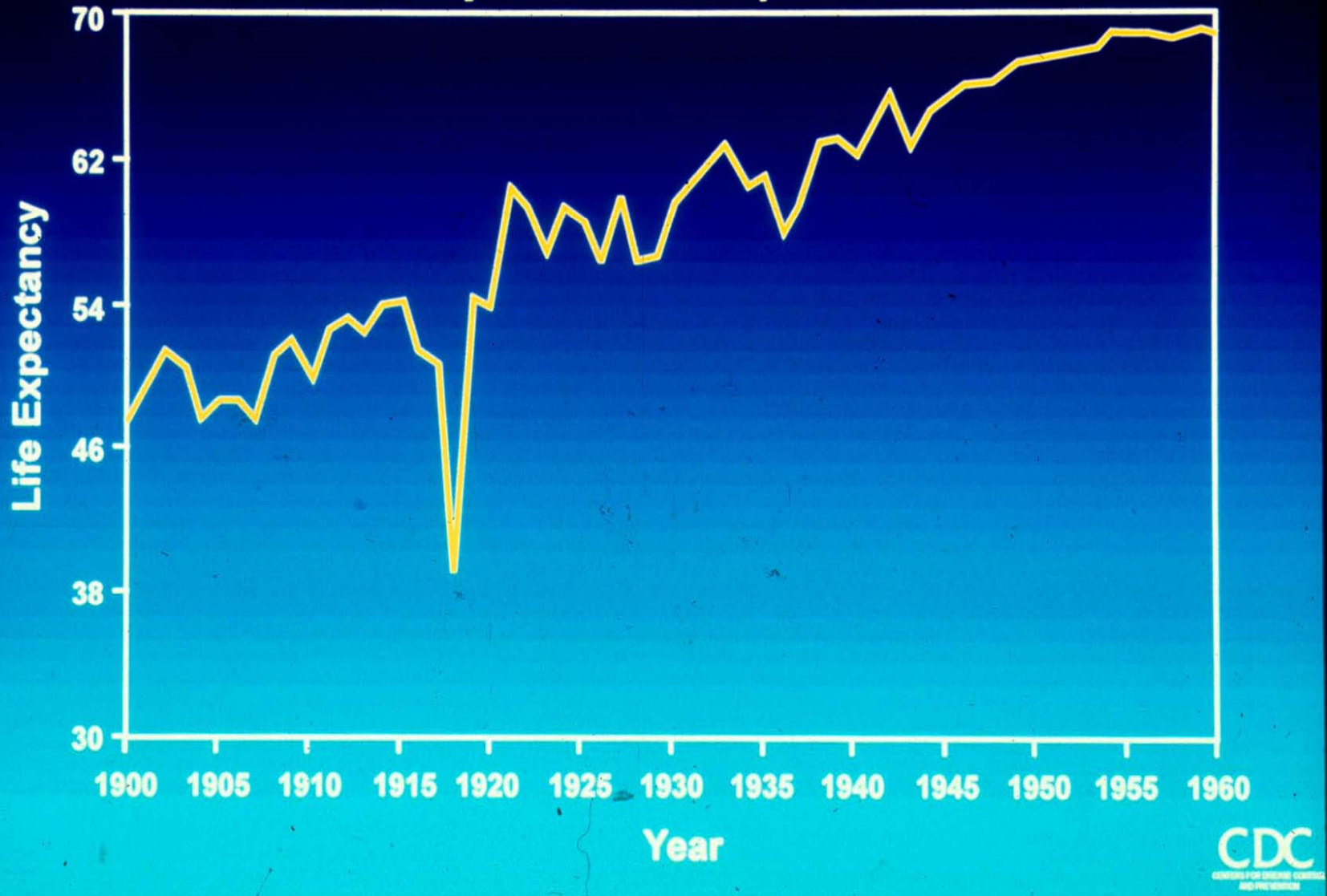




15 HA subtypes

9 NA subtypes

U.S. Life Expectancy 1900-1960



Influenza Excess Mortality

"Spanish" Influenza September 1918–April 1919	500,000
"Asian" Influenza September 1957–March 1958	69,800
"Hong Kong" Influenza September 1968–March 1969	33,800
Total	**603,600**
Interpandemic years 1957–1990	600,800

Cox NJ, Kawaoka Y. In: *Microbiology and Microbial Infections.* 9th ed. 1998;413.

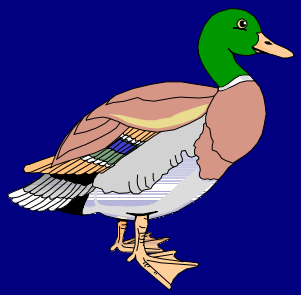
Recent Outbreaks of Avian Influenza in Poultry

Year	Subtype	High pathogenicity?	Location	Birds killed
1983	H5N2	yes	Pennsylvania	17 million
1995	H5N2	yes	Mexico	?
1997	H5N1	yes	Hong Kong	1.6 million
1999-2000	H7N1	no	Italy	13 million
2002	H7N2	no	Virginia	4.7 million
2003	H7N7	yes	Netherlands	>30 million
2004	H5N1	yes	Asia	>100 million
2004	H7N2	no	Delaware	?
2004	H5N2	yes	Texas	?
2004	H7N3	yes	BC/Canada	?

Avian Influenza Viruses Infecting Humans

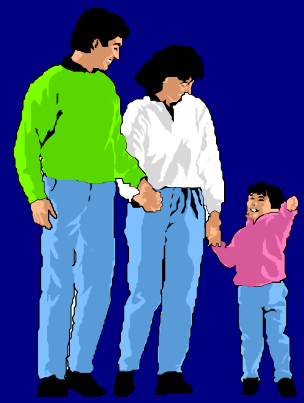
- H5
  - Hong Kong 1997: 18 cases, 6 deaths
  - Hong Kong 2003: 3 cases, 2 deaths
  - Vietnam, Thailand 2004: 33 cases, 22 deaths
- H7
  - Case reports
  - Netherlands 2003: 79 cases of conjunctivitis, 13 ILI, 1 death, 3 person-person transmissions
- H9
  - Hong Kong and Southern China 1999: 7 cases
  - Seroprevalence in poultry workers 1999
  - Hong Kong 2003: 2 cases

Birds

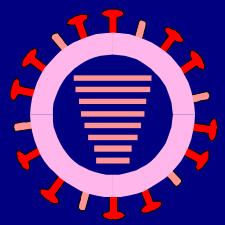


A) Direct infection

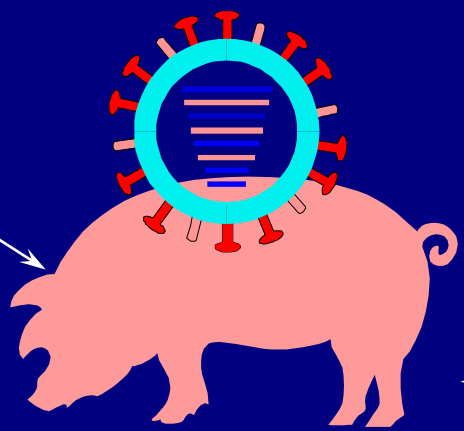
Humans

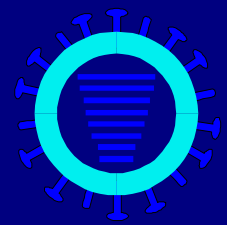


B) Passage in an intermediate host without reassortment



Avian influenza A virus





Human influenza A virus

C) Reassortment in an intermediate host

Potential Social and Economic Impact of an Influenza Pandemic

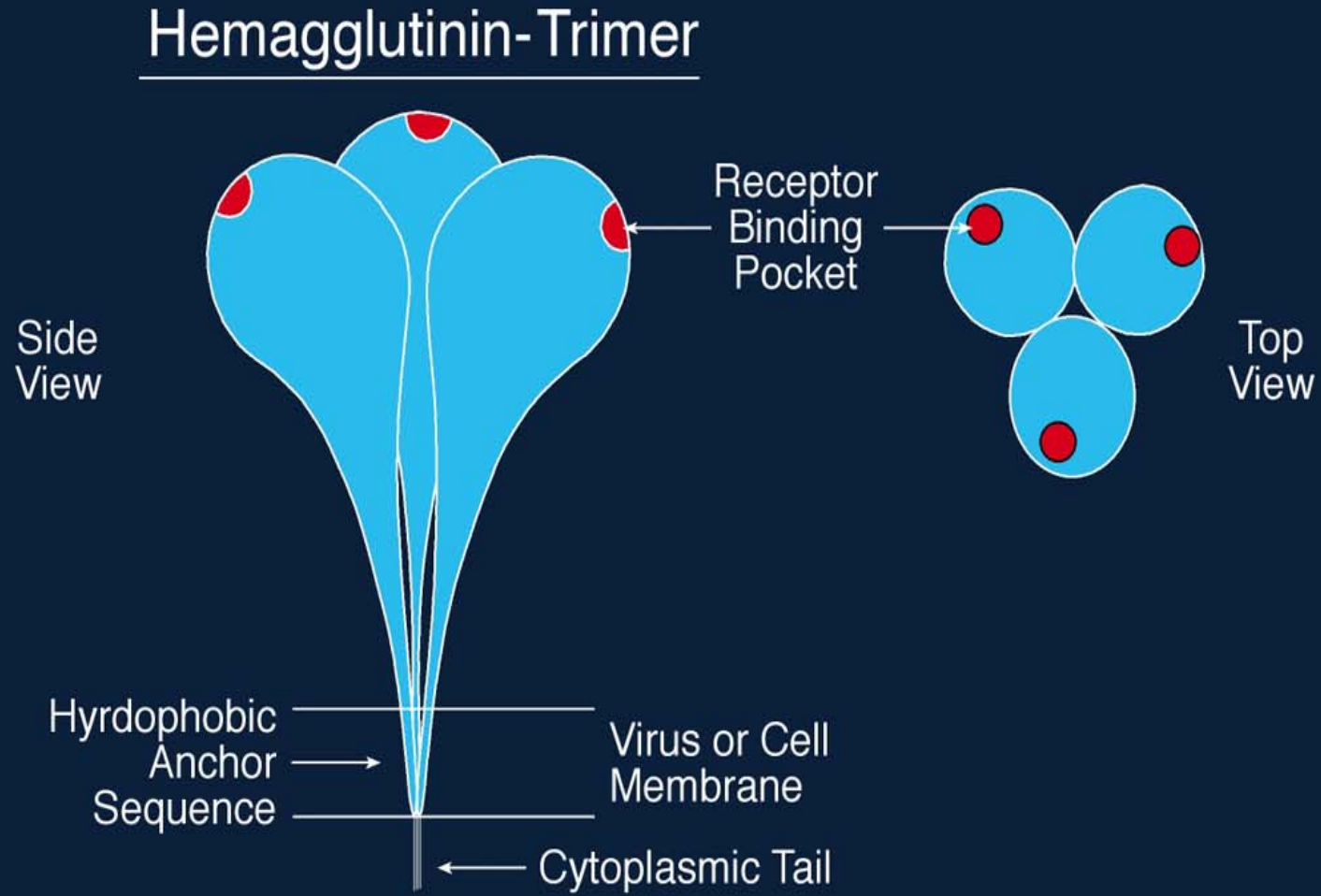
- Mathematical model* estimates for first year of a pandemic in absence of effective interventions:
  - 89,000 - 207,000 in the U.S.
  - 314,000 - 734,000 hospitalizations
  - 18 - 42 million outpatient visits
  - additional 20 - 47 million illnesses
  - economic impact: $71- $166 billion

** Meltzer et al. Em. Infect. Dis. 1999; 5:659-71*

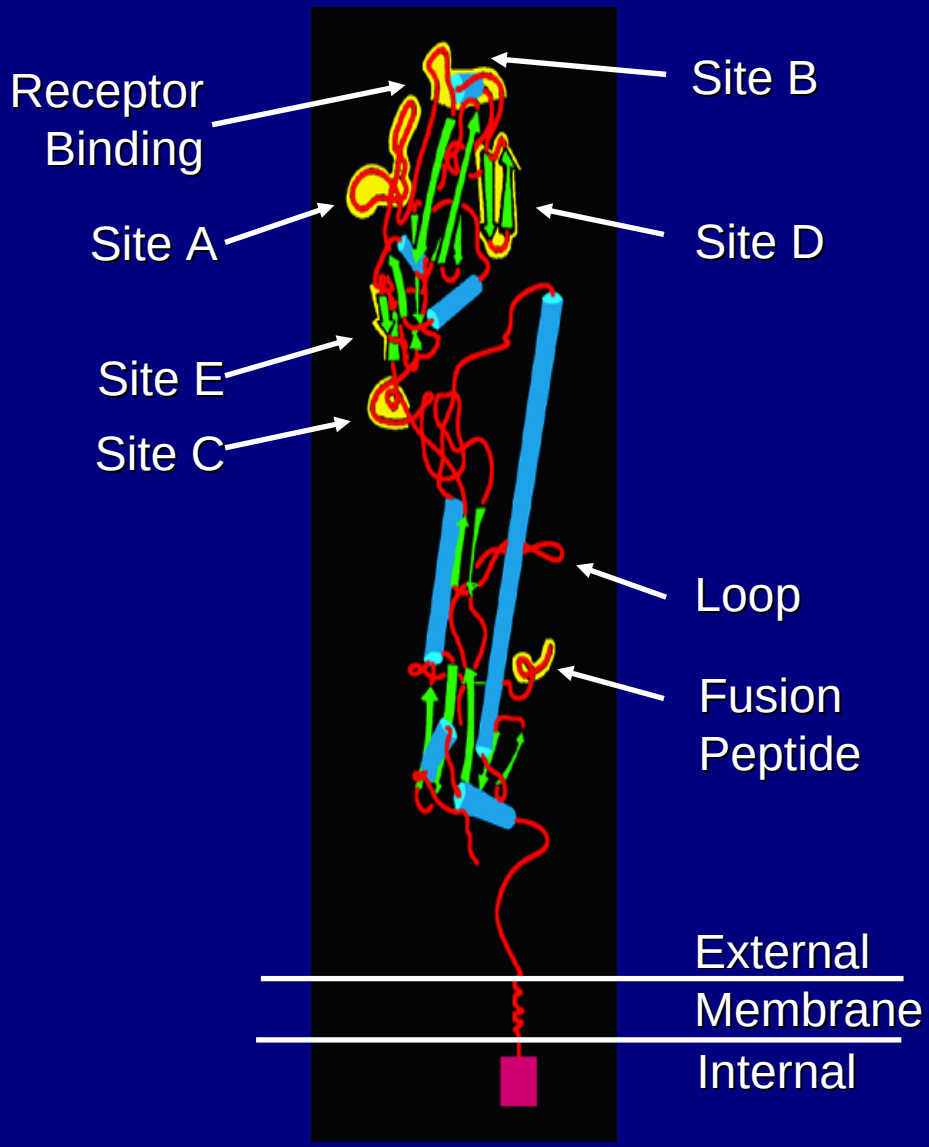
Antigenic Drift

- Gradual alteration of the influenza surface proteins (mainly HA) within a subtype resulting in the inability of antibody to previous strains to neutralize new viruses.
- Antigenic drift results from point mutations in the HA and NA genes.
- The composition of the influenza vaccine has to be updated annually as a consequence of antigenic drift.

Structure of Influenza A Virus Hemagglutinin

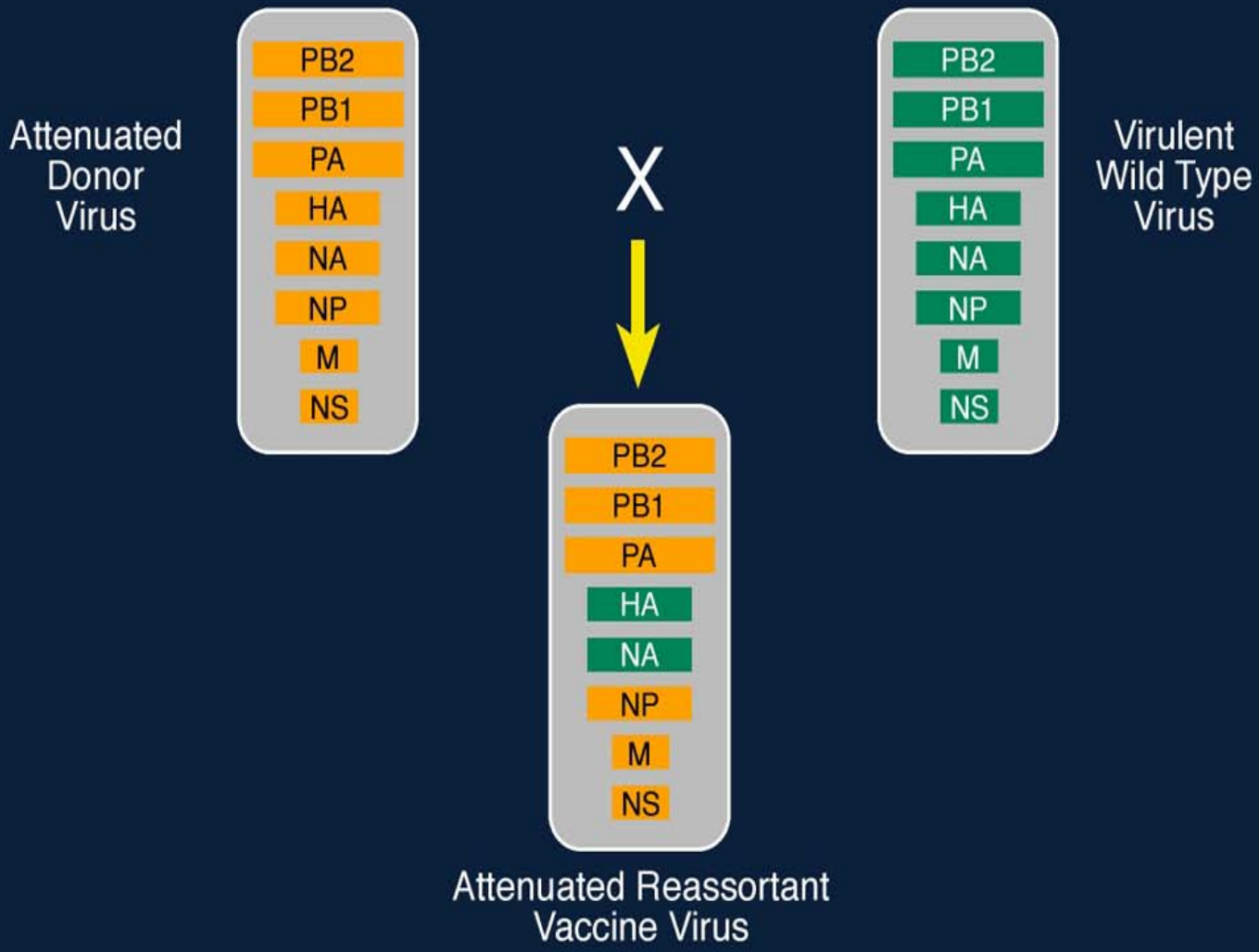


Antibody Binding Sites of HA

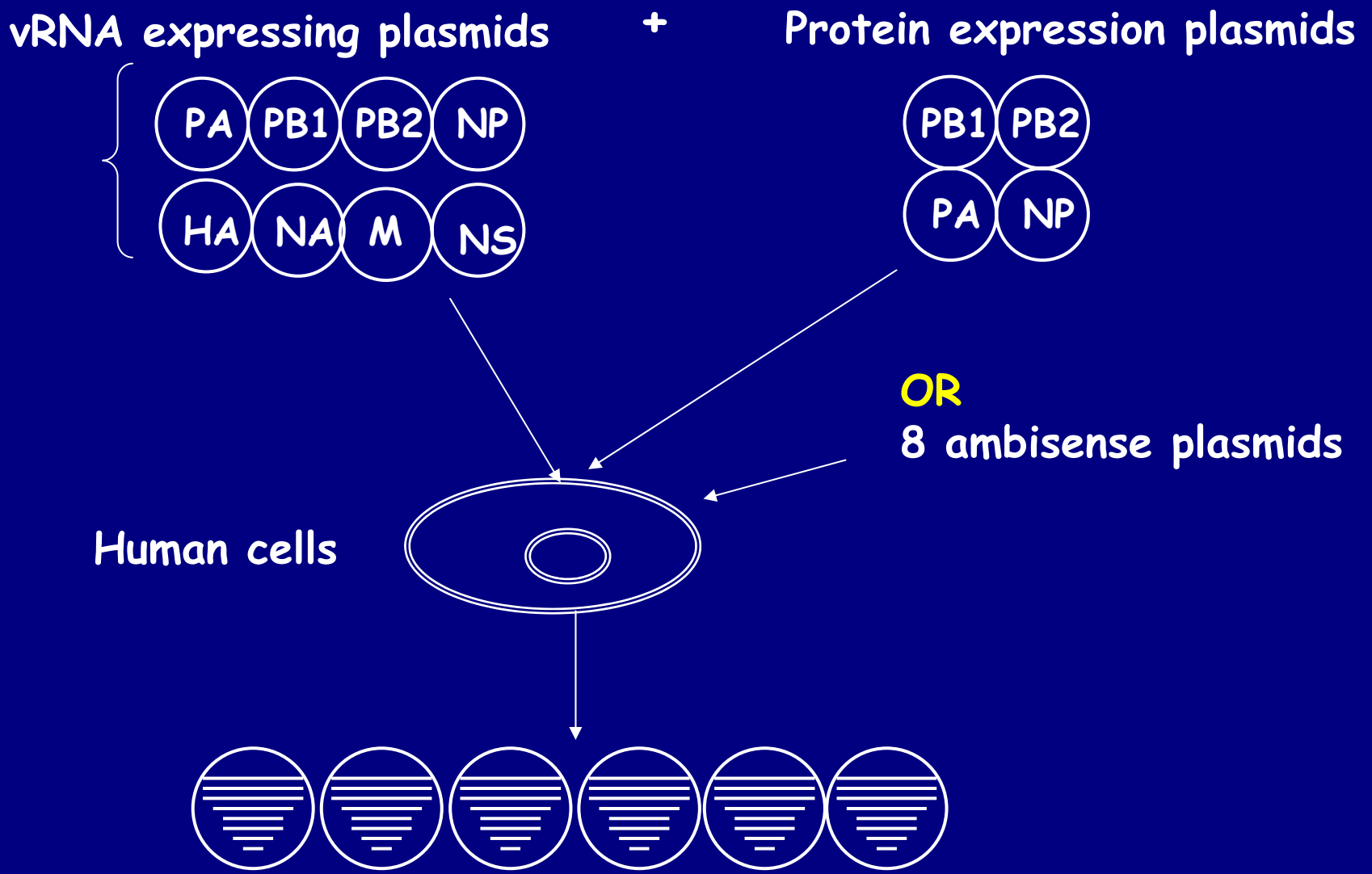


Wiley DC, Wilson IA, Skehel JJ. *Nature.* 1981;289:373–378.

Strategy for Production of Live Attenuated Influenza Virus Vaccine

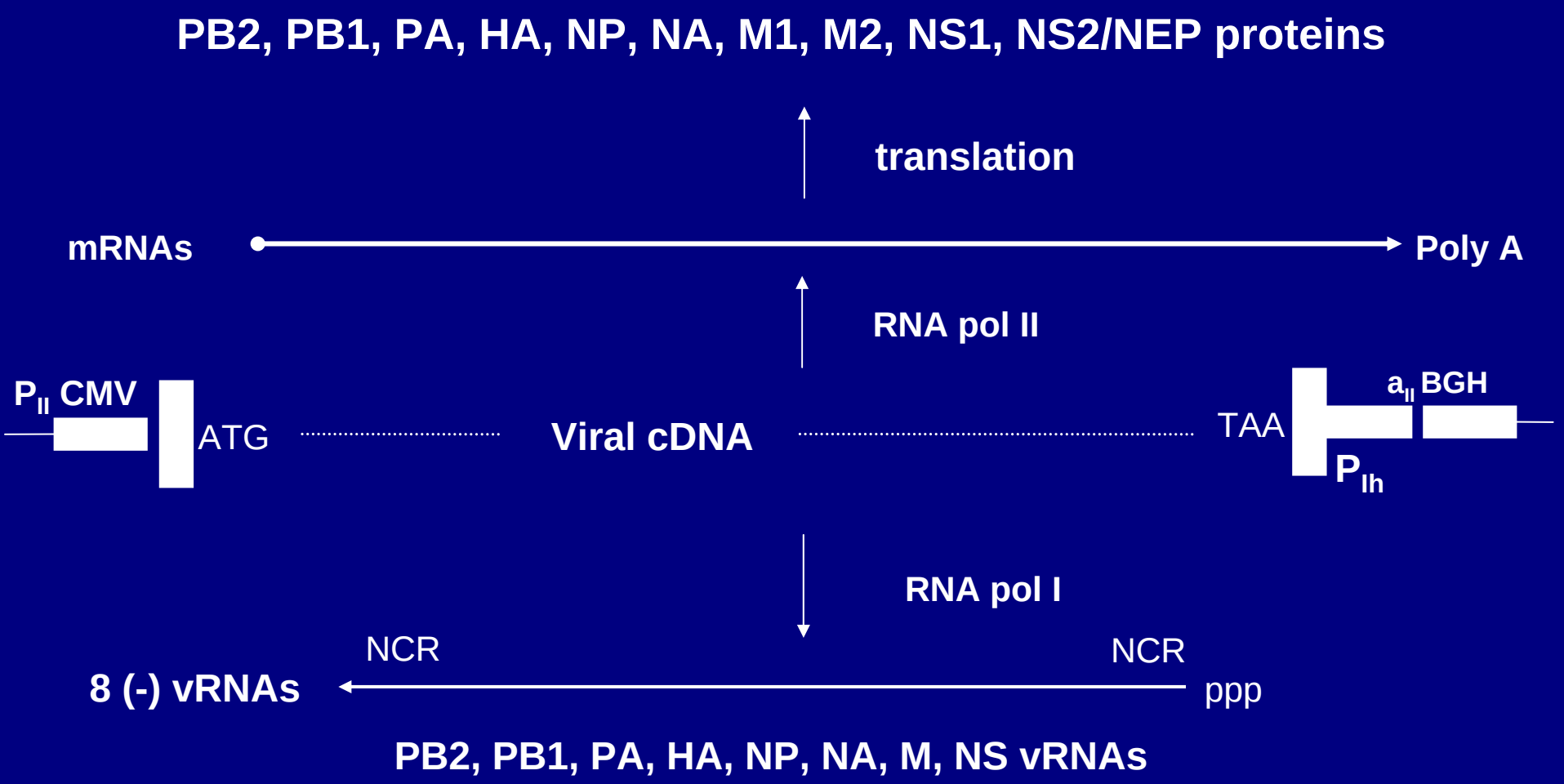


Plasmid-based Reverse Genetics



Fodor et al 1999; Neumann et al 1999; Hoffmann et al 2000

Plasmid-based Reverse Genetics: 8 plasmids



Hoffmann et al. PNAS 2000; 97:6108-13

Molecular Determinants of Virulence and Host-range

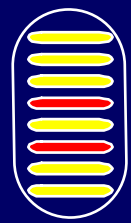
- Specified by >1 residue in >1 gene
- Virulence: HA, NA, M, PB1 and PB2 genes
- Host-range: HA, NA, PB2 and M genes
- Determinants in the HA:
  - Receptor specificity:
    - Humans: N-acetylneuraminic acid-α 2,6,Gal
    - Birds: NeuAc-α 2,3,-Gal
  - Sequence of the connecting peptide: presence of multiple basic amino acids
  - Glycosylation sites

Strategy for the generation of a candidate reassortant H5 influenza vaccine

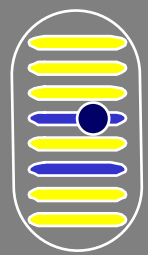
Modify the cleavage site of the H5 HA gene

N1 NA

Influenza Vaccine Donor Strain A/AA/6/60 ca



RERRRKKR ⟶ RETR



Reassortant modified H5N1 vaccine

Li et al JID 1999; 179: 1132-38

Program to generate and evaluate pandemic influenza vaccines

- Generate 2 or 3 candidate vaccines against influenza A viruses of each subtype
- In vitro phenotypes: ts and cold adaptation
- Pathogenicity in chickens (USDA), mice or ferrets
- Efficacy of protection and cross-protection against challenge with wild-type viruses in mouse model
- Determine the importance of antigenic drift among avian influenza viruses e.g. 1997, 2003, 2004 H5N1 viruses
- Proceed to clinical trials to evaluate immunogenicity and infectivity


EQUIPMENT
FIRE EXTINGUISHE


PURPLE NITRILE
Protective App


EXIT


EXIT
6240
COLD
ROOM
50
AIR-MATE
HEPA

Replication of SARS Coronavirus in Animal Models

Our Approach

- Small animals
  - Mice
  - Hamsters
- Non-human primates
  - Rhesus monkeys
  - African Green monkeys
  - Cynomolgus monkeys
- All animal studies are conducted in an ABSL3 facility and all laboratory work in a BSL 3 lab; personnel wear positive air purifying respirators

Conclusions and Implications

- Virus replication models were established in mice, hamsters and monkeys; the level of replication in hamsters>mice>monkeys.
- Findings in mice and hamsters were more consistent and reproducible than those in African Green monkeys.
- Primary infection protects the respiratory tract from challenge in all 3 models.
- Antibody alone appears to protect mice from pulmonary virus replication.
- Vaccines that induce neutralizing antibodies against the SARS spike protein limit pulmonary viral replication and are efficacious in animal models.
- Hamster, mouse and non-human primate models have been used to assess immunogenicity and efficacy of vaccines.
- We can now compare different vaccines.