Influenza across Mammalian Species and Links with Avian Influenza

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Questions the Emergence of Mammalian Influenza A Viruses

- What environmental/ecological conditions favour the interspecies transmission of avian Influenza?
- What role does receptor mediated host range restriction play in interspecies transmission of avian influenza?
- What viral determinants are required for efficient transmissibility among mammals?
- What are the optimal gene constellations and subsequent genetic changes that permit interspecies transmission of avian influenza?
- What selection pressures facilitate the evolution of stable mammalian lineages?

Avian Influenza Viruses in Harbour Seals (*Phoca vitulina*)

New England Coast of USA

- **H7N7 – October 1979**
- **H4N5 – January – March 1982**
- **H4N6 – January 1991**
- **H3N3 – January – February 1992**

Avian Influenza Viruses in Whales

Pilot Whale (*Globicephala melas*)

H13N2
H13N9

Maine 1984

Balaenopterid whale

H1N3 1975 & 1976 South Pacific

Environmental/Ecological Factors

Intraspecific & Interspecific Diversity

Host Density

Host Dispersal

Predation Risk

Aquatic Phase

Avian Influenza Virus in Pigs

Subtype	First Isolation	Location	Epidemiology
H1N1	1979	Europe	Endemic – wholly avian
H1N1	1993	Asia	Endemic
H1N1	2002	North America	Single epidemic – wholly avian
H2N3	2006	North America	Two epidemics – HA, NA and PA genes of avian lineage, remaining genes of TR H3N2 swine lineage. Both farms used raw surface water for cleaning barns and watering animals.
H3N2	1978	Asia	Repeated isolations
H3N3	2001	North America	Two epidemics – wholly avian; one epidemic associated with the use of raw lake water, the second epidemic which was located ~30 km from the first, used well water. No movement of animals between the farms
H4N6	1999	North America	Single epidemic – wholly avian , associated with use of raw lake water
H9N2	1998	Asia	Endemic

???

HPAI H5N1

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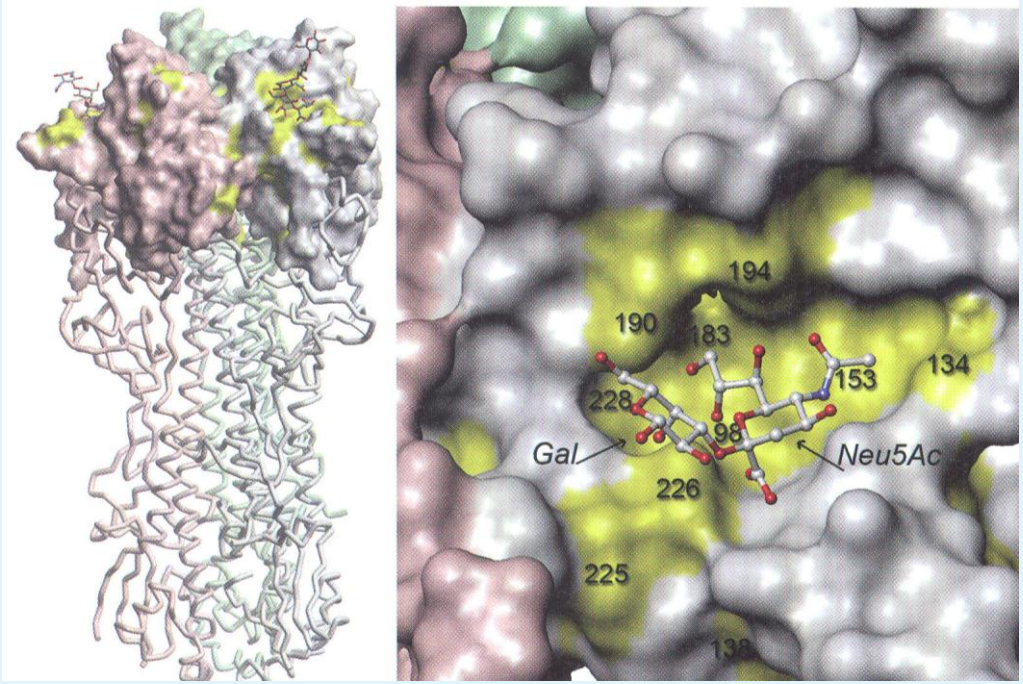
Viral Determinants of Host Range

- HA
- NA
- Polymerase Complex
- NS1

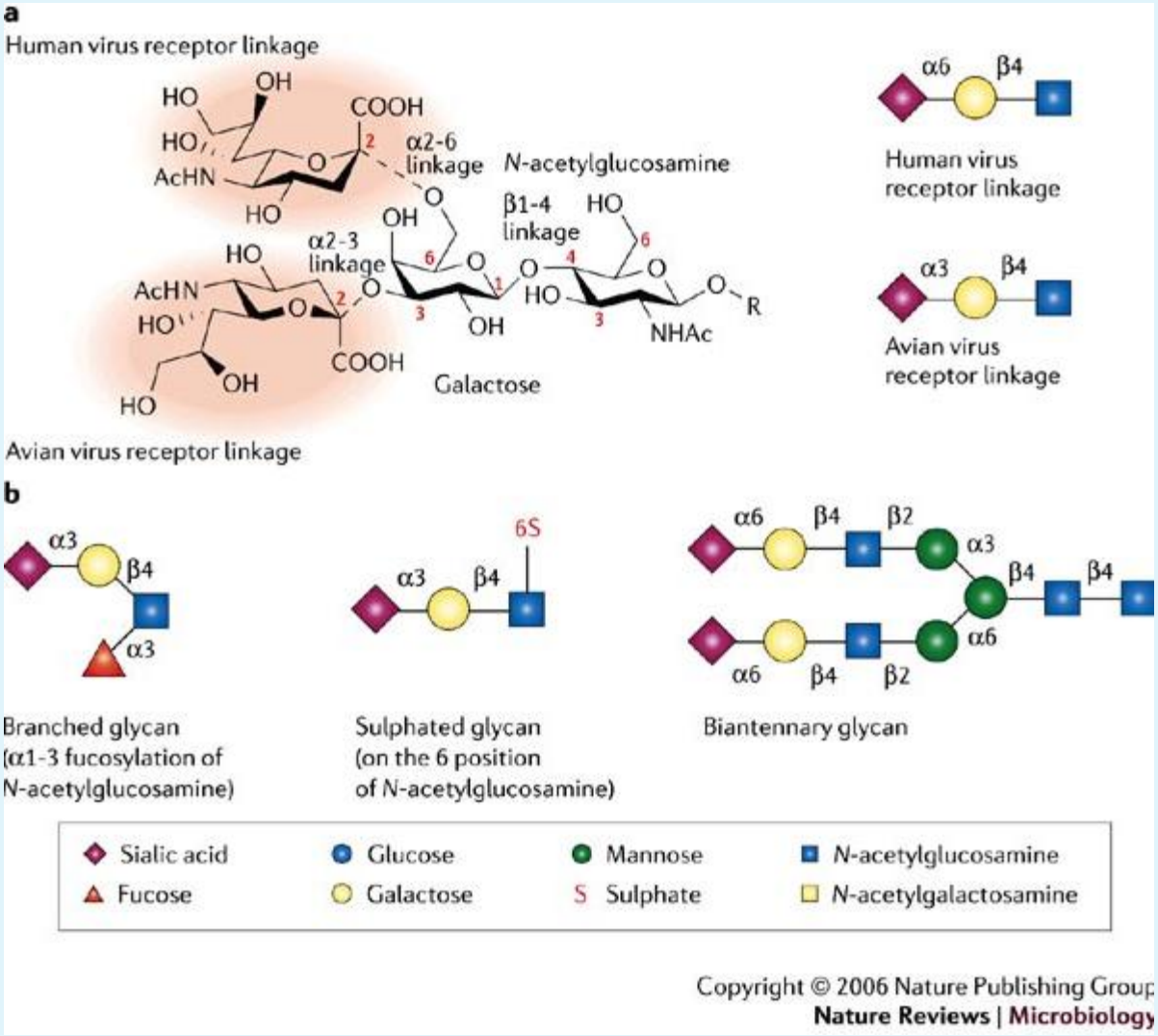
- Host-switching and formation of stable host-specific lineage is polygenic

Receptor Binding Site



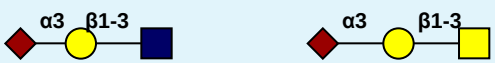
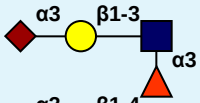
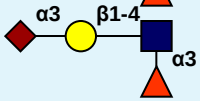
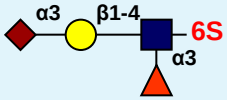
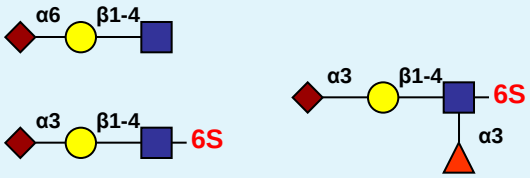
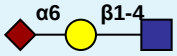
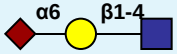
Matrosovich et al. Avian Influenza. Monogr Virol 2008 vol 27, pp 134-155

Receptors



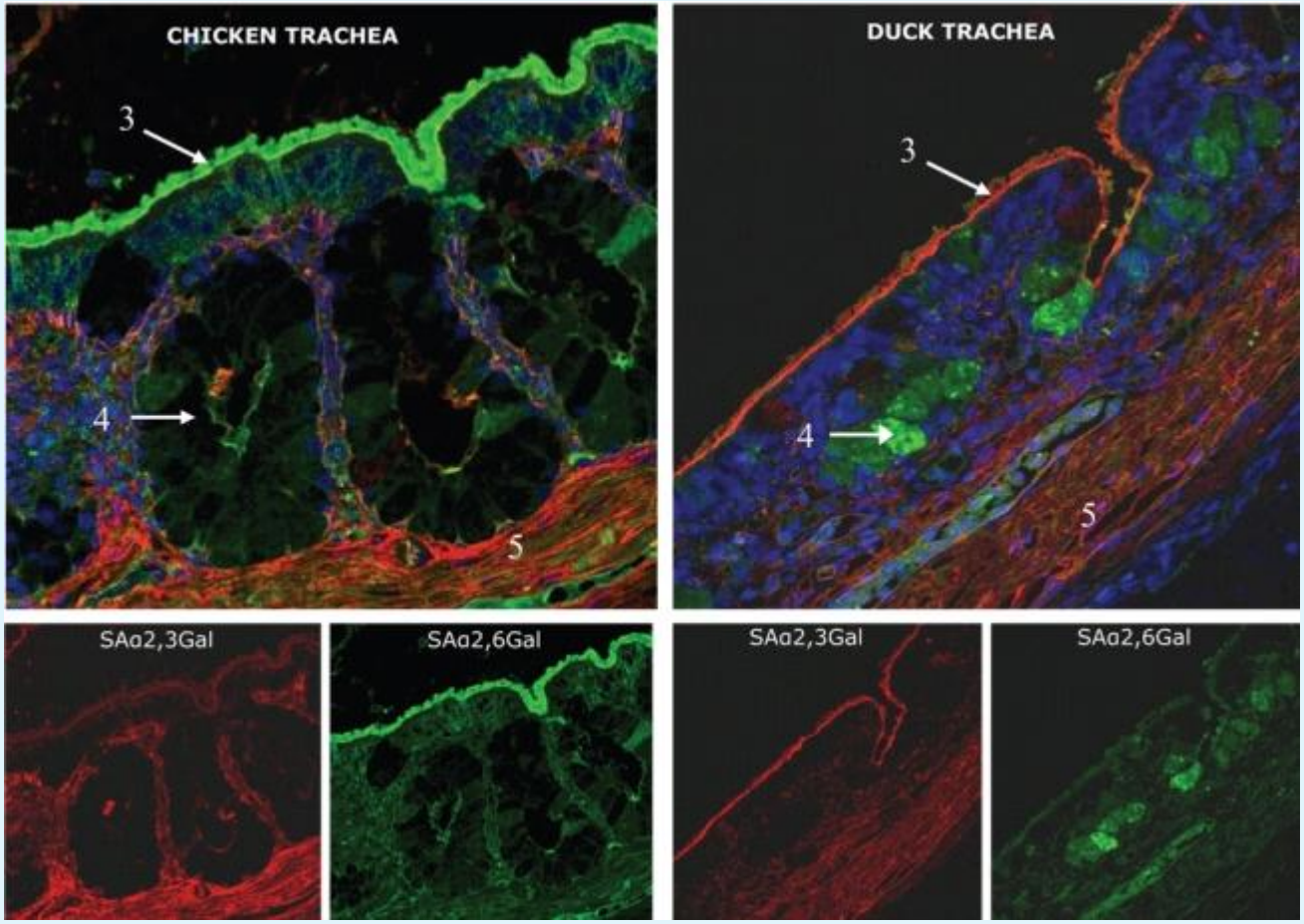
Methods used to Assess HA Receptor Preference

- Hemadsorption assay using desialyated /linkage-specific resialyated RBCs
- Virus binding assays
- Glycan microarrays

Virus Host	HA Subtype	High-affinity binding sialyloligosaccharide
Ducks	H1, H2, H3, H4, H5	
Gulls	H4, H5, H6	
	H14	
	H13, H16	
Chickens	H5, H7	
Poultry	H9N2	
Pigs	H1, H2, H3, H4	
Humans	H1, H2, H3	

Adapted from Matrosovich et al. 2008

Expression of SAα2,3-Gal and SAα2,6-Gal Receptors in Chicken and Duck Trachea

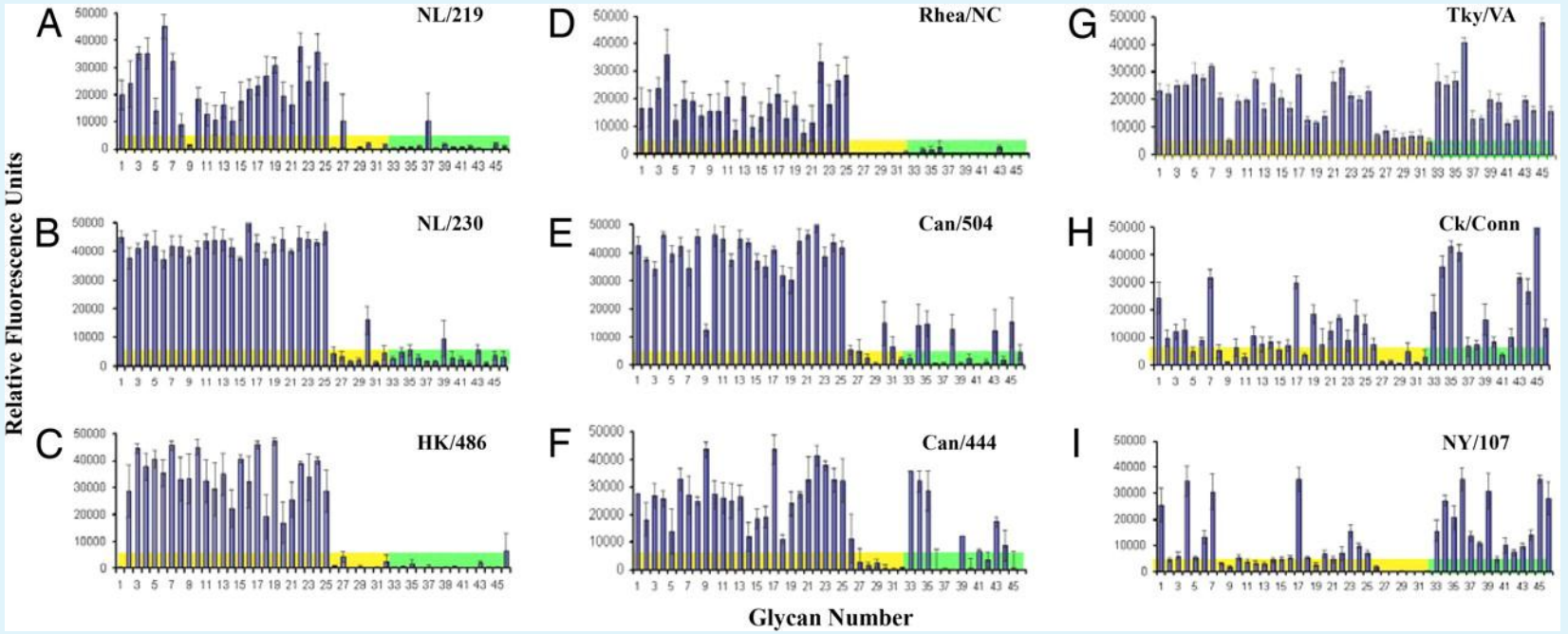


Kuchipudi et al., 2009. Journal of Molecular and Genetic Medicine 3: 143-151.

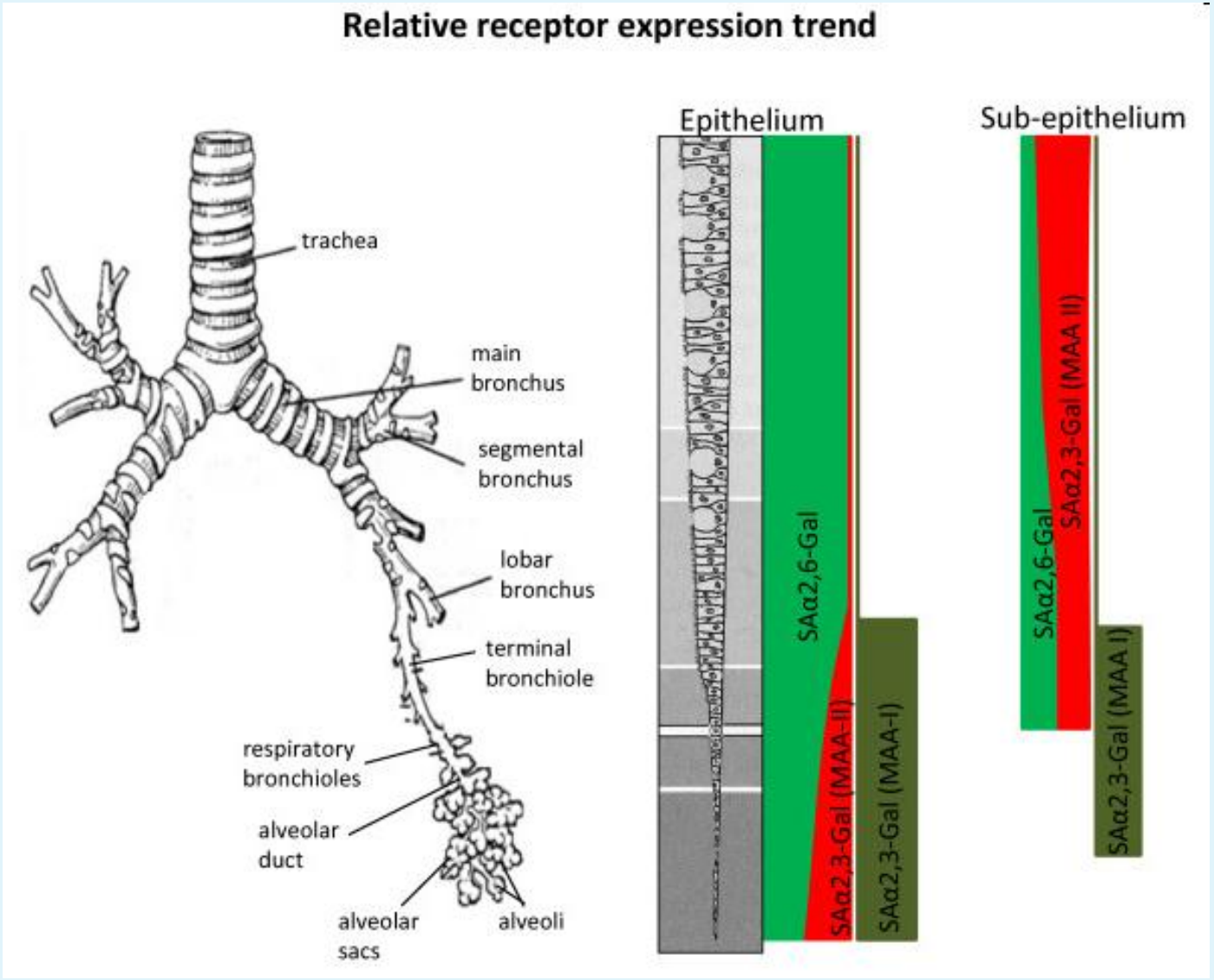
Is passage of AIV through gallinaceous poultry a prerequisite to mammalian adaptation?

- North American LPAI H7 viruses replicate to higher titers in the upper respiratory tract of chickens and turkeys compared with the gastrointestinal tract
- The upper respiratory tract of chickens express more α2-6 SA receptors compared with wild birds
- An increase in α2-6 SA binding may need to coincide with a decrease in α2-3 SA binding in order for mammalian adaptation to occur
- Sialylated secretions or mucins in human airway contain α2-3 SA

Glycan microarray analysis of Eurasian and North American lineage H7 influenza viruses.

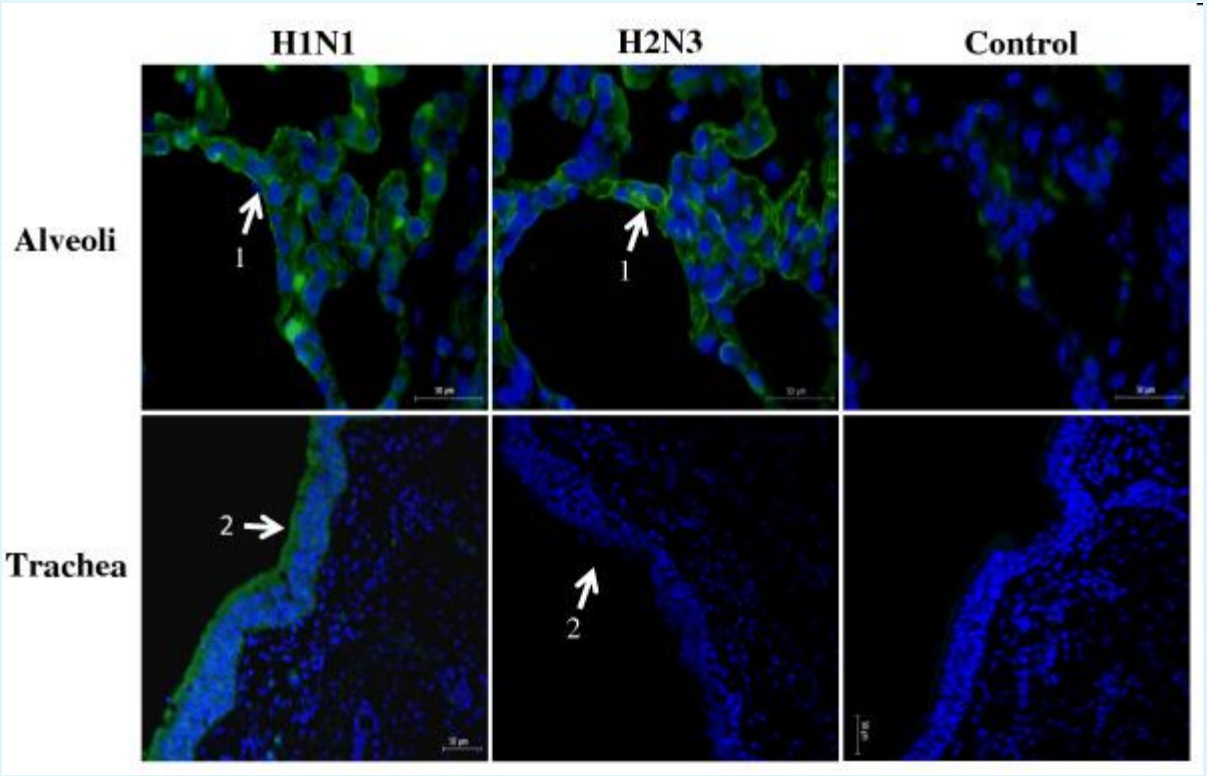


Belser J A et al. PNAS 2008;105:7558-7563



Nelli et al., 2010. BMC Veterinary Research 6: 4

Virus Binding Assays



Nelli et al., 2010. BMC Veterinary Research 6:4

Amino Acids in HA Receptor-Binding Site of Human Avian and Swine H2 Influenza Virus Isolates

	HA receptor-binding residues					
Virus strains	**138/148**	**190/200**	**194/204**	**225/235**	**226/236**	**228/238**
Avian consensus	A	E	L	G	Q	G
Mallard/2003/H2N3	A	E	L	G	Q	G
Sw/4296424/H2N3	A	E	L	G	L	G
Sw/2124514/H2N3	A	E	L	G	L	G
Human consensus	A	E	L	G	L	G/S
Davis/1/57	A	E	L	G	L	G
Albany/7/57	A	E	L	G	L	G
RI/5+/57	A	E	L	G	L	S
Albany/6/58	A	E	L	G	L	S
Ohio/2/59	A	E	L	G	L	S
Berlin/3/64	A	E	L	G	L	S

Ma et al., 2007. PNAS 104: 20949-20954.

Is a Co-Factor in Addition to SA Involved?

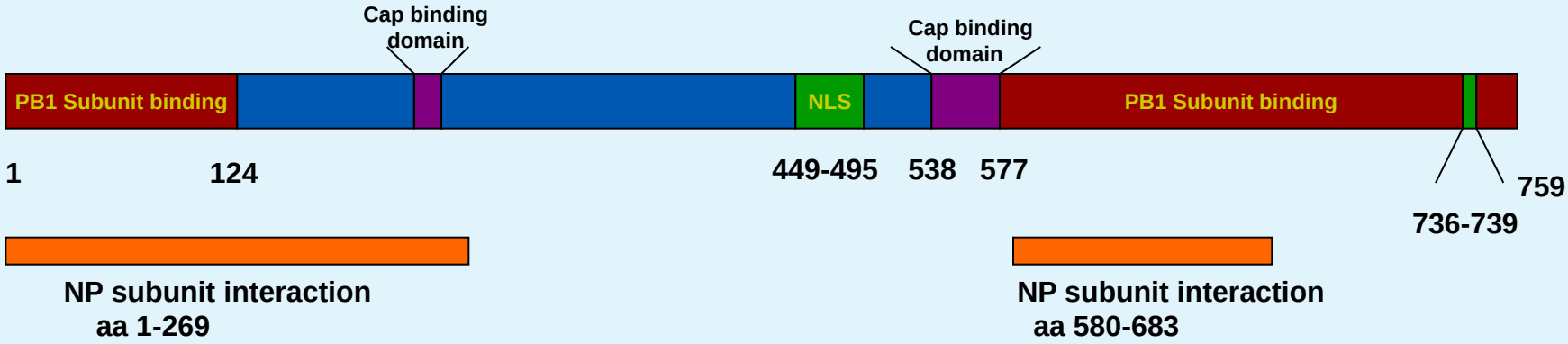
- HA1 binds SA with low affinity (mM range)
- Human tissues lacking the SA receptor can be infected with H5N1 viruses
- An N-linked glycoprotein may be involved in human influenza virus entry

Neuraminidase

- NA can cleave both α2-3 and α2-6-linked SA
- But all influenza NAs have a marked preference for the α2-3 linkage
- Viruses with suboptimal NA activity may aggregate due to HA binding to SA on adjacent viral particles
- NA may be required for virus entry by destroying decoy receptors such as 2,3-linked SA on mucins secreted by goblet cells
- Matched or balanced HA and NA specificities are required for optimal infectivity

Highly Conserved Host-Specific Residues in PB2

Virus	81	199	271	475	567	588	613	627	674	702
Avian	T	A	T	L	D	A	V	E	A	K
Human	M	S	A	M	N	I	T	K	T	R



Virus Mutations Associated with Mammalian Adaptation

Protein	Changes	Effects on Transmissibility	Effects on Virulence and/or Host Range
PB2	E158G T271A G590S/Q591R E627K D701N	Increased titers in nasal washes from infected mice	 Enhanced polymerase activity in mammalian cells
PA	T97I		Enhanced polymerase activity and replication in mammalian cell lines
HA	Q226L/G228S Loss or gain of glycosylation/sialylation sites	May increase transmissibility – often additional adaptive changes are required	α2

NS1

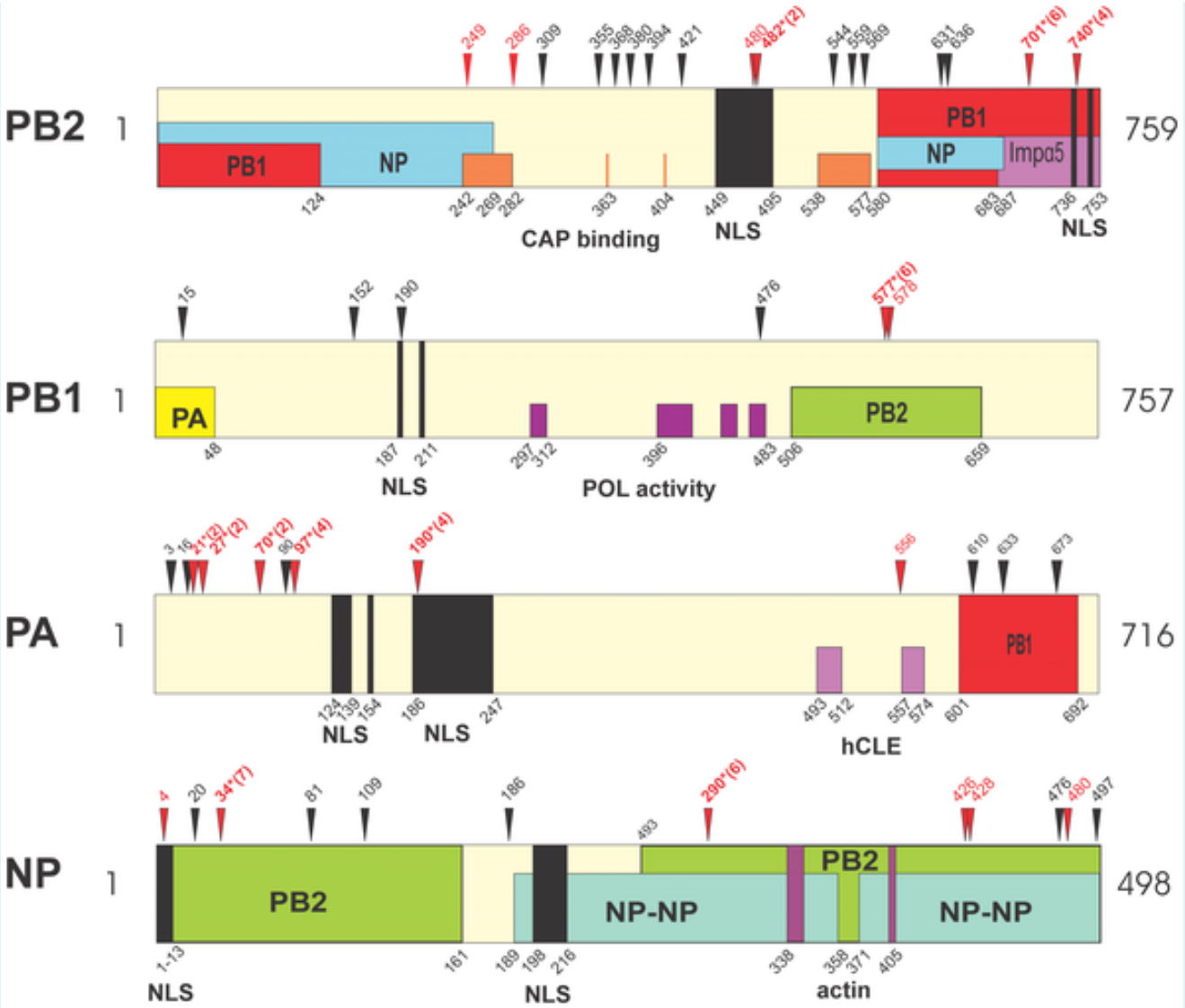
- **Multifunctional role:**
  - Selective translation of viral genes
  - Limit IFN-β production by pre-transcriptional and post-transcriptional processes
- **NS1 proteins divided into 2 major groups:**
  - Alleles A and B
  - Allele A → avian and mammalian viruses
  - Allele B → avian viruses only

The Mouse as a Model to Study Adaptive Evolution

- Multiple genetic pathways likely exist for host switching
- Adaptive evolutionary theory states that phenotypic variation and speciation is explained by selection of biological variants that function to increase replicative fitness
- Adaptation to increased virulence in mice is associated with mutations that increase fitness and replication in mouse-adapted variants

Adaptive Evolution of Human Influenza Virus in the Mouse as a Model System

- A/HK/1/68 (H3N2) underwent 12 and 20 mouse-lung passages (Ping et al. 2011. PLoS ONE)
- Polymerase and HA mutations are most prominently involved in mouse adaptation
- There is a trend toward increased fixation of mutations within viral populations with increasing passage number
- Adaptive mutations were primarily located in regions of interaction with the host and in several instances involved sites of viral subunit interaction or oligomerization



Ping et al., 2011. PLos ONE 6: e21740

Adaptive Evolution of Human Influenza Virus in the Mouse as a Model System

- **Evidence for convergent evolution**
  - PB2 D701N mutation has been demonstrated in mouse adapted variants of A/Hong Kong/1/1968 (H3N2)
  - PB2 D701N has also been found among 154 human HPAI H5N1 infections
  - PB2 D701N appears to have been important for the adaptation of avian H3N8 viruses to equines and has been maintained on adaptation of equine virus to dogs with further evolution to PB2 D701N + D740N
- **A relatively small number of mutations were responsible for mediating mouse adaptation and increased virulence**

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OIE/FAO Network of Expertise on Animal Influenza

Thank you for your attention

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