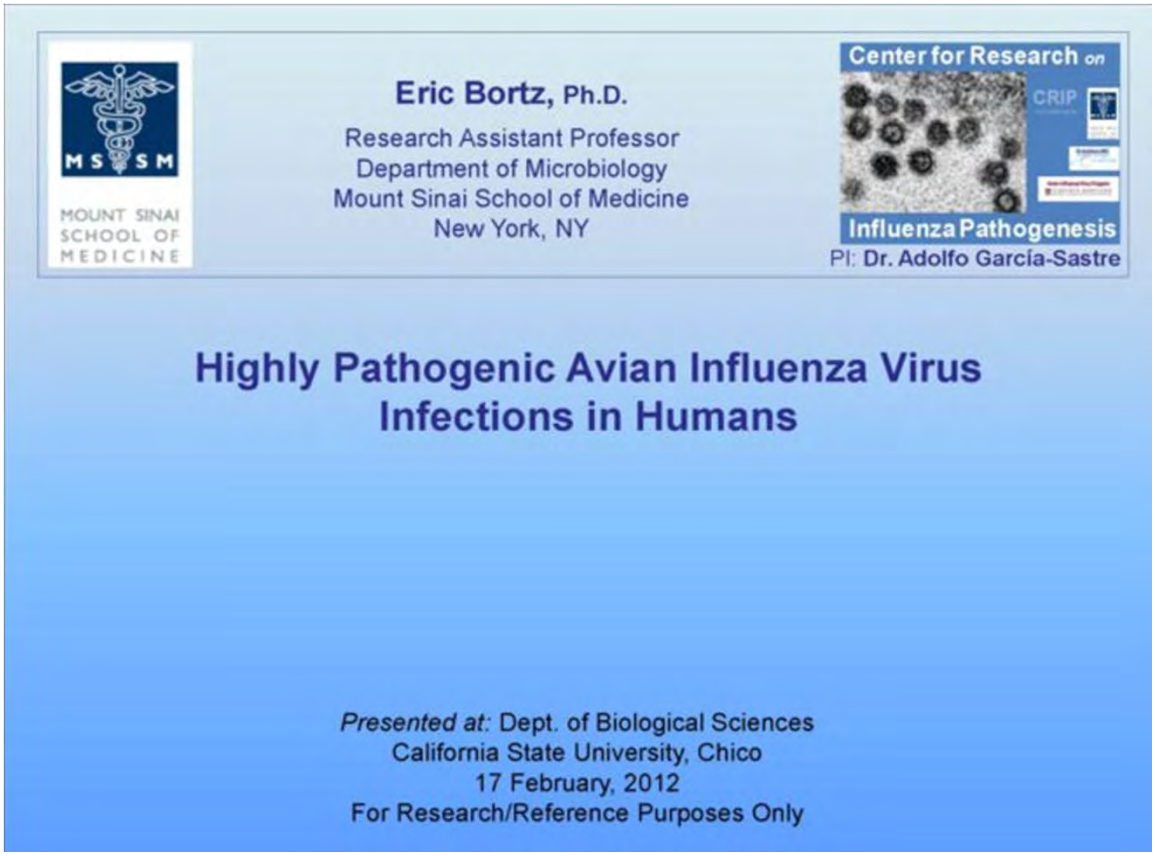
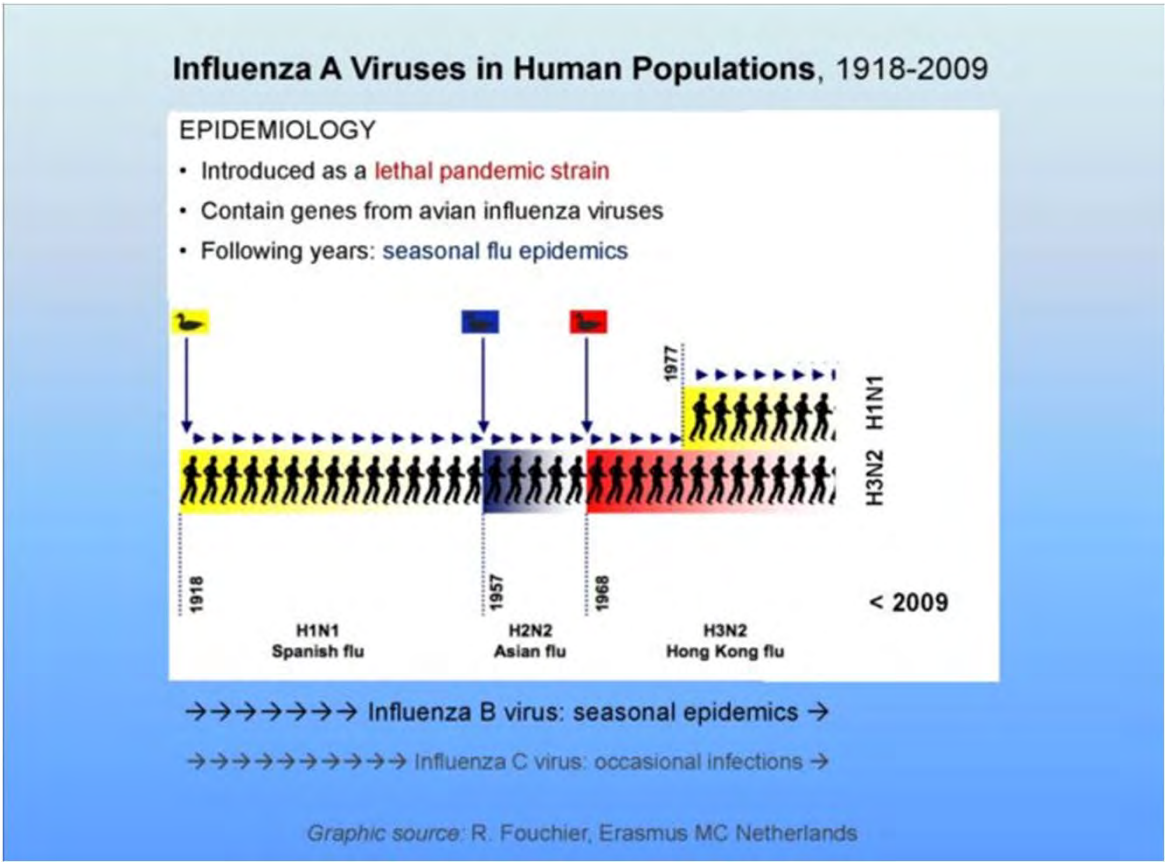
Okay, I'd like to thank the faculty for inviting me specifically and giving me the chance to talk here and it's Friday afternoon before a holiday, so I'll try to go through this without too much really intense molecular detail by the end of it, but we'll have a little bit in the middle.



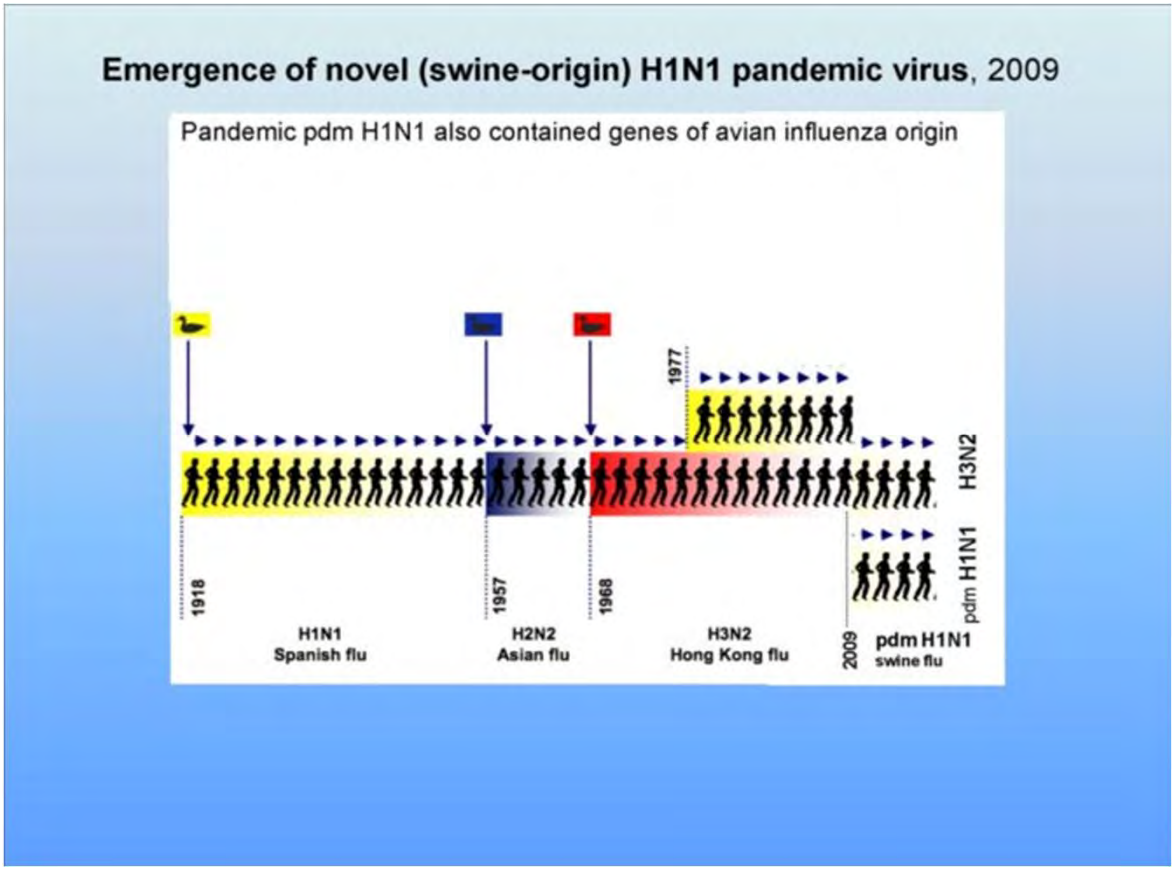
So, I work on highly pathogenic avian influenza virus and particularly, I'm looking at the infection of these, how these pathogens, which are really viruses that infect birds, are able to infect human beings and to infect mammals.

And you might think that, why would that be, and it seems that every time you turn around or turn on the news, there's another pandemic, there's another influenza virus, there's another emerging infection that's some kind of virus and influenza is one of these that has popped up over the last century or so, and including most recently, the H1N1, the novel swine origin H1N1, which arose in Mexico. It caused a public health emergency; people were taking temperatures in airports and so on. And maybe recently you've heard about avian influenza as well or bird flu, highly pathogenic avian influenza, also called HPAI. H5N1 is the, the most well-talked about subtype of this virus as I say. I'll go into that a little bit more, but it really emerged in 1997 in Hong Kong. And in live bird markets in Hong Kong, and in 2003, these highly pathenogenic avian influenza viruses re-emerged in southeastern Asia, in southeastern China and spread with wild birds into the domestic live bird markets in other countries, and the backyard farms in places like Indonesia, Egypt, throughout Vietnam, throughout southeast Asia and into other areas. So they both migrate with wild birds and they also go transmit with the chicken, the chicken trade. I'm not going to say too much more about the ecology of this, but I would encourage, if you have questions during, during this seminar, or at the end, or you'd like to talk more about it, we can talk a lot about this, especially for people who are interested in, in the avian ecology of these viruses and the high path ones.

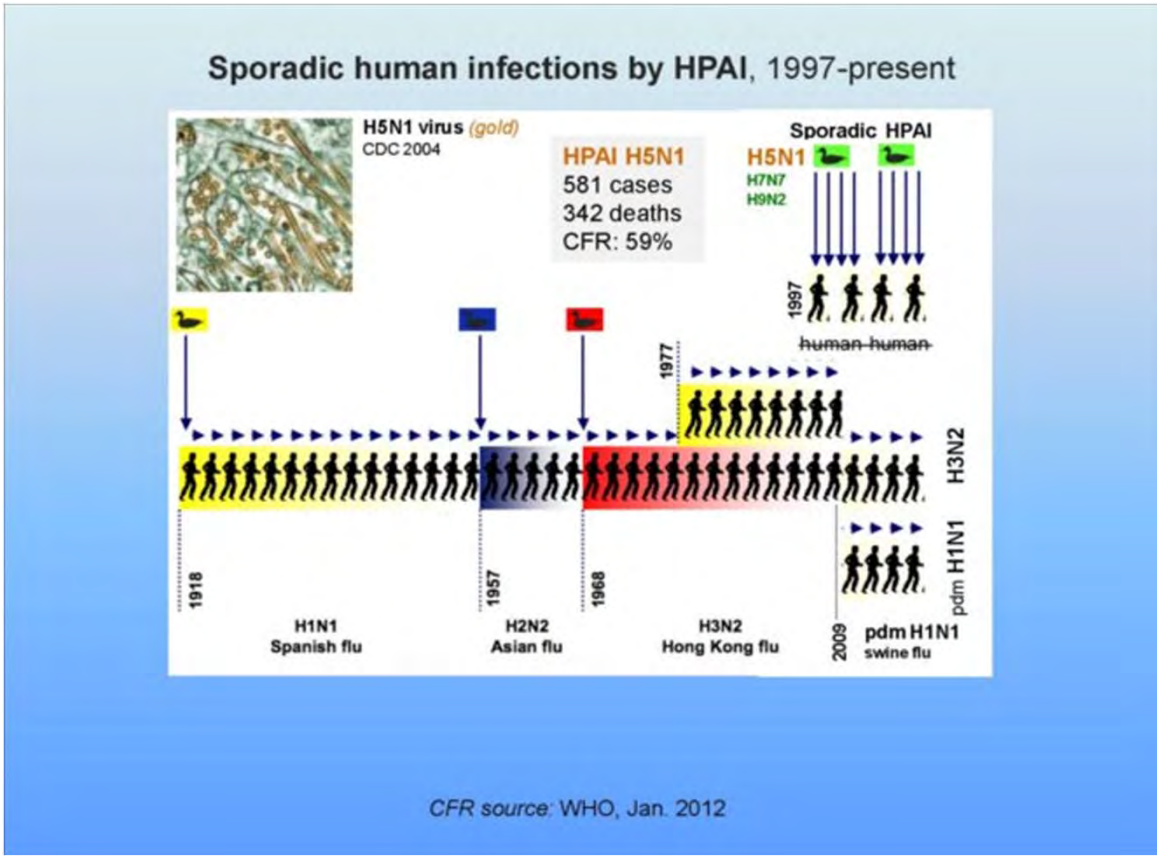
So, this is what I will say about it, is that, in case you didn't know, maybe everyone does, that flu viruses are natural pathogens of wild water fowl. They're natural pathogens of birds and the viruses that we see in humans or in mammals are all thought to originally, sometime in evolutionary time, to have an avian origin. And the reservoirs are geese, ducks and swans, for example, mallard ducks particularly, and they can spread to chickens, as I mentioned before and to domestic ducks. This is a domestic duck farm. This is a little girl next to some ducks on a frozen lake, but we also eat duck, I eat, I love duck, so in southeast Asia and China, for example, ducks are also a source of direct infections for avian influenza viruses to go into a species that humans come in contact with and primary mammals, humans and pigs are the predominant mammals that are infected then by birds. You also get poor little colds, puppies and seals also can be infected sporadically by viruses that have come from particularly from pigs or maybe even from humans or birds. So all of these, all of these species have influenza, even though this is a natural reservoir, the wild water fowl, you do have large influenza outbreaks pneumenatic outbreaks in birds, in domestic chickens and then in, in humans with avian influenzas, and then you have influenza viruses that have been in the human or pig population or a horse population for so long that they've become essentially viruses that are restricted just to those species. So the human influenzas, they are only infecting humans at that point, for the most part. And pig influenza, swine influenza is just affecting swine and so on, and they're not going back to birds, although there is evidence that it can still cross some species various. So we'll get into the microbiology of how this all happens.



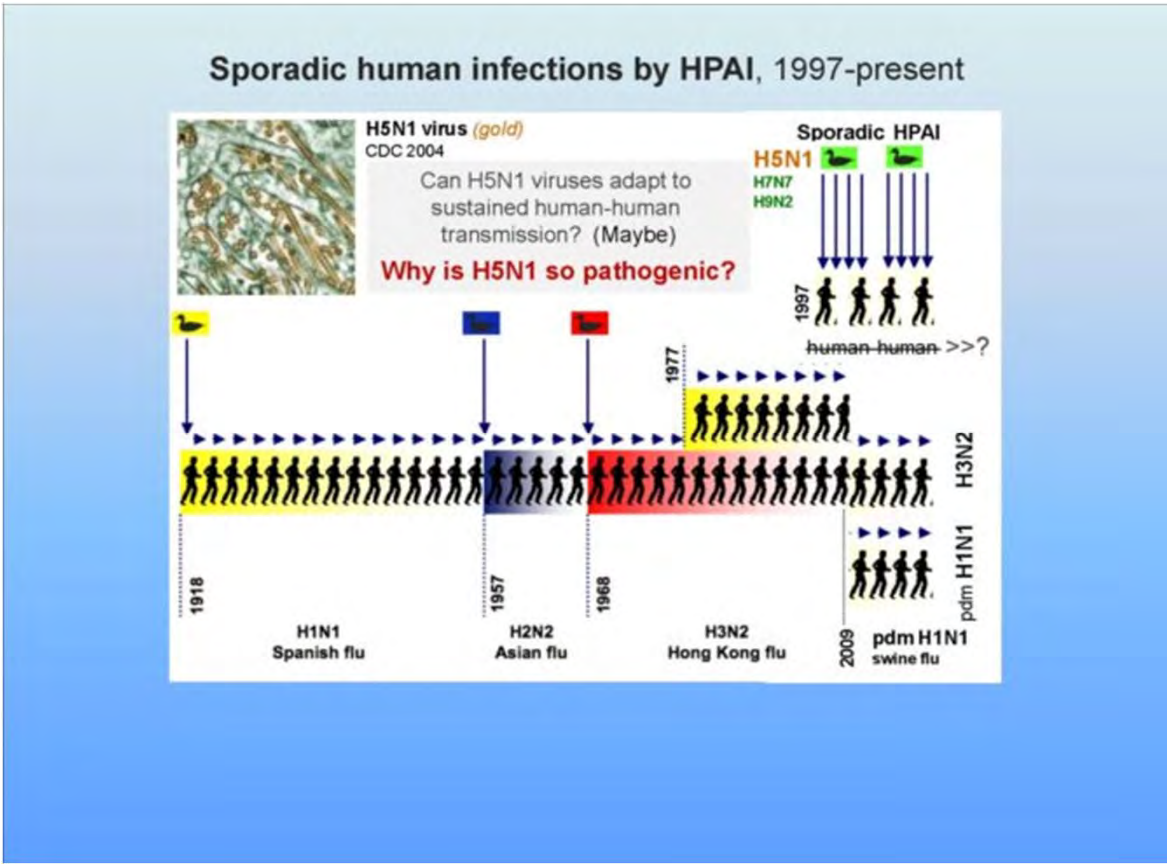
So, typically, in the human population, a new subtype of influenza A virus; there's 3 types of influenza: A, B and C. So A viruses are the ones you probably heard most amount, about, that cause these pandemics, HD, bird flu virus, all the avian viruses are A type influenza, have the greatest diversity. These viruses which have a contribution from avian genes will end up in human population which is immunologically naive, and cause a pandemic. Often the pandemic like in 1918 which killed 20 million people, the Asian flu and the Hong Kong flu in the 50's and 60's which killed 1 or 2 million people each, well, are pretty lethal. And they all can not only contain genes from avian influenza viruses, they are capable of transmitting from human to human and eventually become human flus that they're just capable of infecting humans and no longer really part of the avian reservoir. So they're restricted at that point to human beings, and they become seasonal flus with less pathogenicity. It happens maybe for 2 reasons: the virus becomes less pathogenic; the viruses survive in the human population, and also the human population as it experiences it longer becomes more immunologically competent, more resistant to that particular subtype of virus, so H1N1, you know, lasted until about 1957 and basically disappeared. In '68 you had H2N2 displace the H1N1, '60, I mean '57-'68, you have H2N2 and so on. And H1N1 has this curious capability of coming back, like it did in 1977; H1N1 reappeared and most of the population, except for people of a certain age; people over a certain age still had immunity to one of these earlier viruses, but people who were born in this period do not.



And in 2009, the same thing happened again with the swine origin. Or the pandemic H1N1 came in from an outside species, in this case, directly, probably from swine, but again, with avian gene segments in it, which I'll go into in a little minute, in a little minute what those gene segments are, but they went into the human population and then you had a new pandemic virus of which, to we had little resistance. And for some reason, resistance to this H1N1 over here, the seasonal one, wasn't sufficient to block infection to the new pandemic, so we have 2 strains now in 2009-2012 of influenza in the human population. You also had, which is not at the bottom of the slide, but there is also an influenza type B, which is just restricted to humans and maybe was an avian virus many decades or centuries, maybe several centuries ago and became restricted in human virus, the human population. Nobody's quite sure, and influenza C is the same thing. So this is basically the epidemiology of influenza.

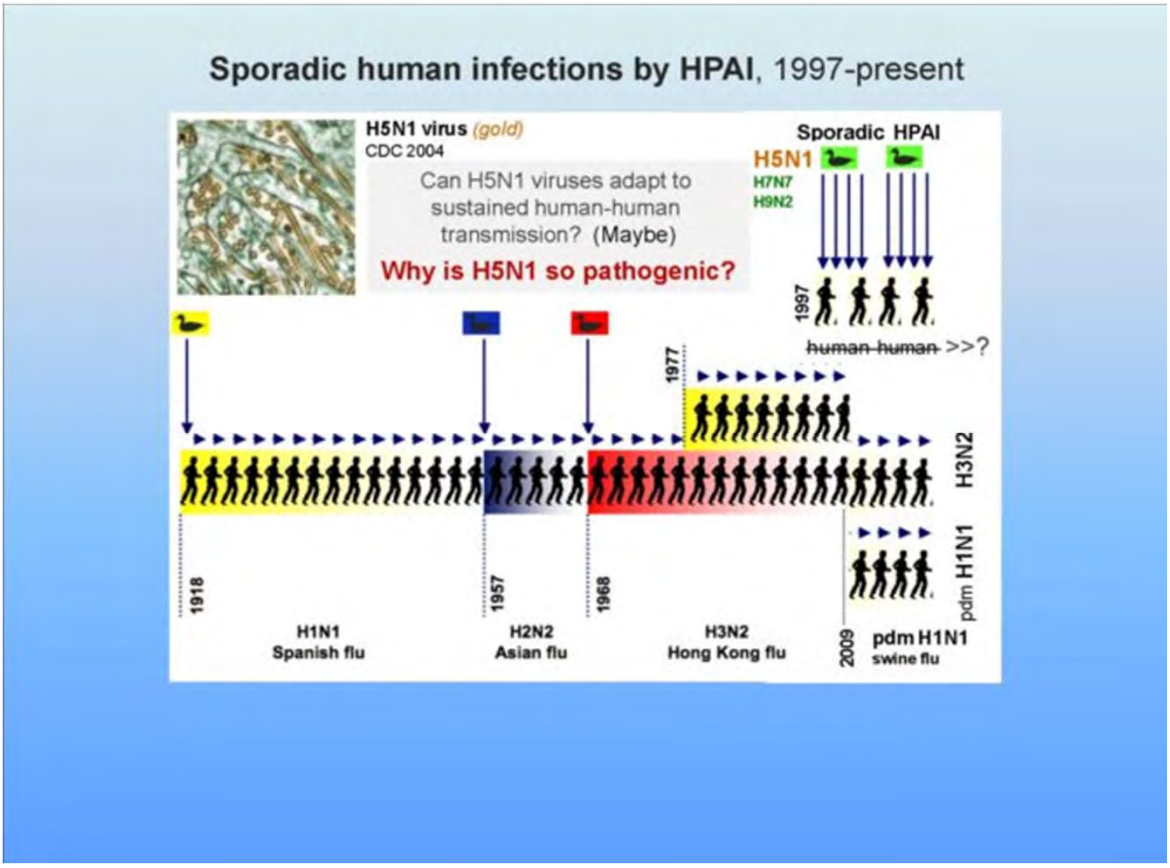


Now high path flu has only caused high path avian influenza, H5N1 subtype, for example, has only caused sporadic infections since the Hong Kong incident in 1997, and continuing into today, where you have these avian viruses will infect human beings and cause infections that are restricted to one person at a time, or 1 or 2 people who are directly working with dead birds, cooking an infected chicken, for example, slaughtering and cooking an infected chicken from a live bird market, but the virus is not capable, seemingly, of spreading from person to person to person to person, and thus creating epidemic or a new pandemic strain. It has not done this yet in nature. But in laboratory confirmed cases, it is a very, very lethal influenza virus. Case fatality rate is about 59% out of 500 and some laboratory confirmed cases of this infection. We don't know, maybe the denominator is a lot larger in this equation and there have been a lot of these symptomatic infections, but the evidence on that is pretty weak at this point. Most influenza, even the 1918 pandemic which killed, like I said, 20 million people, had a 1-2% mortality. So this thing may be 10-20 times more lethal than even the 1918. But, on the other hand, it's really difficult to say because we don't know the number. We do know that it's a high path virus. This is a electron micrograph of the virus, they're from MDC case files from the CDC.



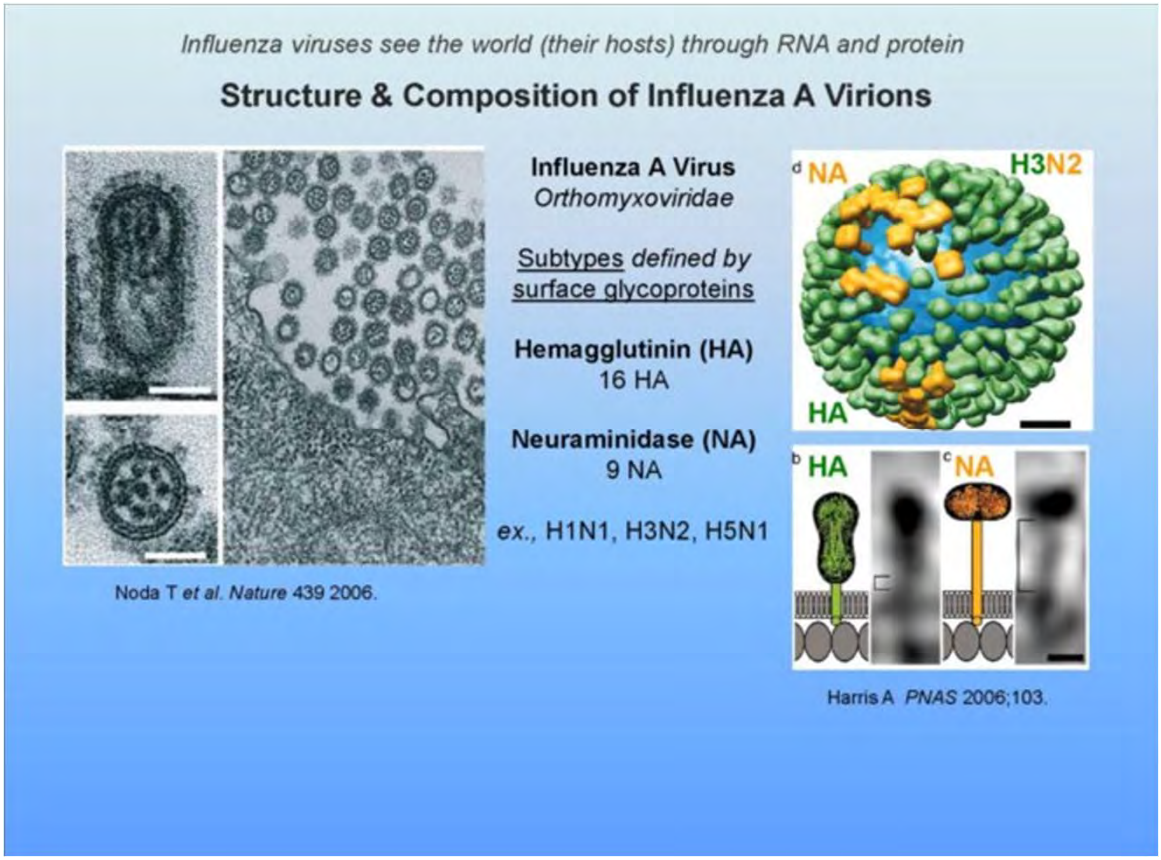
So the question, the questions that are raised, 2 important questions among the many questions you might ask about H5N1 and these high path viruses is: one, can H5N1 viruses adapt to sustain human-to-human transmission or sustain to mammalian to mammalian transmission as opposed to just birds? Can they infect one person and then pass on to the next? Because if they can, then we may be dealing with a virus that's capable of becoming a pandemic; and second, why is it so pathogenic? Seasonal flu doesn't seem to have a 50% mortality rate; why does this virus have one? Okay, so the first question, I'll only discuss very briefly because maybe you've heard about it, and I don't really focus my research on transmission, but you've heard about it in the news lately maybe. And the question is: can H5N1 the experiment to do that will address this, can H5N1 transmit from mammal to mammal in the lab? And there are 3 models for influenza virus research animal models, 3 dominant animal models: the mouse, the guinea pig and the ferret and the mouse is good for pathogenic studies. You see symptoms, you see disease symptoms, but mice don't transmit flu between themselves, it's just, haven't been able to accomplish that in the lab. The guinea pig is the opposite. The guinea pigs transmit flu from one guinea pig to the next, but they don't get sick. You give them a very highly pathogenic virus, they barely get sick and they don't die. So they both have their advantages for certain kinds of studies. The ferret, on the other hand, seems to replicate human influenza viruses pretty well, so does the H5N1? Can you find in H5N1 either in nature or by making slight change, genetic changes to it in the laboratory that may occur in nature that would be able to infect one of these species and cause both pathogenesis and transmission and Yoshi Kawoka at U of Mass, University of Madison, Wisconsin, Madison. He's also got a lab in Japan, had a pretty interesting point about the rationale for this kind of research, which is to understand the

molecular determinants…

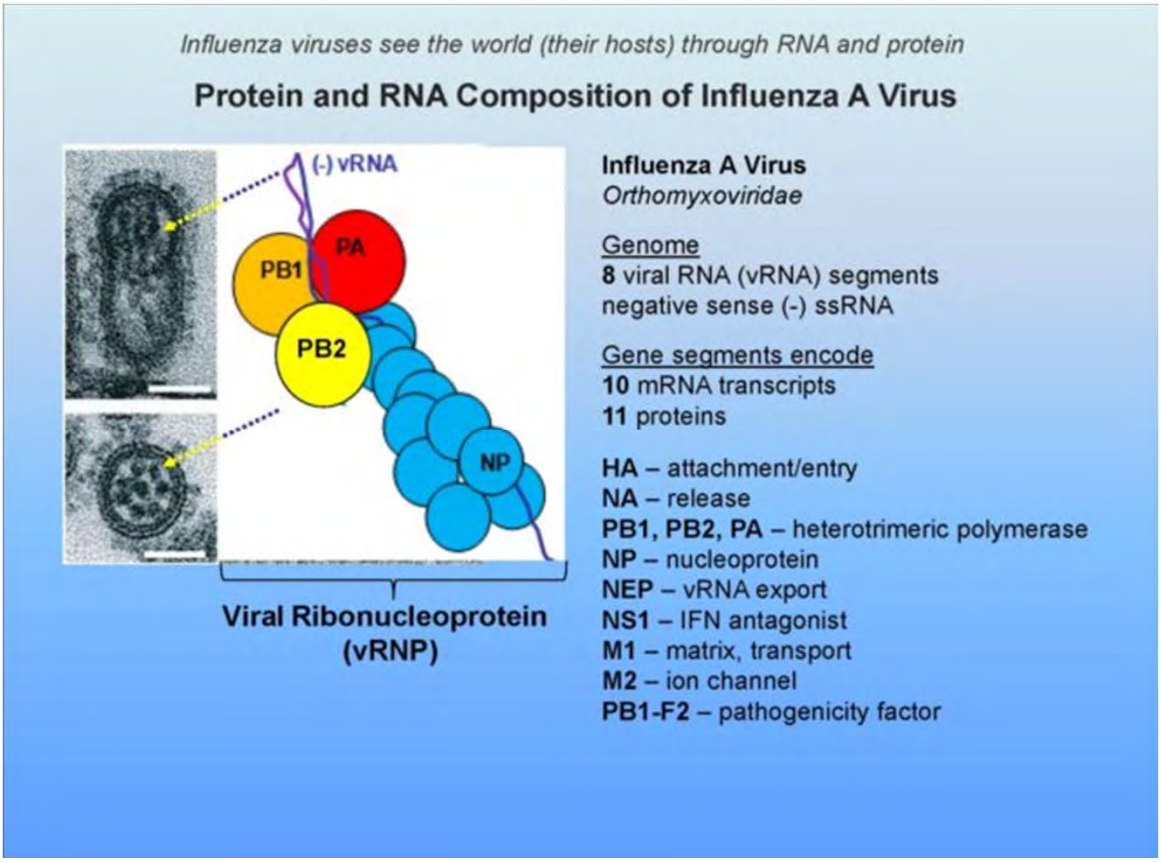


…that would allow this to happen because nature is conducting this experiment in southeast Asia. There's been 500 something cases that's were recorded, right? So we need to know. Preliminary studies by both Yoshi and another group in the Netherlands, Ron Fouche has suggested that yes, H5N1 can mutate to retain high pathogenicity and acquire the ability to transmit between ferrets. This is unpublished and I know there's been a big debate in the media about this, about whether it should be published at all. And I'm not going to say really too much more about that other than that now, I'm really afraid of these viruses. I'm not, [laughter] not because they're going to escape from the lab, because they may be able to do this, and like I said, nature's conducting this experiment. So I'm worried about what's going on out in the world with this and really, it's important to know what are these molecular determinants. But this was part of the reaction scientists developed new strain of H5N1 that could kill millions, so, and then there's a lot, a lot of debate about censorship and this is...I work for Adolpho Garcia Sastre [phonetic]and Peter Polizzi [phonetic]at Mt. Sinai School of Medicine. Adolpho is my post op mentor and I'm still affiliated with this group as a research assistant professor and he's the department chairman and they're taking a very reasoned approach over this issue and about what's called the schism by thumb about whether, you know, how this research should, should progress. You know the influence of virus in eggs. You can grow laboratory strains or low path strains with, in a BSL2 or a regular tissue culture hood environment that you make sure has a good HEPA filter, or something like that. When you're dealing with high path viruses, you use higher containment, even up to BSL3 or 4. For example, for this experiment is conducted in BSL3. There are a lot of safeguards on the laboratory level for doing it. Okay, the second question is what I study fortunately from a media perspective

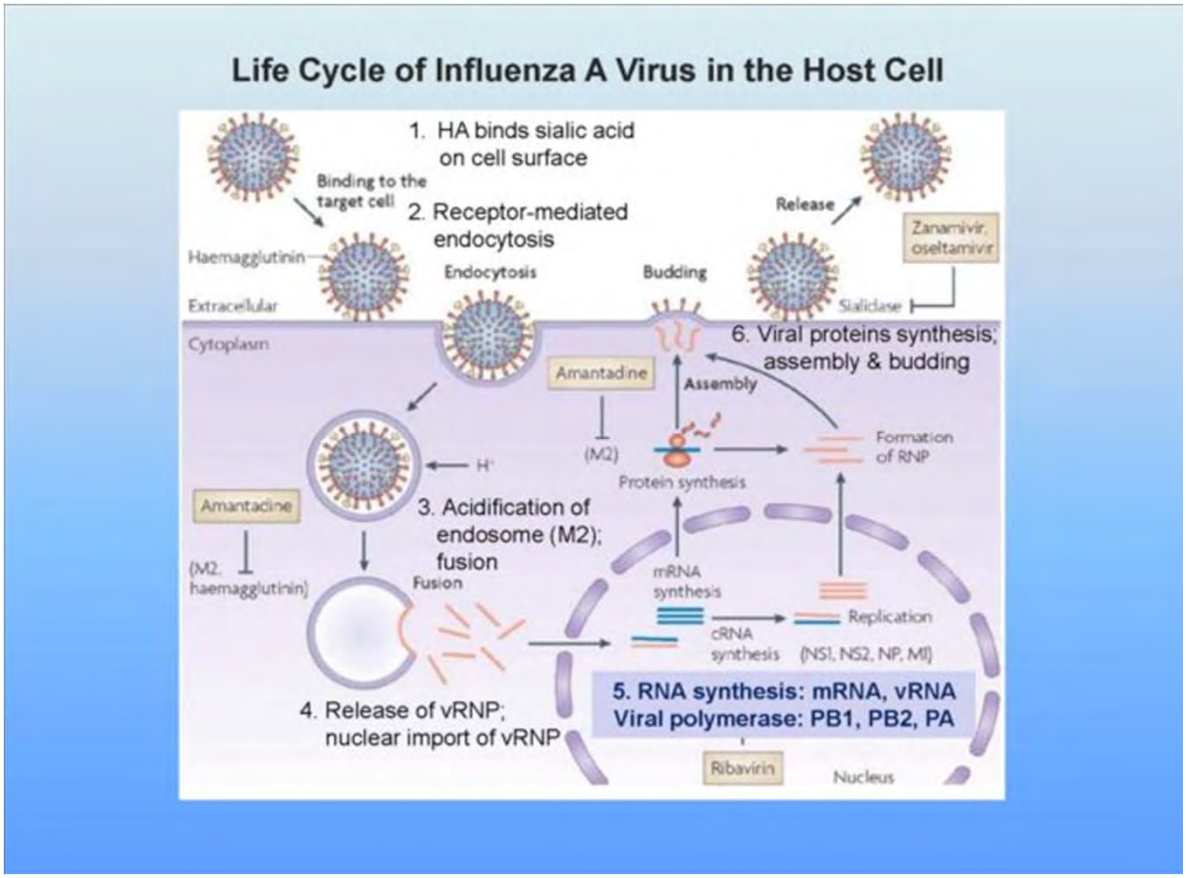
and that is, why is it so pathogenic?



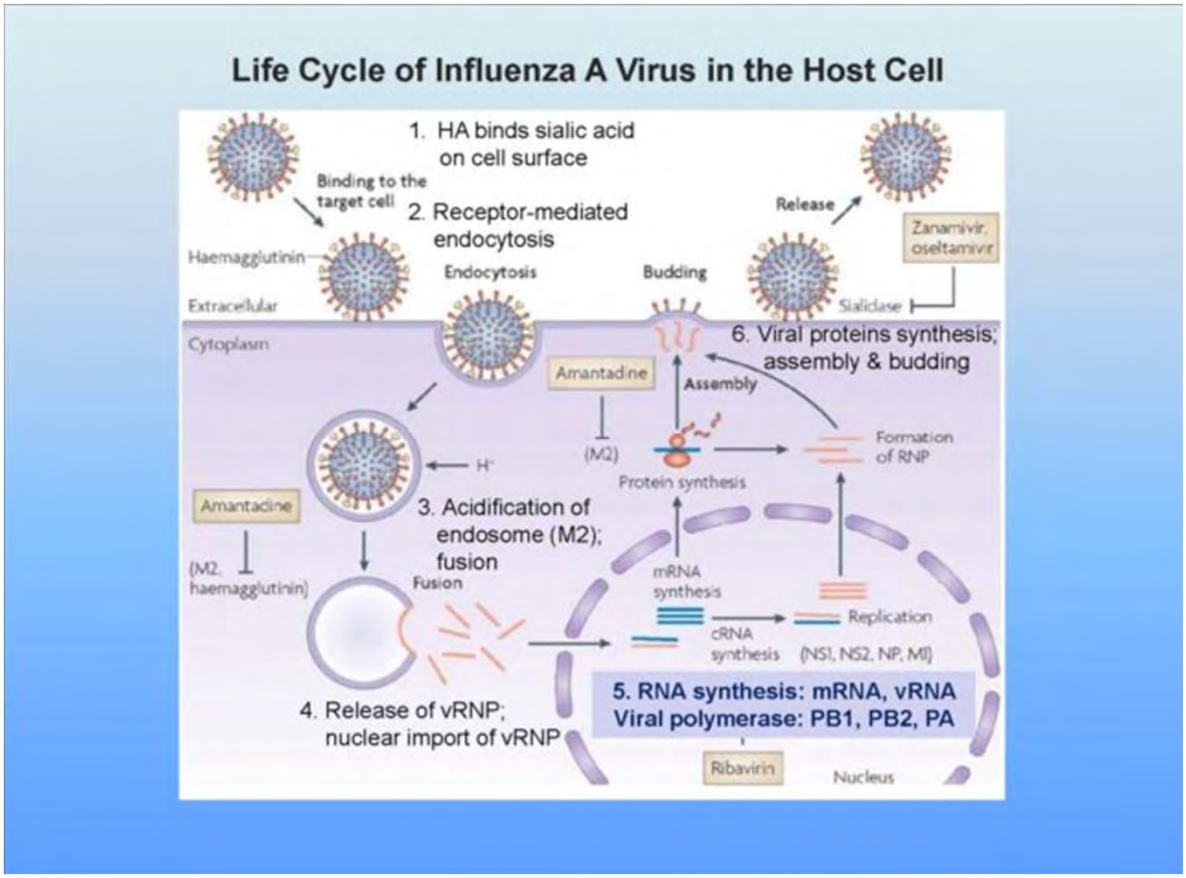
Why is H5N1 have such a high rate and to understand this, we're going to look at a virus and we're going to look at influenza through the way it sees the world, which is its host and that's through its RNA and proteins. And influenza are a mix of orthomyxoviridae, they're a subtypes defined by the surface proteins in [inaudible]. The hemagglutinin in the HA or 16 different kinds, 16 different serotypes and there are 9 different serotypes of the neuraminidais NA, these two proteins can be seen on the electromicrographs of the virus, so this is from the top, see these like, spikes on the outside. You see them here on the side as well. And then if you get in kind of a closeup, you'll see, this is a 3d structure of the HA of the NA, superimposed on the TEM images, which are not very high resolution at this point. But we're talking, you know, sub, very, it's about 3 nanometers as it is. So, these, these 2 proteins are the outer coating of the virus on a, over the lipid envelope and they are what interacts with the cell, they're what's seen by the immune system and they are what's defined as subtypes of the virus. They have a lot to do with the immunological characteristics of the virus.



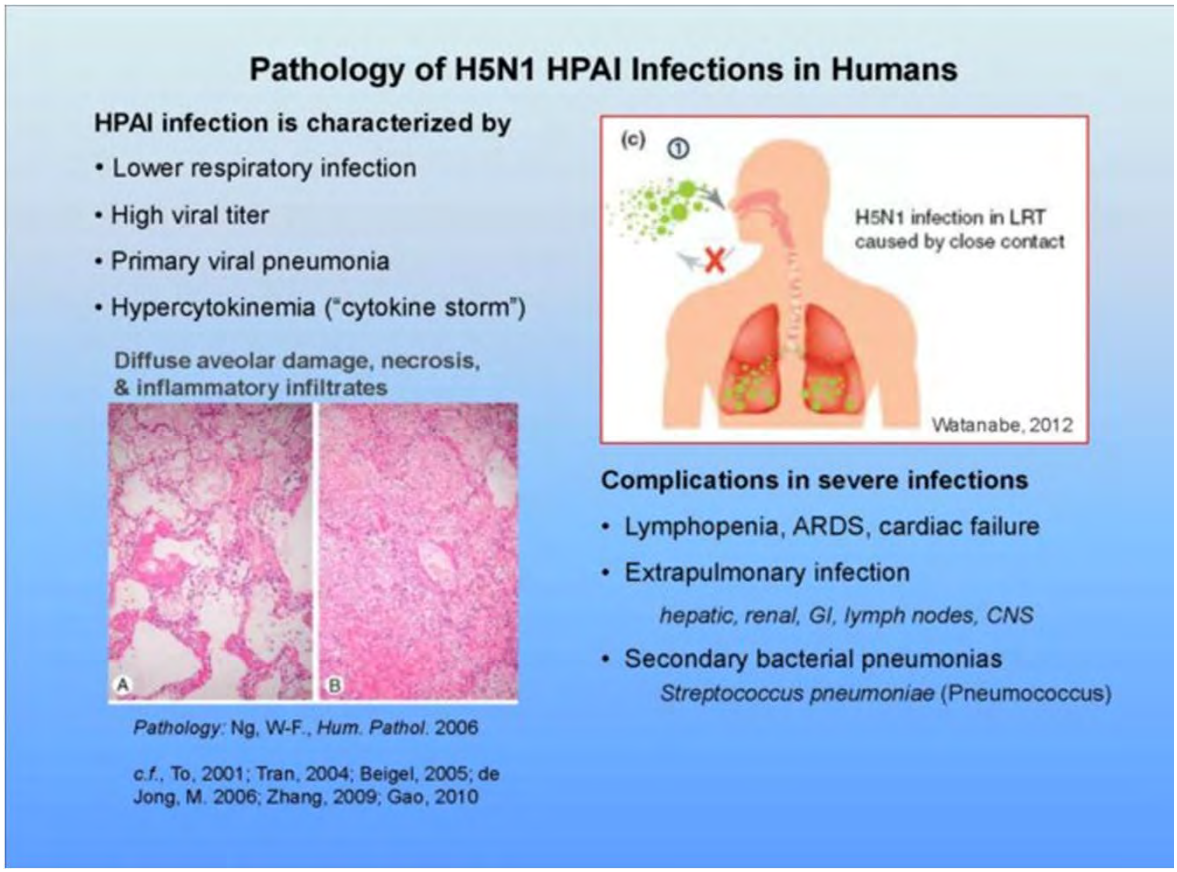
And then the inside, there's RNA and protein, the viral RNA genome segments. There are 8 segments in influenza; there are negative, negative single stranded RNA so they encode, they encode both viral proteins through a number of transcripts, the viral proteins are here. And they also are the viral genome and they are these dots, basically, on the inside of the virus or from the longitudinal perspective, you see these long filaments of these RVNP, rivonuclear proteins, which are the viral RNA, covered by the nucleoprotein, and there's also a polymerase complex, the head of trimetric polymerase PB1, PB2 and PA which are bound to the, bound to the ends of these VRNP segments. So, each influenza virus has 8 of these, give or take; there's some you'll find 7, some have 10, you know, because they'll have some duplicates, but generally, they have 8, and you can also see that if 2 different viruses are infected the same cell, they might be able to mix and match these and get reassortments, which does happen in nature and a lot of pandemic strains we see include reassortments, so they'll switch these out, 2 different viruses can switch these segments out, I'll take your HA and I'll give you mine, basically. All right. So the proteins are the HANNA are involved in attachment, entry and release, the [inaudible] is involved in replication of the RNA, the nucleoprotein is involved in RNA basically RNA interactions and transport, then there's a few others; there's an interferon antagonist, which blocks cellular interferon responses, we'll say a little bit more about that in, in a minute. There's ion channels, a matrix protein, which is on the inside here, which you can't really see on these diagrams, and export protein, and another passage in the [inaudible] called the PB1F2, which is a tiny little 90, 90 amino elastic protein, which seems to have something to do with the pathogenicity of the 1918 virus. Although it's not clear that it really is a factor for the HPAI, the H5N1, which is really unusual.



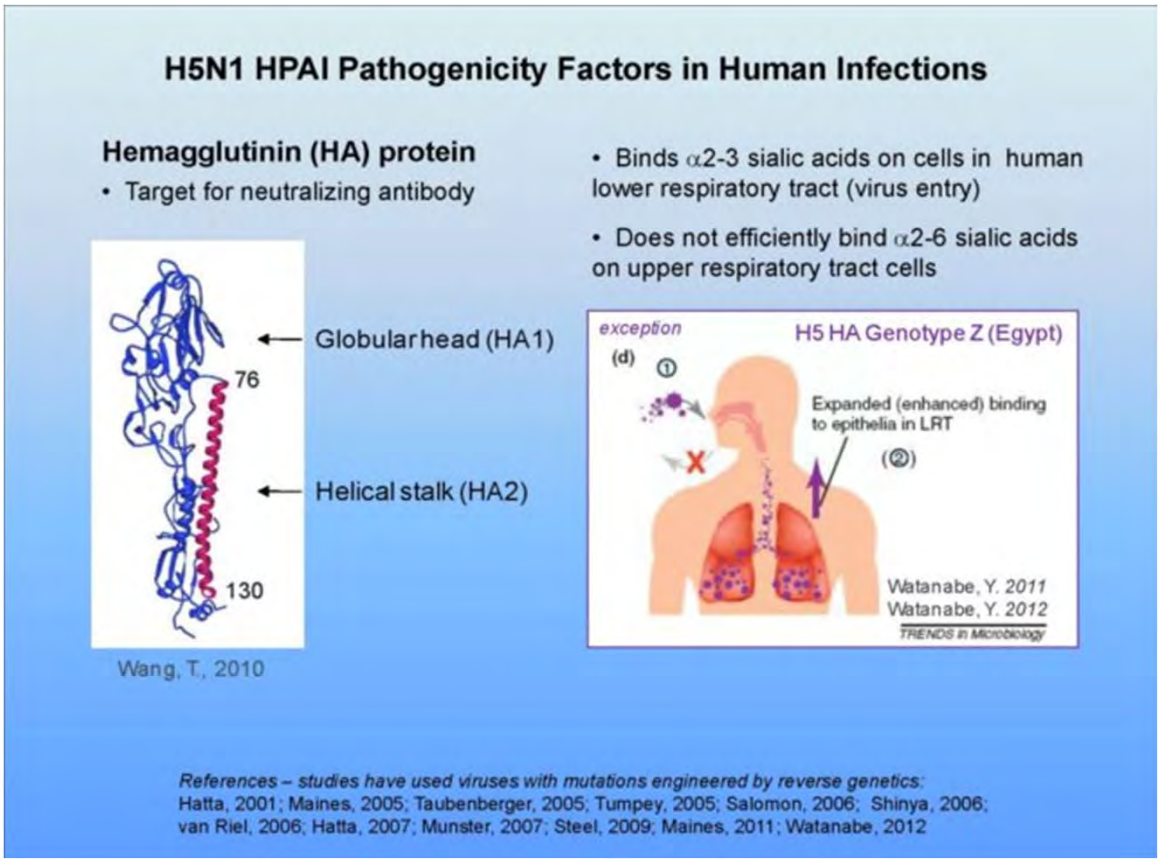
Okay, so the way to understand how these proteins interact with the cell and to understand pathogenesis is to look at, take a look at the basic virus life cycle. So maybe you've seen this before. It's much like in any virus; you have entry of the virus, replication of the RNA and release of new virus. Okay. And some of the viruses might have steps where there's integration, there's long term survival in the cell influence, it's acutely infecting, it goes in and makes the cell, it reprograms the cell into a virus production factory, releases virus and the cell dies. There's no latency, there's no lysogenic phase, like there would be in a phage, it's just a simple, a simple infection. So the HA mediates binding and entry and receptor mediate receptor endocytosis to the cell, the [inaudible] acidify with the help of the M2 protein, which is an ion channel, the acidification results in a change in the HA structure, which creates, which results in fusion of the envelopes in the enzyme and releases this inverts the inside of this fusion of the envelope and hurts the inside of the version releasing the RNPs which are magically transported into the nucleus, with the help of important proteins, but it's not really understood. And inside these VRP's are, and inside the nucleus, the VRNPs are acted on by the polymerase, which they bring a little bit with them and they produce more to produce viral messenger RNA producing viral proteins and also to replicate viral, viral genome, VRNA through a CRNA intermediate, which is a positive. So in a negative sense, we are [inaudible] in a positive. I'll show you that in a minute. And the polymerase is responsible for this. And then, did I go back, yeah, and then finally you get viral proteins essentially, eventually accumulate these viral RNAs are exported from the nucleus which is, involves the M1 protein and the nucleo X4 protein and NS2 and you assemble all the components at the plasma membrane and bud new versions.



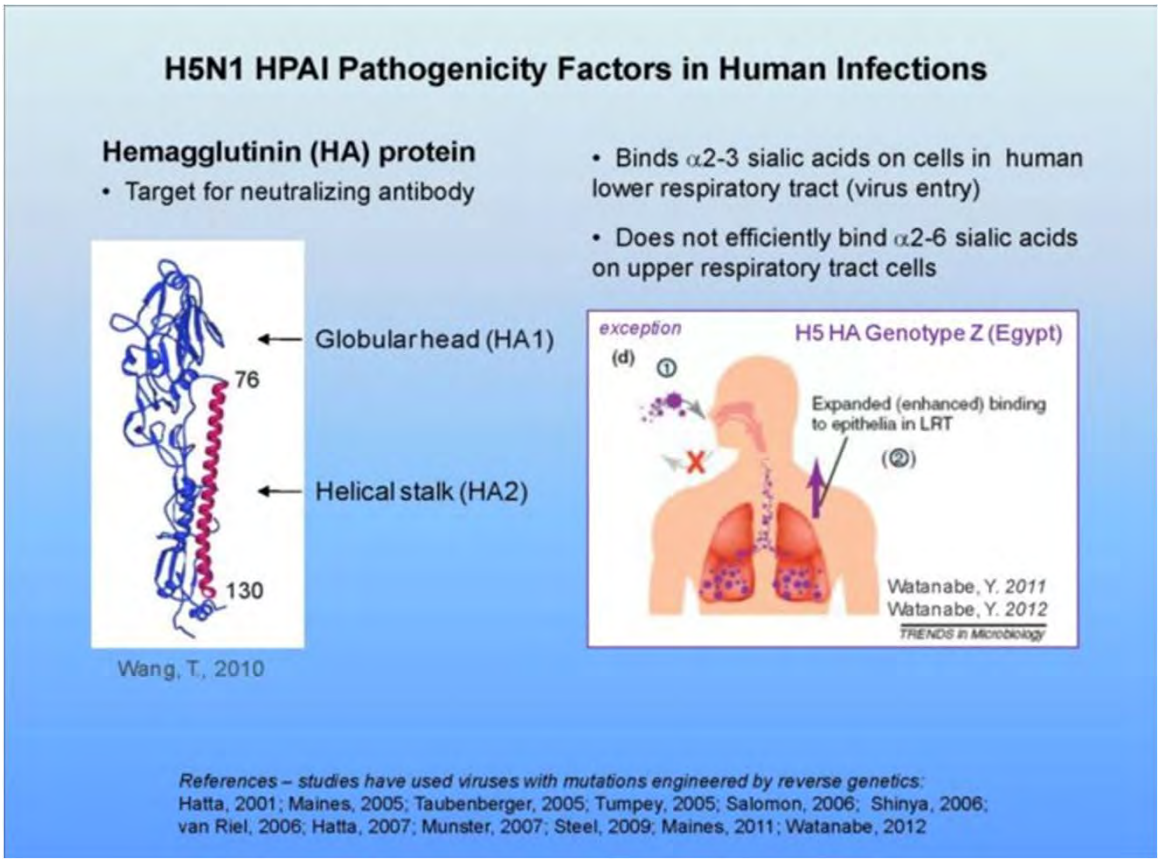
And there's some antiviral drugs that will block specific points in this process. For example, riboviron will block the synthesis of RNA and Tamiflu or Oceltamivir also blocks the maturation of the particles by blocking the neurominidase, which is required to release these, these particles from the surface of the cell, so they're still stuck to the surface because of these transmembrane interactions and neurominidase cuts this, is a, cuts the cialic acid where cellular carbohydrates and releases new virus.



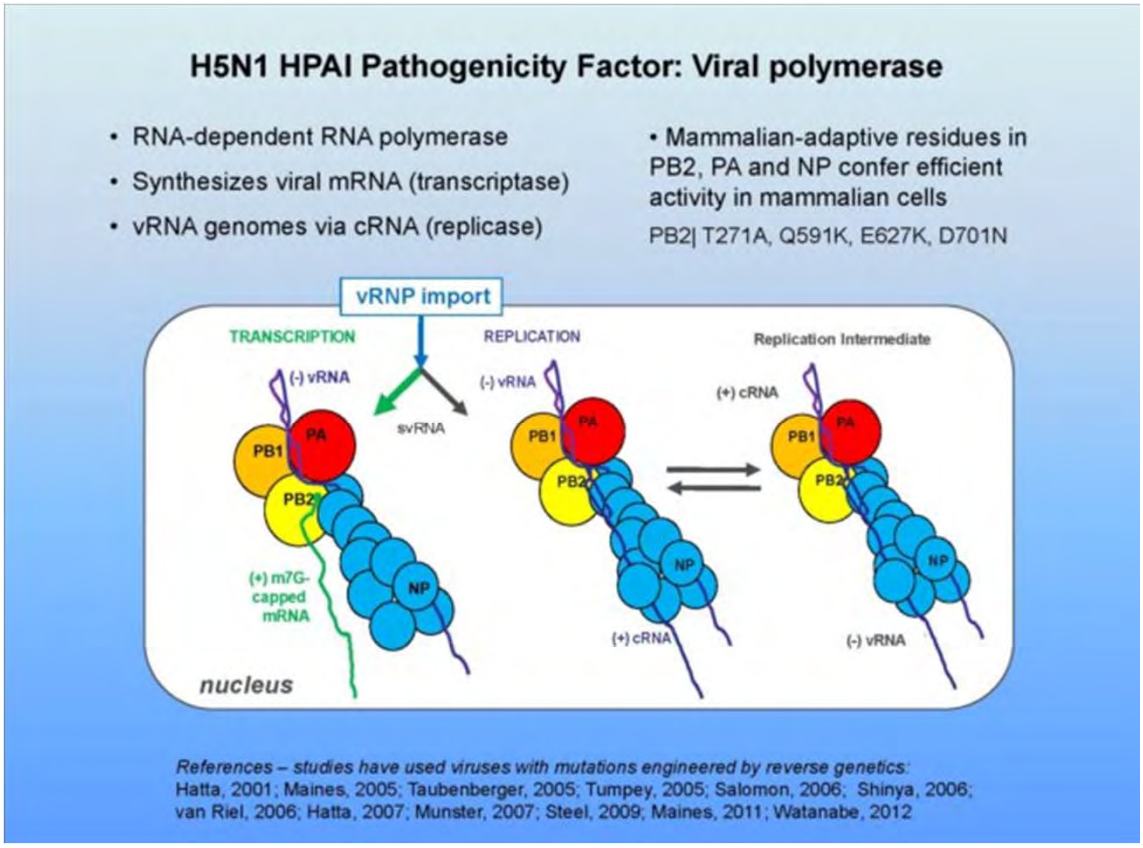
Okay, so when H5N1 successfully does this, and infects a human being, this is what it looks like. Like I said, it's a pretty severe, lower respiratory infection, has a primary viral pneumonia, because pneumonia in and of itself, and also has what they call, or what's been called cytokine storm, a very high bearing production of proinflammatory cytokines and hemokines which seem to result in immunopathological damage, so to speak. So you get a lot of cellular infiltration into the lungs; lungs should not look like this; lungs should have lot of wide open spaces; they should not be completely filled with dead and dying cells like this infiltrating immune effecter cells. So, the H5N1 does this in a lower respiratory tract interacting with the [inaudible], but it is not at present capable of really infecting the upper respiratory tract or transmitting from the lower respiratory tract into a human, as we said before, at least in present form, although, like I said, those experiments in ferrets suggest that it may have the capability of doing this with a series of mutations. But in, there are sometimes severe complications and this contributes to the death rate in H5N1 and other influenzas as well. 1918 has a very similar process, although you can see more cell death in 1918.



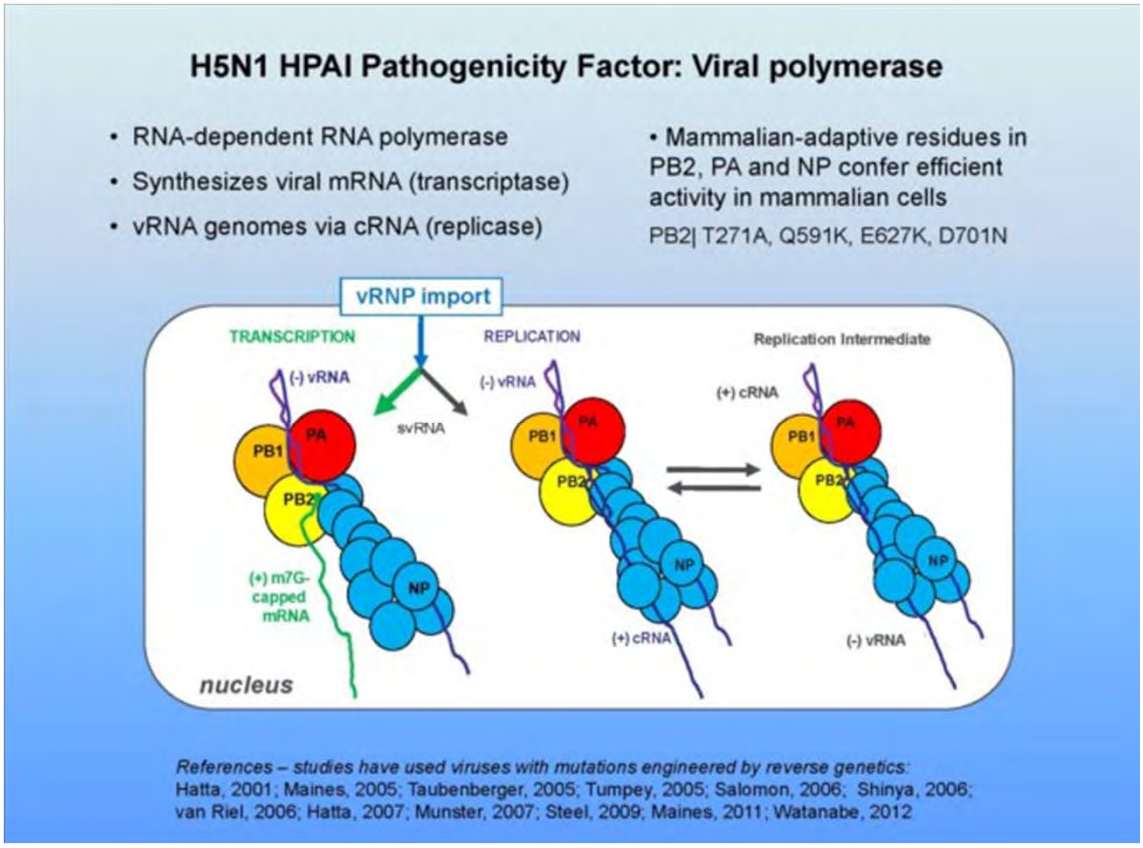
So, the pathogenicity factors, I'm just going to into the 2 of them here that are responsible for this H5N1 here, are the hemagglutinin, the HA, it's on the outside and the other one is a polymerase. The HA protein is a target for neutralizing antibodies. Neutralizing antibodies produced by your body will bind and block the globular head particularly of the HA protein, preventing its interaction with the host cell. It interacts with carbohydrates on the host cell called the alpha 26 sialic acids or alpha 26 or alpha 23 sialic acids on cells, this serves as a receptor for this cell and a neutralizing antibody will actually block this. There is a second domain called a helical stalk. This is more conserved and it's thought this is going to be a good vaccine target, so new generations of vaccine are attempting to target the helical stalk of the HA protein and thus prevent, prevent, maybe, and since these are more conserved, maybe prevent a wide variety of influenza strains with, with, with just one vaccine, because currently, what happens is the globular head has a high level of mutation, a high level of glycosylation and every year, there are variants of the existing strains that have mutated enough to escape immune surveillance, escape antibody and thus you have to get a new flu shot every year. You don't have to get a flu shot, but a new vaccine has to be formulated to be effective against the current, current strain. So, and there is also some indication that in nature that the binding of the H5N1 to the sialic acids in the respiratory tract can expand a little bit from alpha 23 sialic acids, which are cells on the lower respiratory tract, to alpha 26, which are throughout the respiratory tract, including the upper respiratory tract, and maybe that's going to have something to do with the possibility of human, human transmission, but more research needs to be done on this. I might say that maybe you've heard previously that 23 sialic acid, if you heard or studied influenza 4, is confined to birds; alpha 26 is confined to humans and both are in pigs.



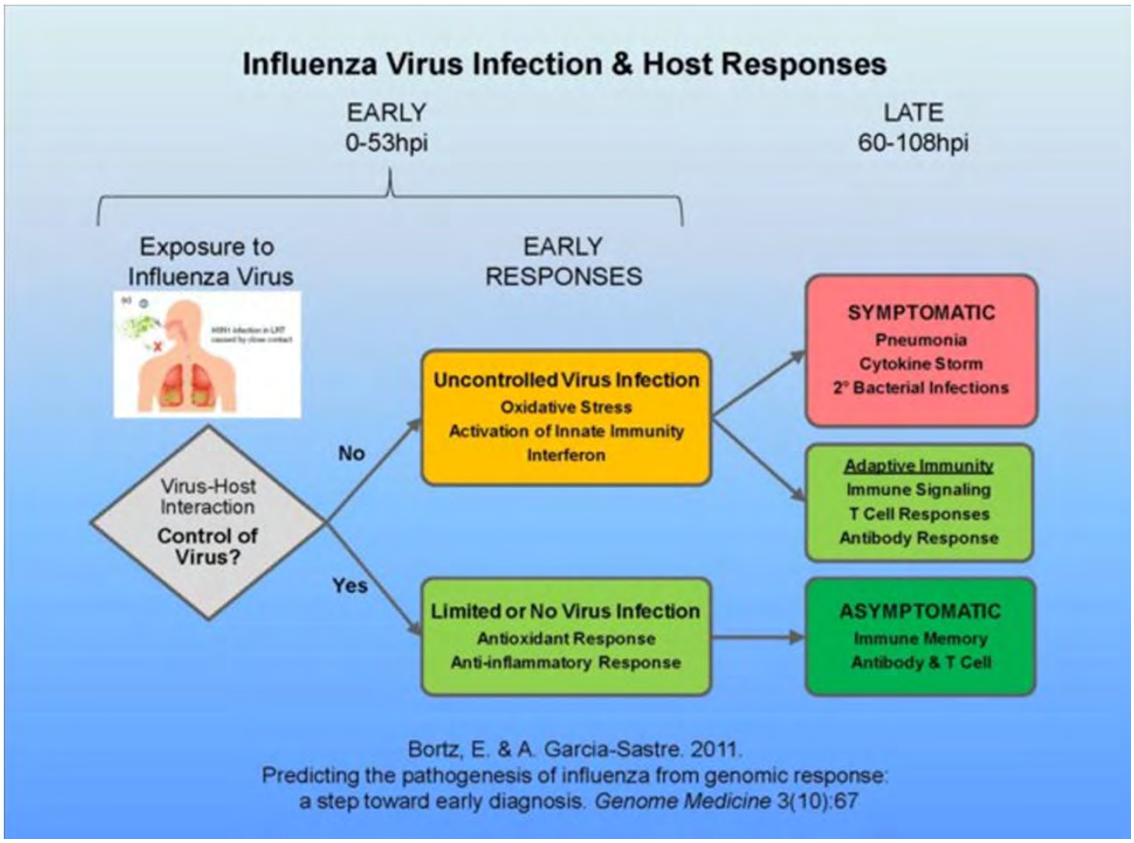
So avian viruses infect humans, they don't infect, they can infect pigs, but they won't, no, avian virus will infect birds and pigs, but not humans because they can't interact with alpha 26. That's all actually not true. None of that is right [laughter]. That's, it's sort of a dogma, I think the pig is back in the equation with the swine flu, but humans have actually both of these receptors in different cells in the lungs. Type 2 pneumocytes , for example, have 2. So that's why H5N1 seems to be able to infect these cells. If not, then we wouldn't have H5N1 infections.



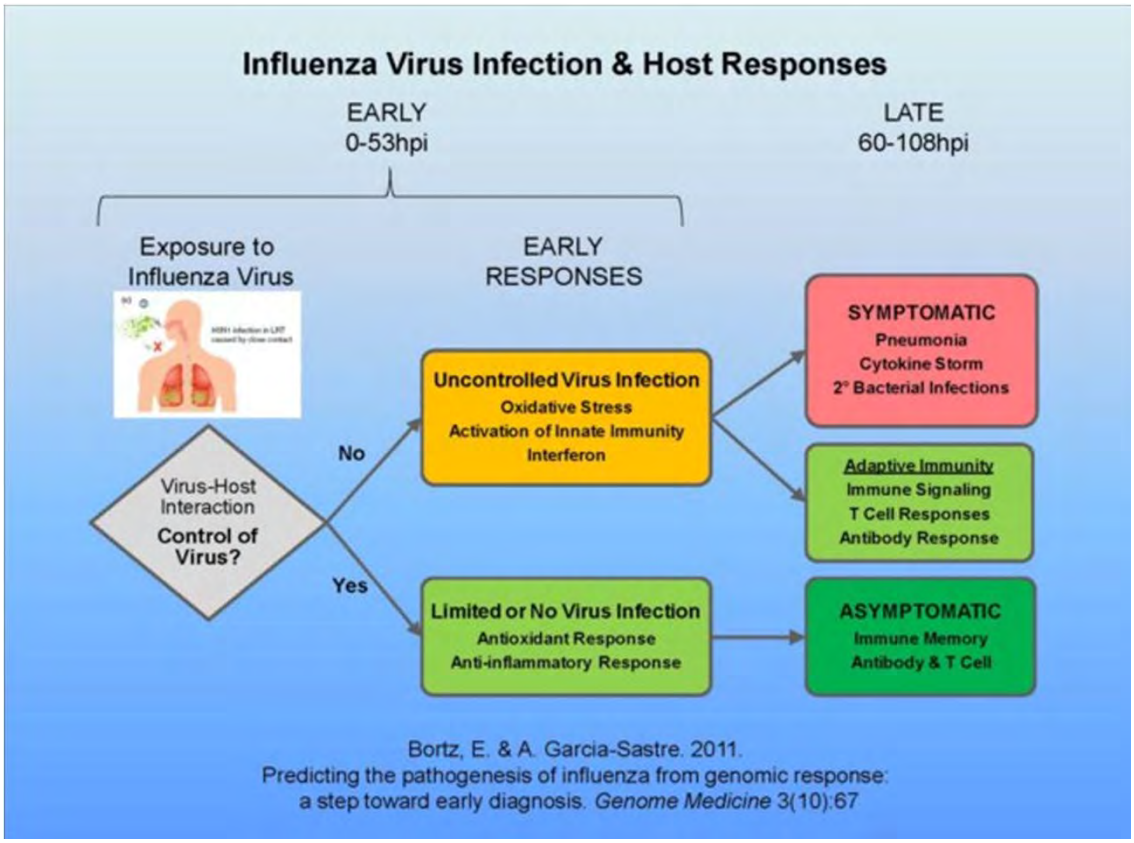
The other factor, the pathogenicity which I focus most of my research on is the polymerase and the polymerase is an RNA dependent RNA polymerase. It synthesizes, it acts as a transcriptase, synthesizing viral MRNA and replicates synsethizing viral complimentary RNA and viral RNA. So inside the nucleus where this takes place, the RNP's are imported and you either get MRNA, either the preliminary synthesized MRNA, which is a 7 cap RNA, it steals the caps for the MRNA from cellular transcripts through a cap-stealing or cap snatching mechanism, maybe you've heard or studied that in the past. Or it creates full length CRNAs, which are then copied back into full length VNRAs, replicating the entire genome. This is not capped. This entire viral RNA is, it's not capped or polyadenylated like the message RNA. There is a switch between these 2 processes. Who says the preliminaries have to make messenger RNA or make VRNA through the CRNA intermediate. Right? Well, there's some indication that there is a small viral RNA that corresponds to 1 end, 3 prime end of the viral genome and maybe it binds and biases this process towards one or the other. So there's, there's some evidence that is possible also, host factors or other nucleotide availability for example, may, may have something to do with this process or the amount of NP. If you get enough NP in this cell, you start to close down the message RNA and you start to replication. This is very clear, this switch, in other viruses, for example, in bacteria phages, but in fluids, still a little bit mysterious how that happens. Okay. The other thing to note is that influenza, avian influenza, H5N1 does not , if you take this complex and you put it, you clone it, you put it on plasmas, you put these 4 proteins viral RNA, you put in the mammalian cells, it just doesn't do this very well. Avian, purely avian viruses don't do it very well. They have a low efficiency in it. Chicken cells are fine. Works great. Does not work very well on mammalian cells without certain mutations.



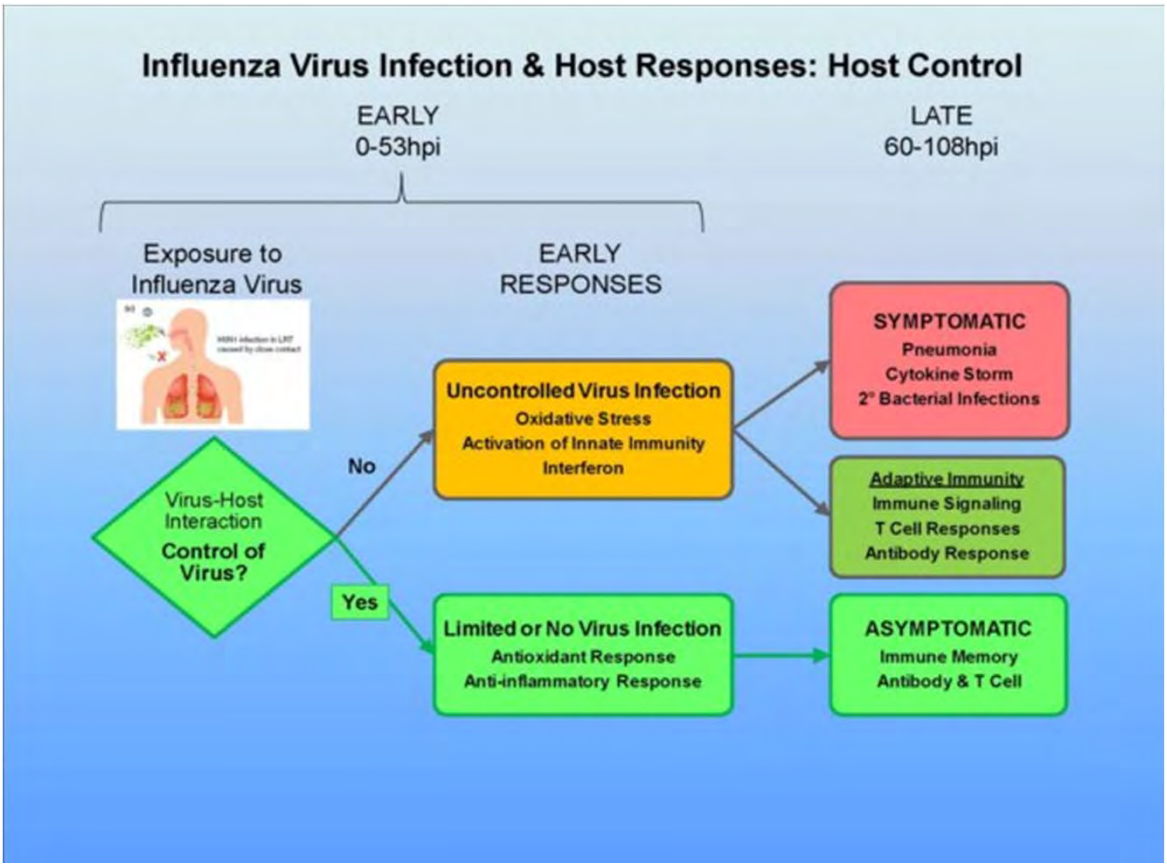
Some of these mutations have prominently been found are in the PB2 subunit of the polymerase, for example, 627701 and these are mammalian adaptive mutations efficient activity in mammalian cells. I say mammalian because experiments are done, for example, in human cells, but then there are in vivo experiments done in mice but this really, mammalian cells in this case seem to be that human and mouse act very similar with respect to the polymerase, although as I said before, not with respect to things like transmission like HA. Okay, so in mammalian cells, you have mutations in H, in avian influenza polymerase subunits that confer high pathogenicity, high levels of RNA replication and these are important for all high path avian influenza, according to sequence studies, for example.



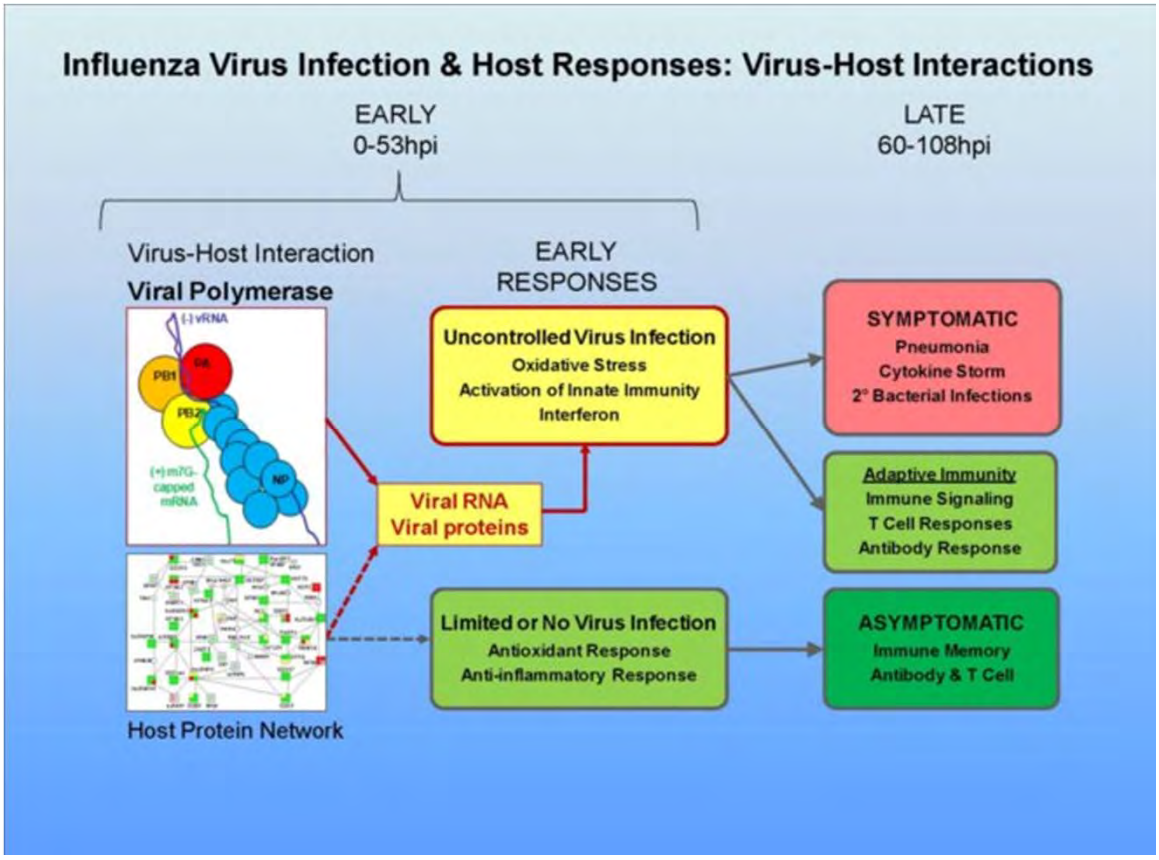
Now when the virus infects the host, one of the things that, one of the, one of the, one of the questions that I sought to get at is: how does this infection, this decision happen, whether you're going to have a high level of replication or, for example, for the avian polymerase, it's not a very high level of replication, right? And we know what the HA does. But it, it's apparent that, for microerase [phonetic]studies, for example, in humans or in mice, if you take them, you give them virus, some infections go very well and some infections for the virus don't go very well. So it seems to be an early control process in the cell. It may be very intrinsic to some replication mechanisms that are happening inside the, inside the cell, and whether the virus is able to, to, to efficiently replicate, leading to disease or leading to no virus replication. In the case that you have host control, you have very limited virus replication, the virus, for example, if you take the flunaise, the flunaise vaccine, it's actually an attenuated virus; it's cold adapted. It works in the upper respiratory tract in the nose. Once it goes down in the lower respiratory tract with higher temperature, it no longer functions. So it's a simple temperature depending control. You get limited virus infection; you get anti-inflammatory, anti-oxidant responses, according to microrace [phonetic]studies, and, but you do establish a memory response. The body does see the virus and creates a new memory antibody and t-cell memory. So this is in the case where you get either a very weak infection or early host control. On the other hand, if you do not, the virus is able to enter the cells and successfully start making enough viral RNA and protein, it's not really clear in vivo how this happens, what controls this, but you do see uncontrolled virus infection, at least for a period of time until the immune system can kick in, and this is characterized by oxidative stress and activation of innate immunity and interferon responses.



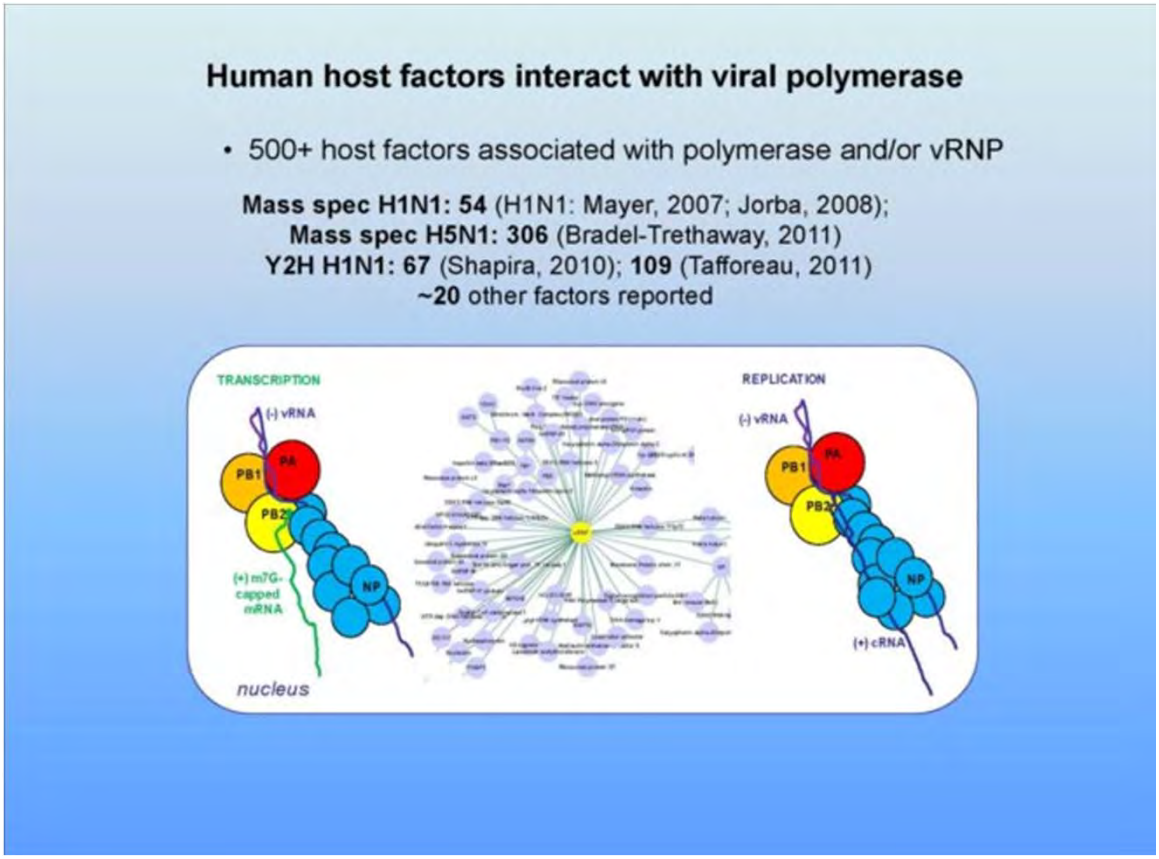
The, these will lead to a symptomatic infection within pneumonia, cytokine storm, I said in the case of H5N1 secondary bacterial infections which have caused a lot of disease in past pandemics, like in 1918, I was talking about that this morning. Somebody mentioned that this morning, that's interesting. Also, the adapted immune response kicks in. You get immune signaling t-cell responses and antibody responses. Also in this case, which you're not only going to go back and control the infection but also lead to a memory response.



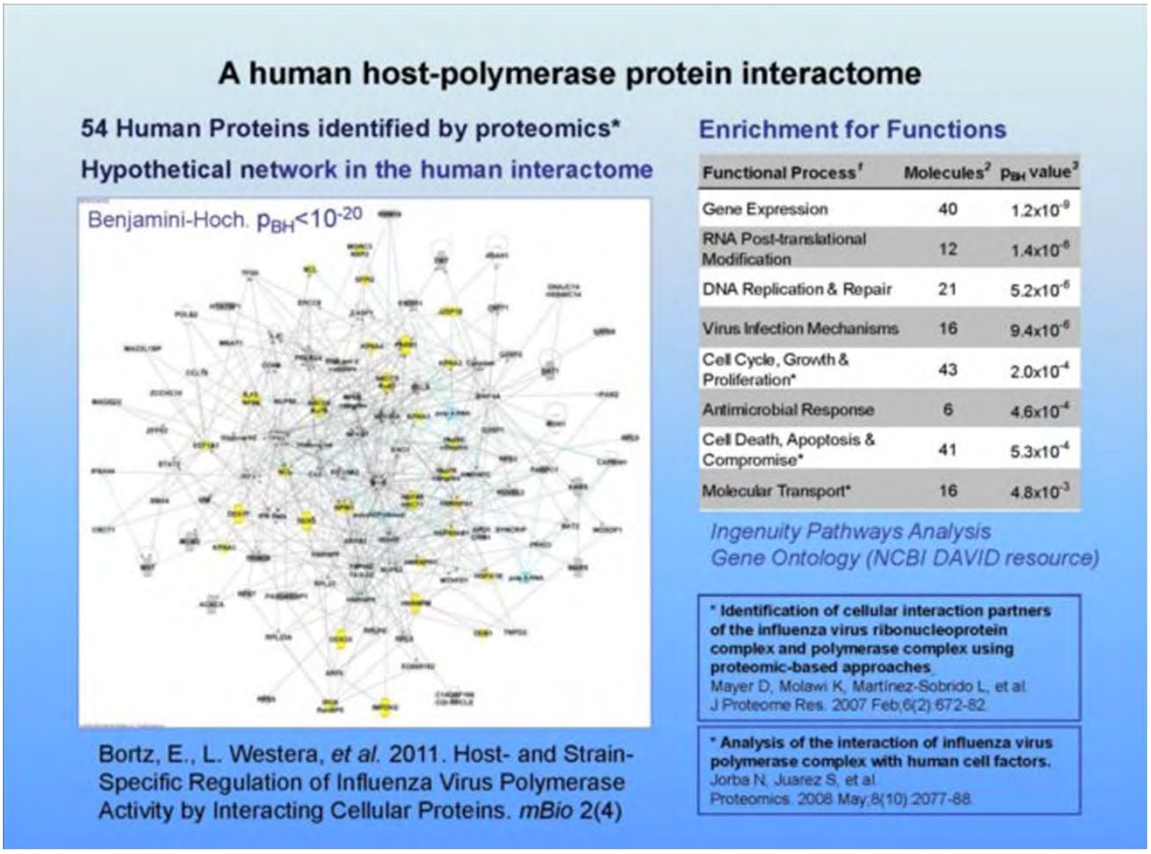
So what is this control mechanism. What happens early on? Virus infects the cell, let's say it has the right HA, it gets in, what helps this polymerase? What determines whether this polymerase is actually going to produce lots of viral RNA and proteins?



And what I've studied is the interaction of viral polymerase with host cell factors that control the replication, the adaptation of avian influenza viruses to human proteins in the nucleus, as well as possibly the activation of some of these innate immune responses, so these viral RNA and proteins are not simply produced by the virus, they're produced in conjunction with host cell proteins. And as you know, that's a paradigm in all virus, in all virus infections, the viruses need the host cell machinery, but in this case, we're actually able to study what this host cell machinery is and this is, this is, this is, this is being done for a lot of viruses right now.



So in polymerase, if you use Proteomics experiment, as spectrometry, and this is mostly been published, H1N1 viruses, if you take this complex, you put in the cells and then you identify all the proteins that interact with it, you find, you know, 50 something pro-, at least 50 something unique proteins by mass spectrometry that interact with an H5, H5N1 virus recently, 300 something proteins were found that interact with this. With the more sensitive mass spectrometry technique. Also, yeast 2 hybrid studies or other biochemistry studies have picked up a number of individual proteins.

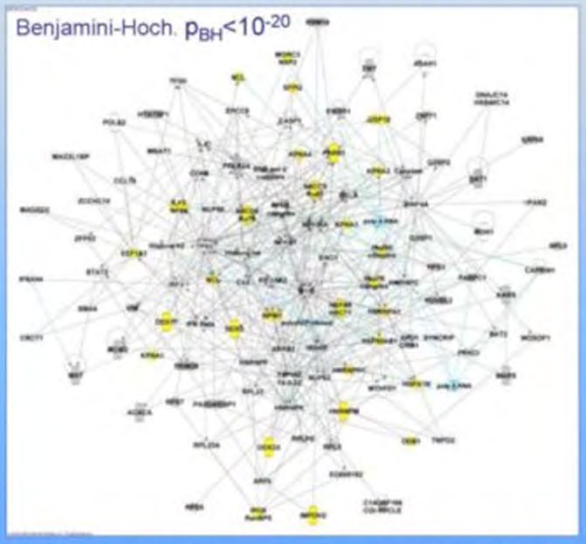


So the experiments that I originally sought with this was if I take the entire complex and I throw it in here, and I look at this set. So these are proteins that interact with the whole complex and interact with an intact complex, because it's replicatively competent, we can test that. And it's also, we're pulling down proteins that exist in their native abundances in the cell and they were over expressing them, like in the yeast 2 hybrid, these are just querying those cellular protean with the viral polymerase and the VRMP complex and the MP and seeing what it interacts with and it pulls down these proteins. And what are these proteins? What is, is this a random collection of proteins? It turns out it isn't, all right? So if I did a quick find for an informatics analysis and found that with a P value of 10 to the minus 20, it's called a Benjamin Hochberg value for false discovery, it's used for networks, but it's similar to a regular P value. The P's protein is highly interactive with each other. This is not a random collection of 50 something proteins, it's a very tightly knit structure of proteins that have been, have many described interactions between them. And in fact, they're not only interactions, they have enrichment for certain viral, certain cellular functions, gene expression, RNA post-trans modifications, DNA replication repair, viral infection mechanisms, which is a pretty vague category, but, cell cycle, molecular transport, cell depth, for example, that there is an enrichment for proteins in this grouping, so it's not a random collection of proteins that don't interact, it's a collection of proteins that interact with each other, and they interact with each other in known cellular processes. Now this is a pretty broad brush to paint this with, so what I then looked at is can we do some more phenotypic analysis of this. Can we see if these proteins are really having anything to do with influence of virus replication, particularly for high path influenza viruses. All these were found with H1N1 viruses.

A human host-polymerase protein interactome

54 Human Proteins identified by proteomics*

Hypothetical network in the human interactome



Bortz, E., L. Westera, *et al.* 2011. Host- and Strain-Specific Regulation of Influenza Virus Polymerase Activity by Interacting Cellular Proteins. *mBio* 2(4)

Enrichment for Functions

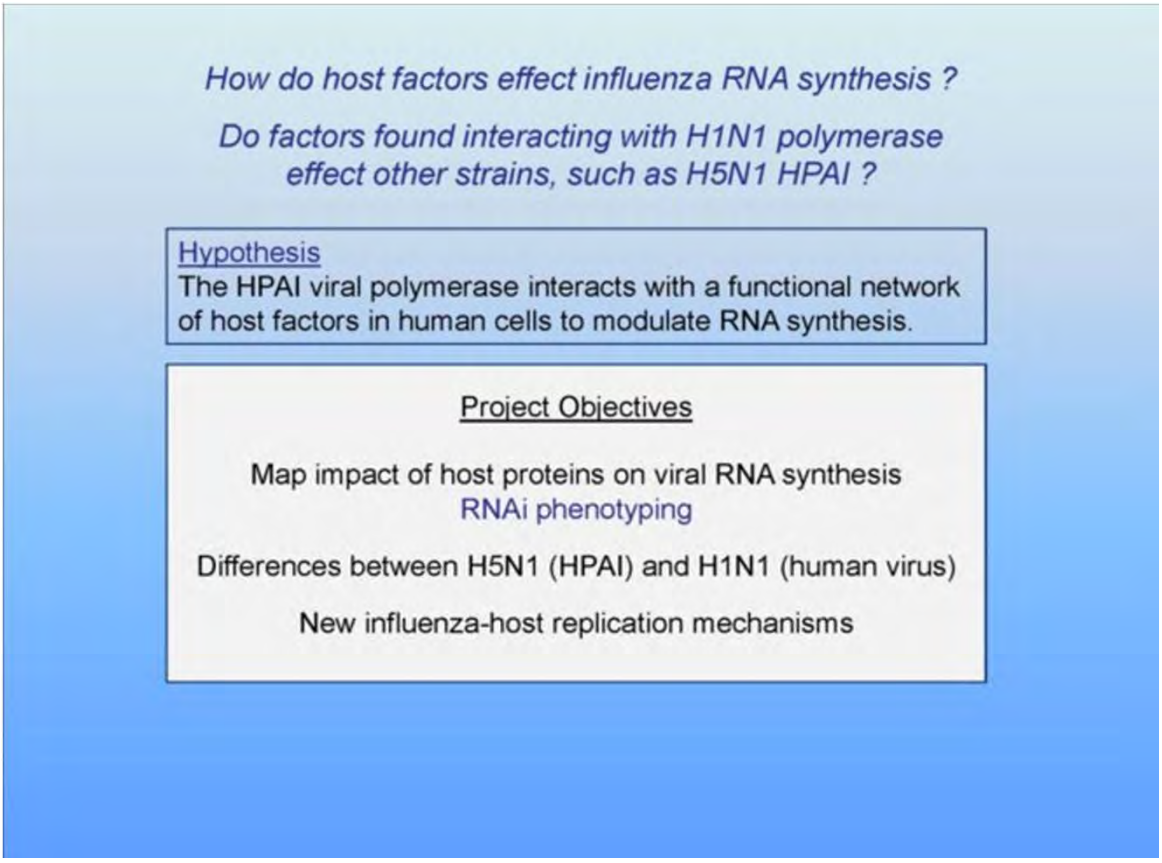
Functional Process[1]	Molecules[2]	p_{BH} value[3]
Gene Expression	40	$1.2x10^{-9}$
RNA Post-translational Modification	12	$1.4x10^{-6}$
DNA Replication & Repair	21	$5.2x10^{-6}$
Virus Infection Mechanisms	16	$9.4x10^{-6}$
Cell Cycle, Growth & Proliferation*	43	$2.0x10^{-4}$
Antimicrobial Response	6	$4.6x10^{-4}$
Cell Death, Apoptosis & Compromise*	41	$5.3x10^{-4}$
Molecular Transport*	16	$4.8x10^{-3}$

Ingenuity Pathways Analysis
Gene Ontology (NCBI DAVID resource)

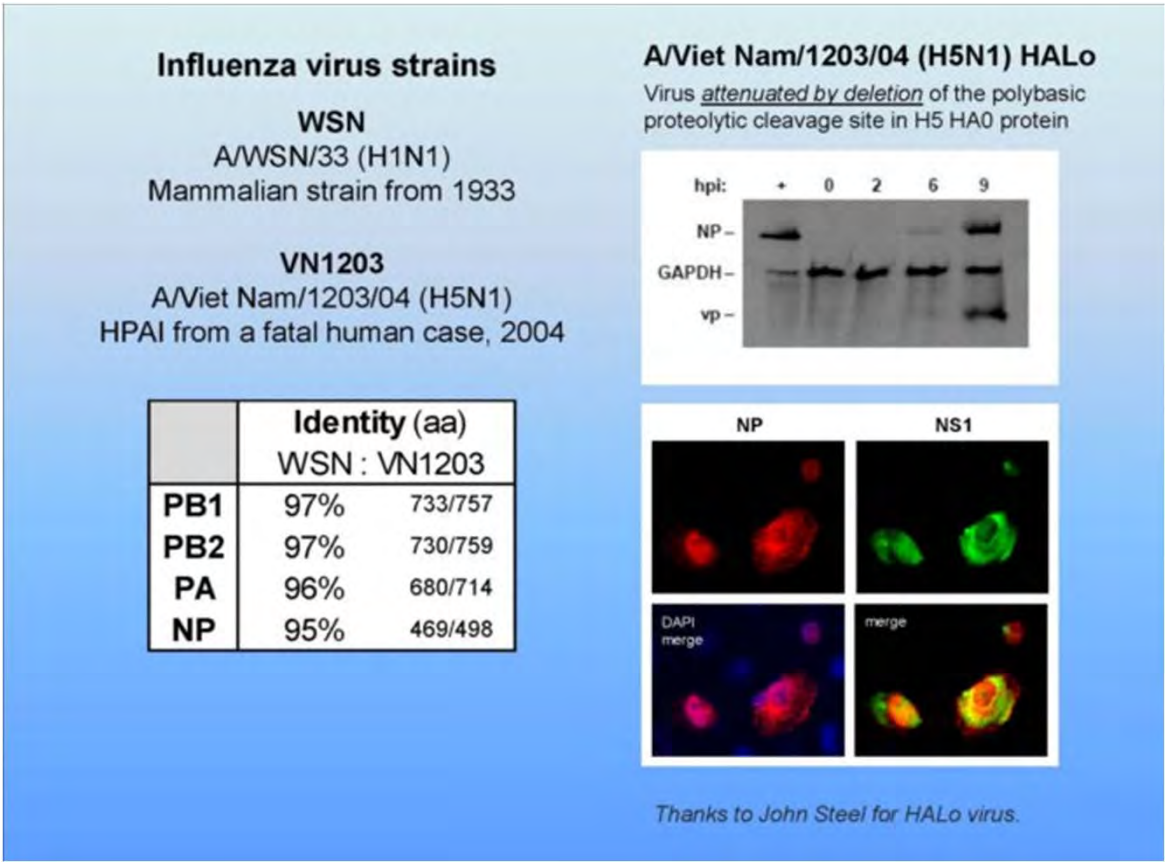
* **Identification of cellular interaction partners of the influenza virus ribonucleoprotein complex and polymerase complex using proteomic-based approaches.** Mayer D, Molawi K, Martínez-Sobrido L, et al. J Proteome Res. 2007 Feb;6(2):672-82.

* **Analysis of the interaction of influenza virus polymerase complex with human cell factors.** Jorba N, Juarez S, et al. Proteomics. 2008 May;8(10):2077-88.

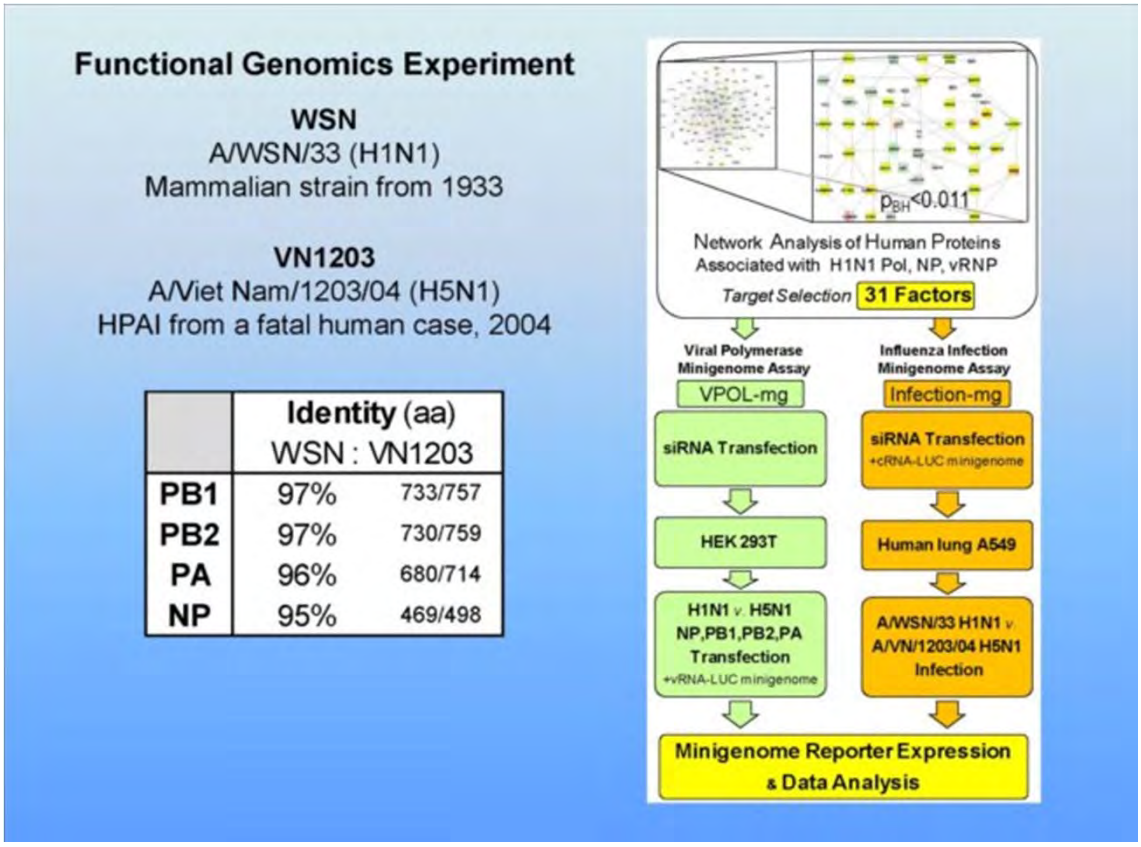
Laboratory strengths basically. So does high path influenza work the same way? Can we just assume that laboratory strains and high path polymerases will work the same? Maybe, but maybe not.



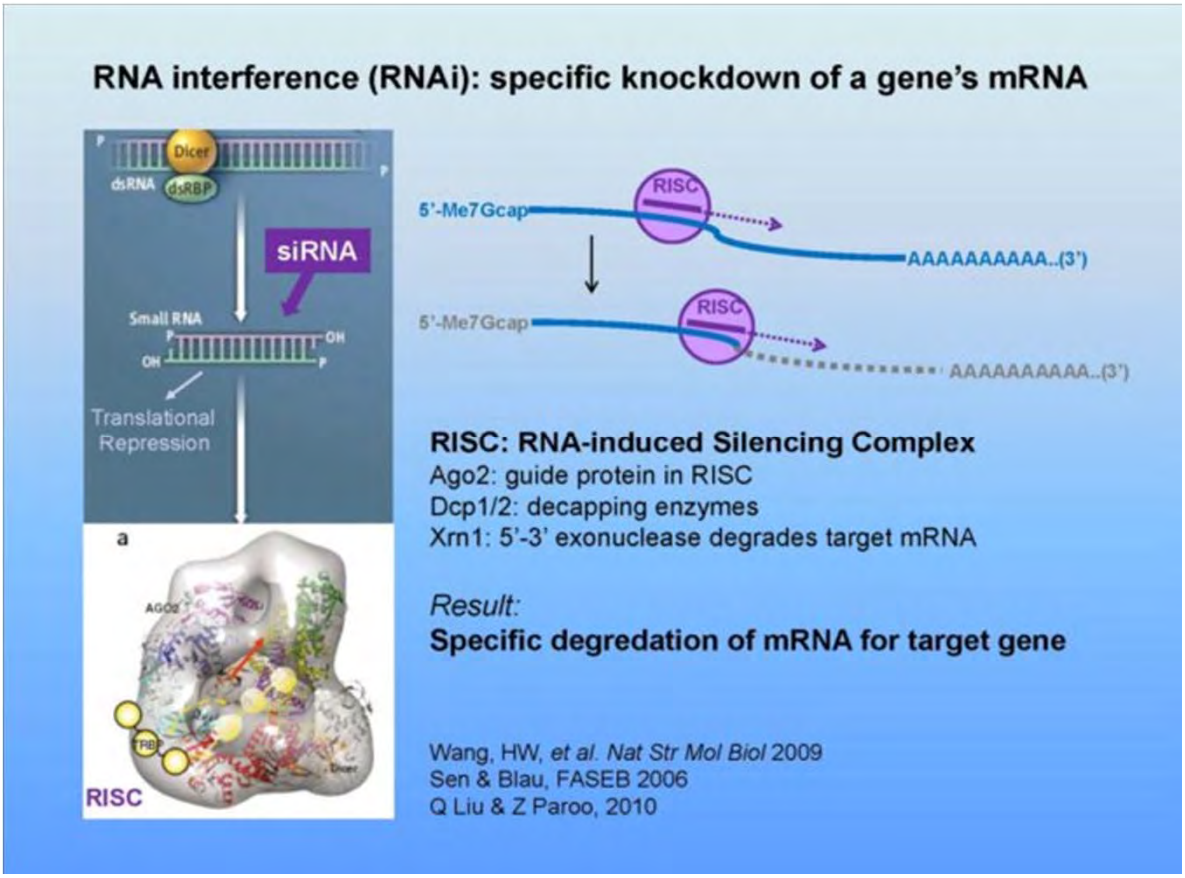
So the project objectives are to test if these factors affect influenza synthesis, RNA synthesis, to look at factors that are interactive with H5 and see if they affect other strains such as H5N1 highly pathogenic avian influenza virus. And the opposite is that polymerase, since we know that it does have the capability of functioning in human cells with those mutations, it interacts with some network or some sub network of those proteins and it modulates in that way, or it regulates to RNA synthesis, so I now, objectives were to map the impact of the proteins on viral RNA synthesis using RNA interferon genotyping, I'll go into that in a second and to look at the difference between HPAI and the human H1N1 virus and to look at new replication and RNA synthesis mechanisms.



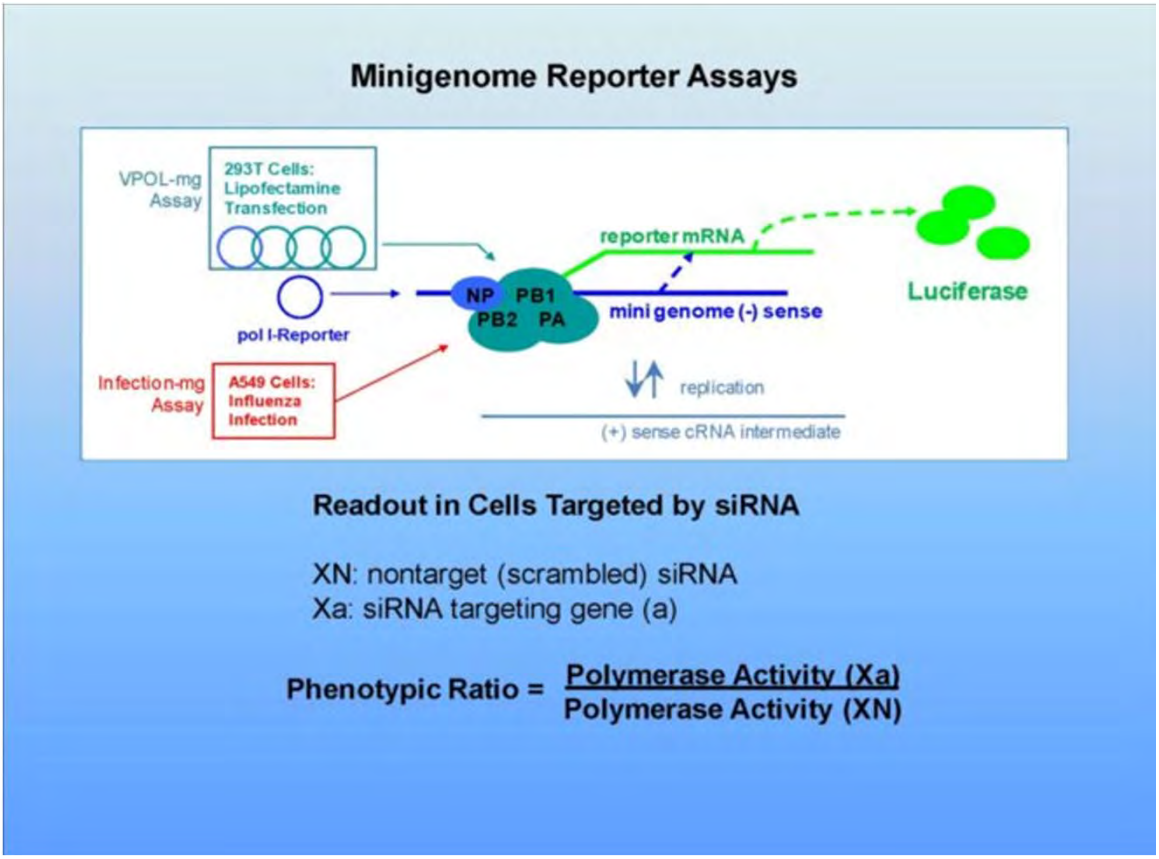
Now, the two viruses that are used in these studies were a laboratory strength, WSN, from 1933. It's been in the lab since 1933. You can find original tubes if you dig deep in the freezer, I think. But -- or strains derive from it, but also an H5N1 from a fatal case in Viet Nam, which is called A-VietNam-1203. And the identify of the pulmonary complexes in these viruses are actually -- between these two are actually remarkably high. It's kind of low for a flu viruses, but just from a basic stand back, you know, do these proteins -- are they amalogous? A perspective, yes, they seem to have a lot of similarities. But as I said before, a couple mutations can convert a avian PB2 from, you know, a high path, a low path to a high path. So, you know, this turns out that this -- these numbers may be significant. Also to note that this can all be done in tissue culture in regular BSL2 conditions because the H5N1 virus is called halo, like the video game, but in this case we're actually attenuating the virus by deletion of a part of that HA protein that makes a virus highly pathogenic. The virus actually replicates, and you put it into A549 which are our lung cell, human lung cell model, human lung cell cancer cell line and you see production of viral proteins like nuclear protein, other viral proteins and on immunofluorescence, the NS1 protein, the NP. You see these proteins accumulate in the cells. And it only goes through one round replication. It might release virus, but this virus is not highly pathogenic because it doesn't have a complete HA, but the replication complex is exactly the same as it is in a highly pathogenic virus.



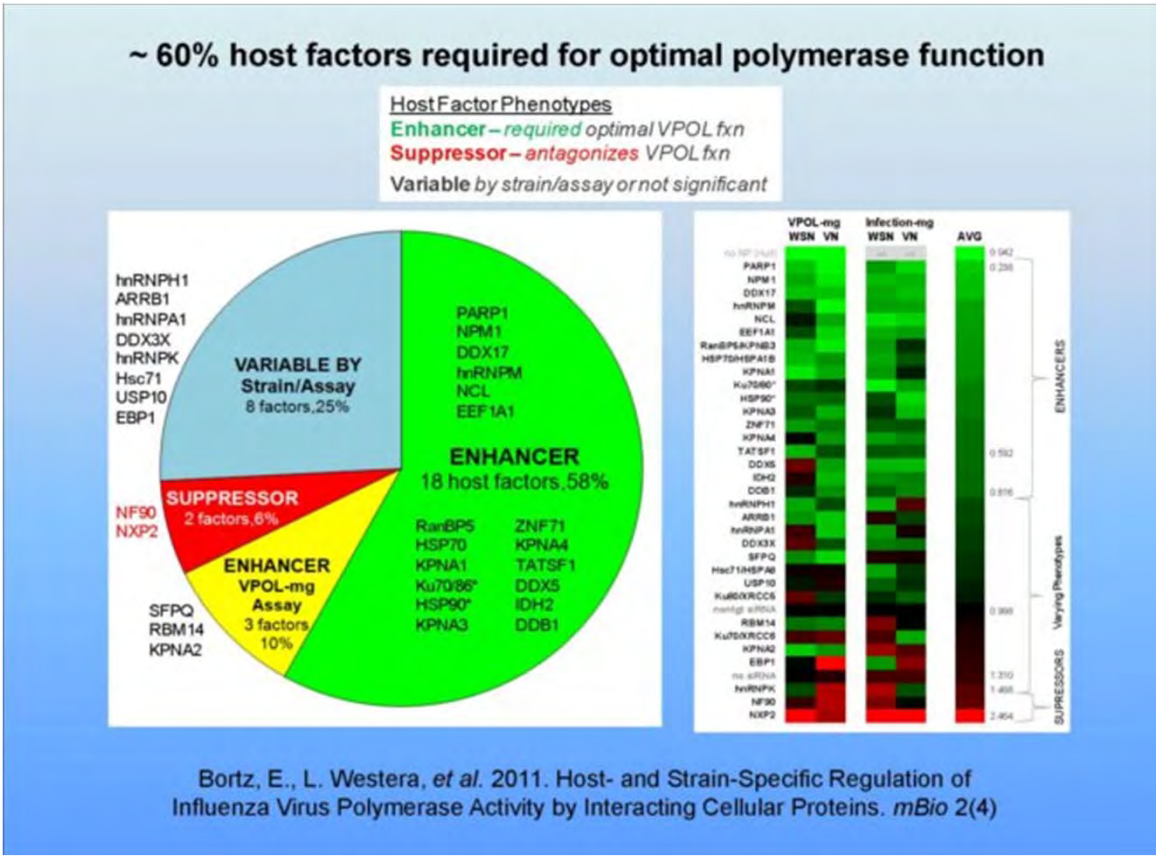
So the experiment was to use, as I said before RNA I see no typing or functional genomics where you look at the genome, you pick out the proteins you want and you do a functional study on them like you target them individually. So I took a number of these factors that were enriched highly in this network for connections with each other and I put them through two assays. One was to look at the polymerase just by itself in a mini genomases. So we transfact the knockdown, we target with SRNA, we target the protein in 293T which is a human cell, we put in the polymerase complex and we measure the amount of preliminary findings. The other one is to knock the target down, to hit the target genetically with RNA interference, as I said, I'll explain in a second in human lung cells infect with either the WSN, H1N1 or the H5N1 halo attenuated virus and to measure polymerase activity.



So RNA interference is this, RNA interference is a way of knocking down a gene specific RNA, messenger RNA, there's basically in a cell you have -- this was originally discovered in plants. Okay. People who got the Nobel Prize for it did some really great work in, but actually it was called post relation of gene silencing in plants. But what happens is basically one of these small RNAs, which are about 21 nucleotides, they're created by a complex called dicer, they find a cognate RNA on a message in cooperation with a number of proteins in the risk complex. This is originally probably an anti-viral mechanism for targeting viral RNAs in plants at least and even in mammals, and this complex results in degradation of that specific messenger RNA causing specific degradation of the target gene. So you can put in a short interfering RNA that matches the RNA of your gene of interest and it will degrade it, at least to some extent, thus knocking down the levels of that protein. So that's the genetic experiment although it's not a knockout so it's not a completely penetrating genetic experiment as a knockout.



The other assay, besides looking at influenza to measure the polymerase activity is simply there's a negative plasmid which encodes a luciferase or a GFP reporter. You can put in plasma either expressing the polymerase complex or the NP, you'll produce RMNA and you'll produce luciferase or you can, in fact, introduce the polymerase complex that way. It works on this reporter and this mini genome reporter as it's called, and produces luciferase giving you an idea of the amount of polymerase activity. And if you look in cells that are targeted for your gene of interest versus cells that are targeted with a not control scrambled SNRA that doesn't target anything in the genome, you see how much you get of phenotype or a phenotype of how much the polymerase activity is affected or dependent on that factor, on that particular factor.



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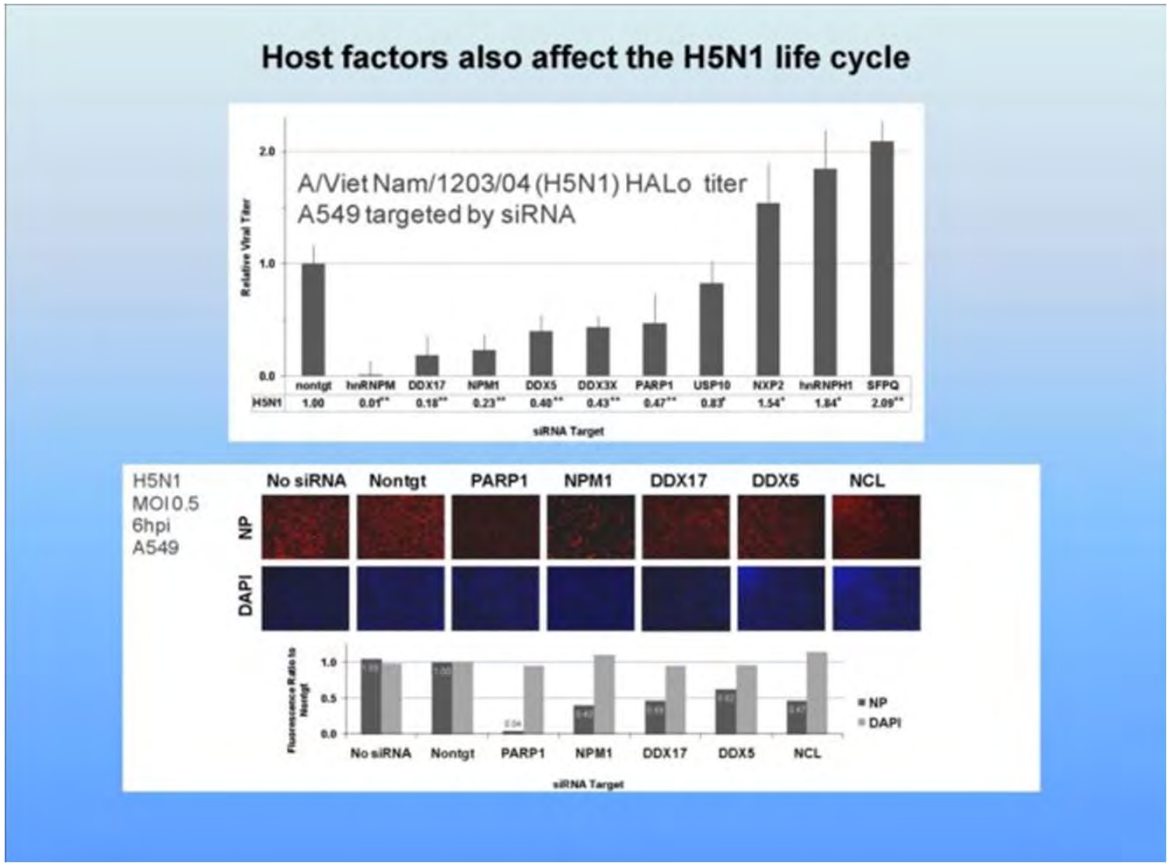
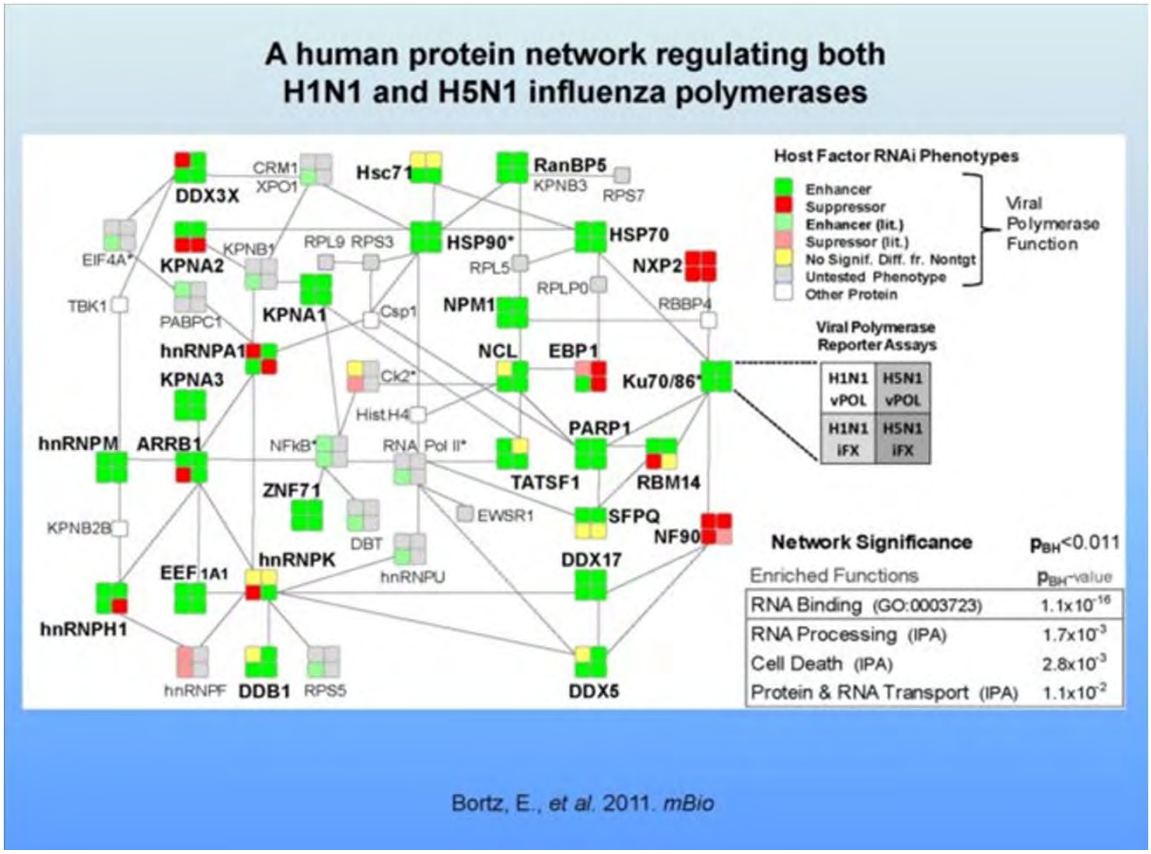
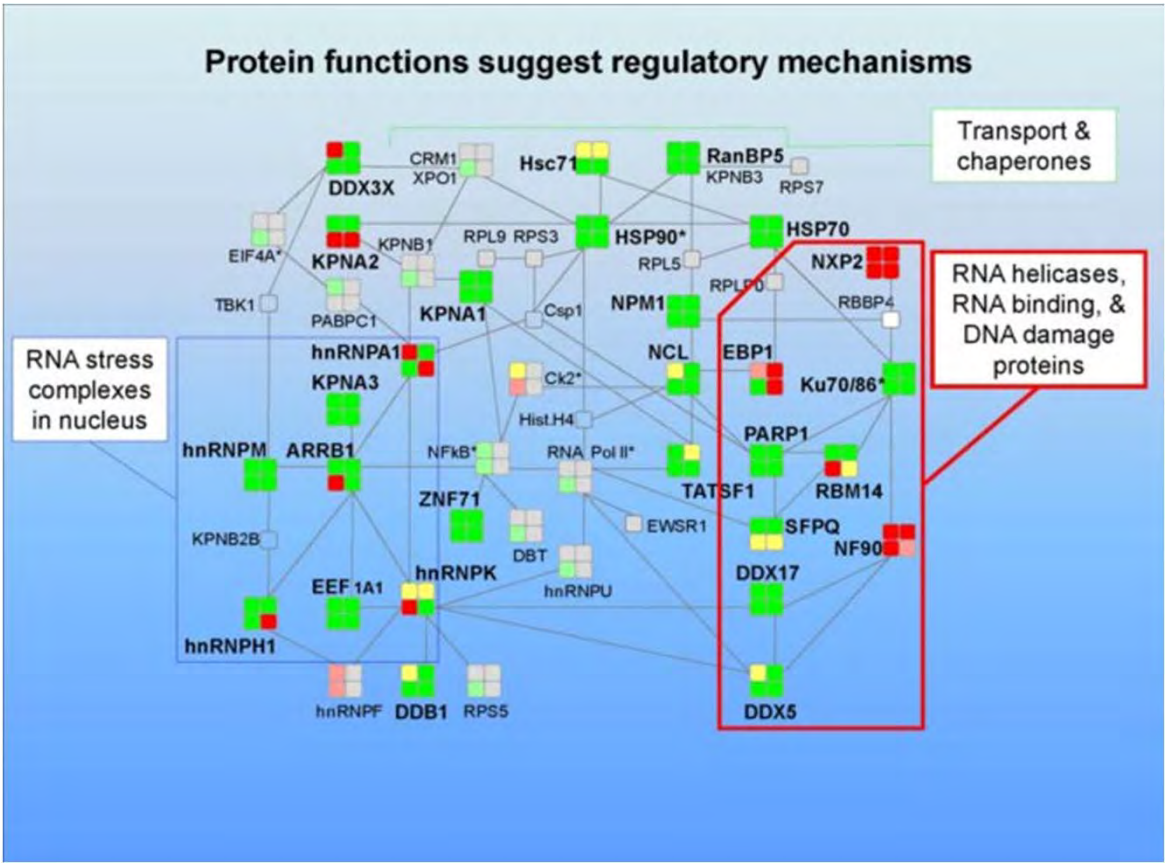
And when we do this, those 31 some factors from mass spectrometry, interestingly enough 60% of them, most of them are required for virus RNA synthesis between H5N1. There's a couple that are inhibitory and there's a few that have variable phenotypes. So on a heat map here if it's green you see that you knock down that target and you less polymerase activity and there's a few that you knock down the target and you get more polymerase activity. Okay? So they're not just not only not a random collection of proteins, they interact with each other, they have cellular function, maybe they're involved in certain cellular functions and not only do they interact with the polymerase they affect the polymerase are in a synthesis capability both in the polymerase only in the mini genomasi and during infection.



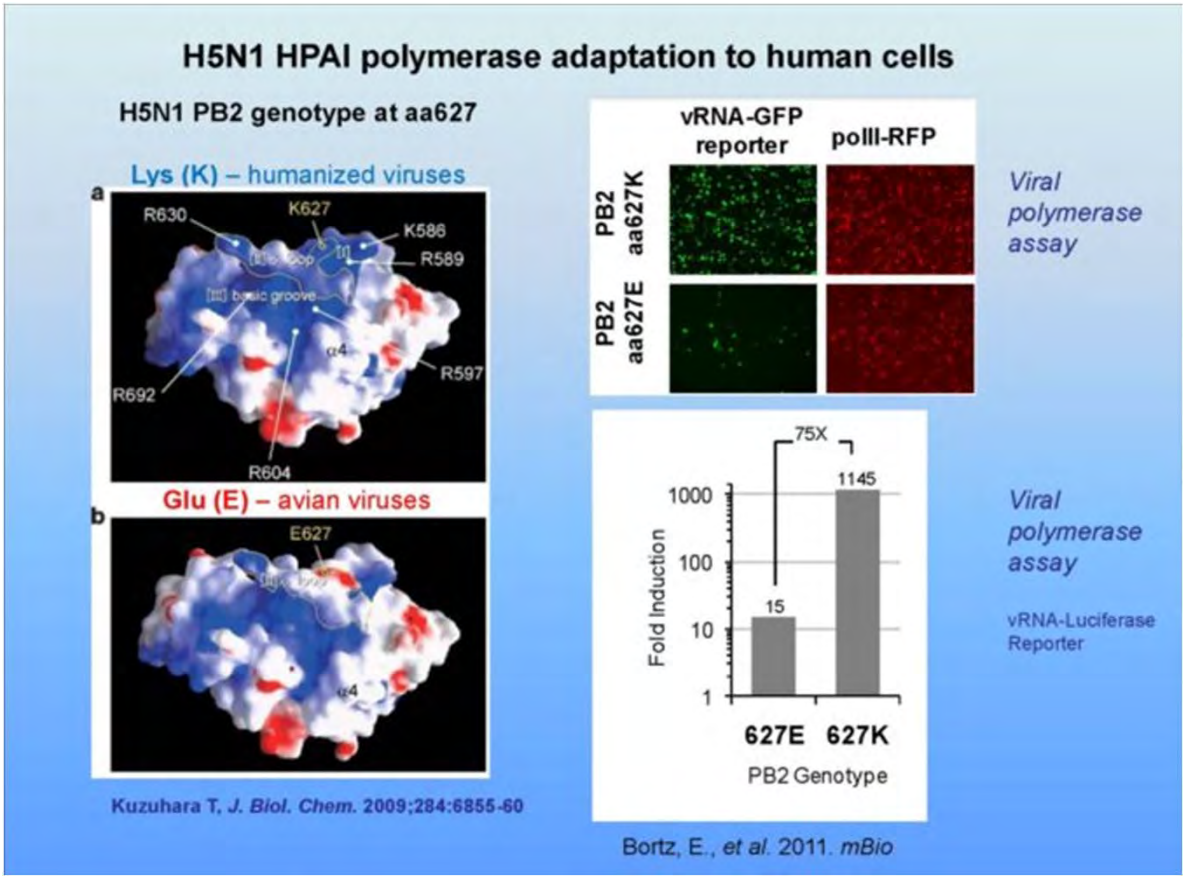
Also if you look without any genomes at all and you just look in the H5N1 lifecycle, if you knock down some of these prominent factors, for example these RNA binary proteins, HNRNPM, DDX17 nucleophosmin, DDX5, these are dead box RNA hela cases you get less virus replication, you get less virus gene expression as well early on during infection. So they're using a plaque [inaudible] limiting dilution [inaudible] as well as immunflouresence. So it's not just an artifact of mini genomes, it's actually that these things are having a roll. And you see a few are the opposite as well. You knock them down you get more virus. So they're antagonist.



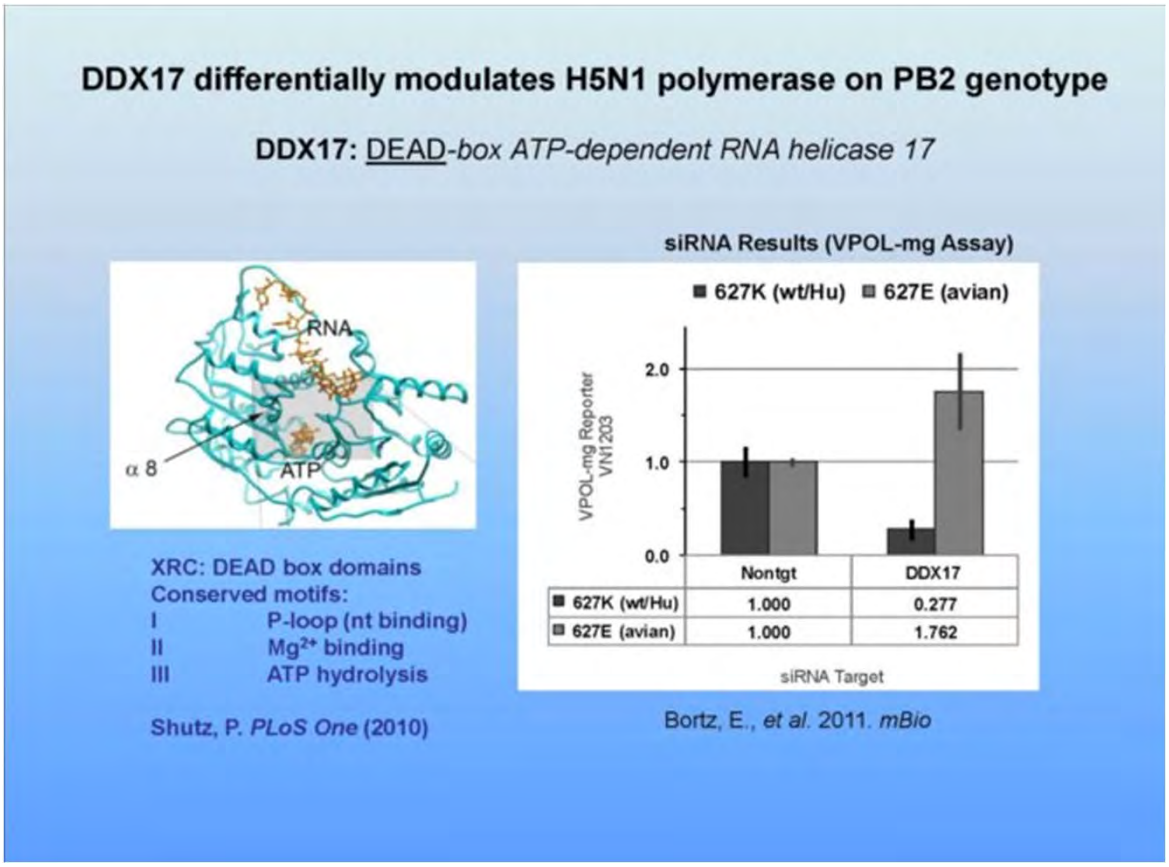
And as I said before, that these proteins all seem to interact, the suction of proteins all have a tightly interacting network or sub-network within the cell and this network as we're proposing as a regulatory network regulating H1N1 and H5N1 influenza preliminary [inaudible]. And where you see green is the phenotypes are the same. For DDX17 H1N1 in the polymerase by itself assay, H5N1 in the polymerase by itself or either of those viruses in infection, this protein is required. NXP2 or an NF90 it's the opposite. If you knock down that protein, if you knock down that transcript and get less of that protein there's a lot more control experiments behind this RTPCR, for example, to show the levels of knockdown. You get more polymerase activity meaning this is probably an antagonist. And this particular grouping of proteins is very highly enrich in RNA binding processing RNA transport as well a little bit of self-doubt. So these proteins may be controlling the RNA synthesis activity.



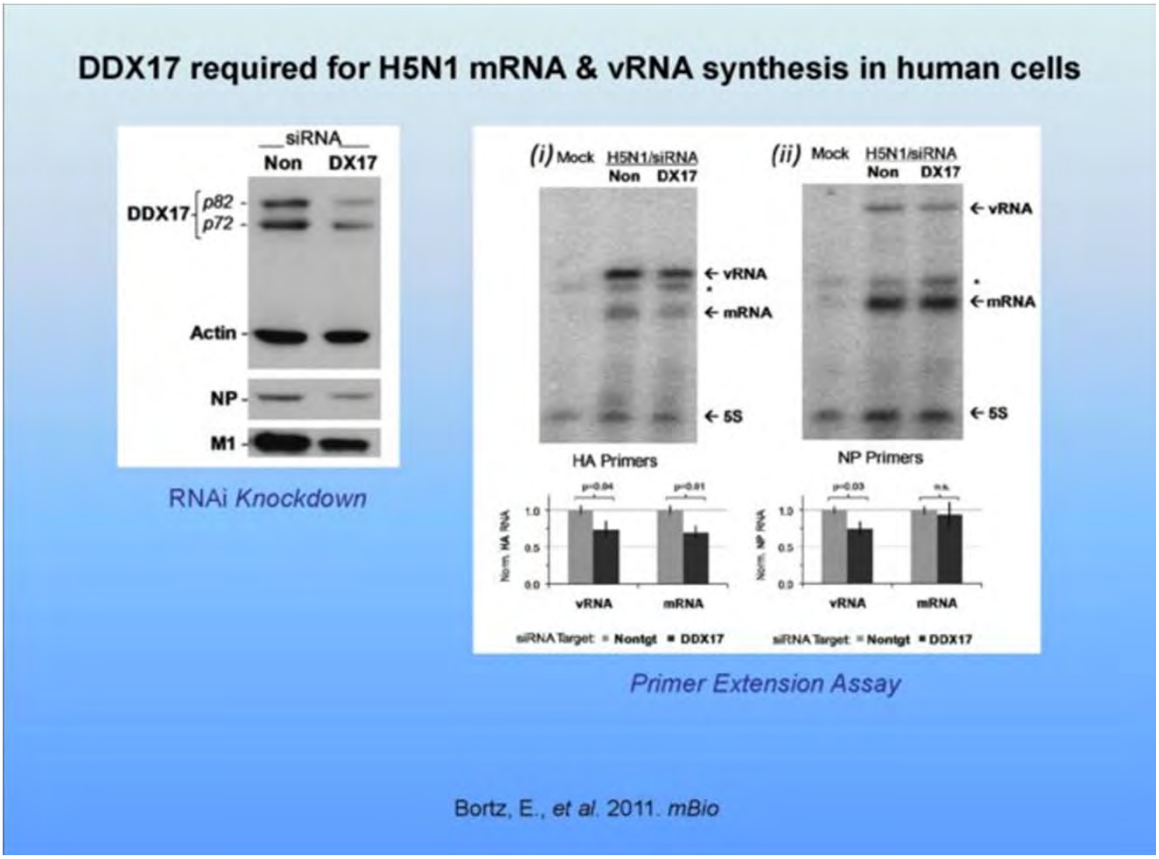
And this is a couple of the areas under investigation right now that I'm looking at. For example, RNA stress complexes in the nucleus involving these proteins which may have something to do with how the cell sees RNA, strange RNAs that are not cellular RNAs and then also RNA hela cases and RNA binding protein such as DDX17 and others maybe have something to do with RNA processing. But it's not quite clear. You can't just point and say all these are stress complexes so this next set of experiments is to show that these are a particular molecular mechanism of how these things are working.



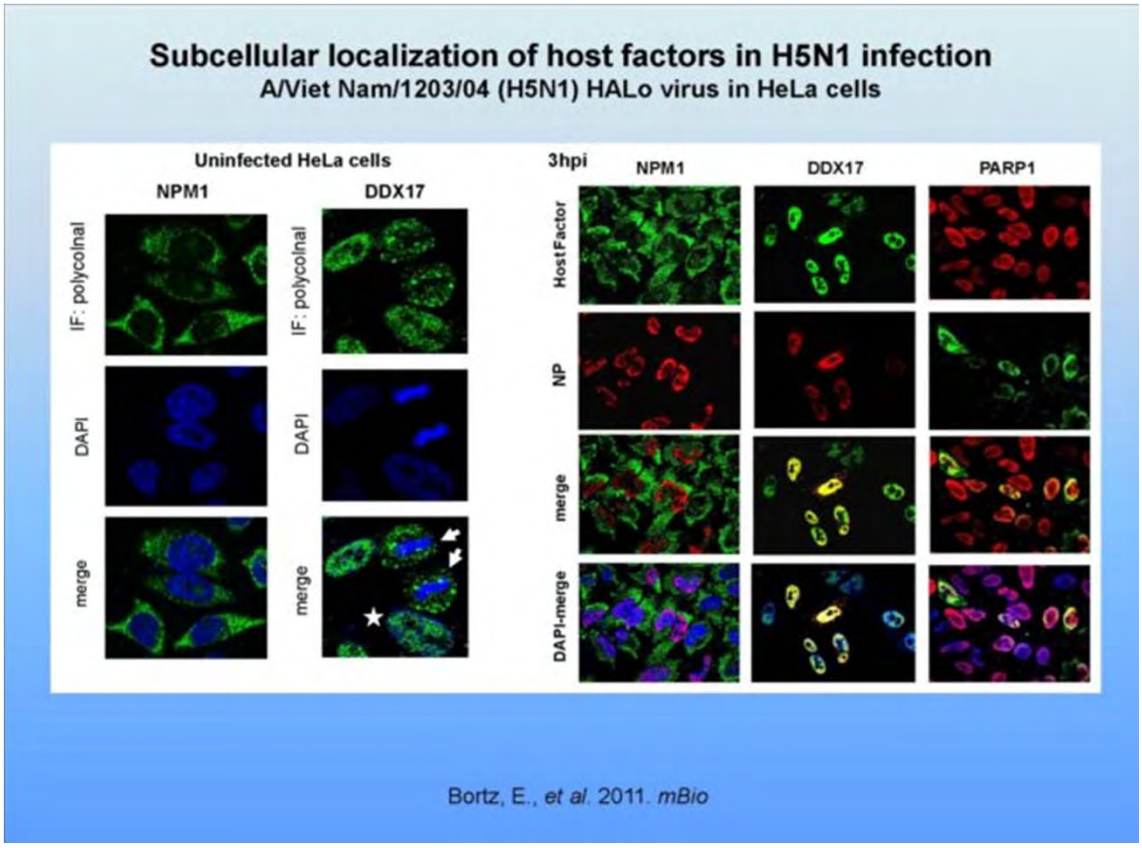
Now for polymerase that are not adapted, avian polymerase is not adapted to human cells. For example, one of the mutations, the consensus they have is in 627. You know after 627 which creates this acidic patch here when you have a glucemic acid, avian viruses have this. This, there's a mini genome assay with 627E, you get very little GFT, you don't get very much luciferase, they don't work very well in human cells. If you make the mutation to 627K which is found in the humanized viruses, that are found in avian influenza, H5N1 viruses isolated from fatal human or any human case that has disease, usually you have this mutation or one in one of these neighboring -- neighboring amino acids which compensates and makes this patch right here into highly positively charged patch. On the surface is a PB2 and you get much more influenza RNA replication. So I took a look at the network and saw if those factors have differential phenotypes between these two, we have 15 full above background here in the 627E, so are these factors affecting this and this or are some of these factors seemingly something that the virus interacts with thus being host factors that control this difference in phenotype because in chicken cells, the K and the E have the same levels of replication or RNA synthesis so there's something in human cells that allows that differentiate these two.



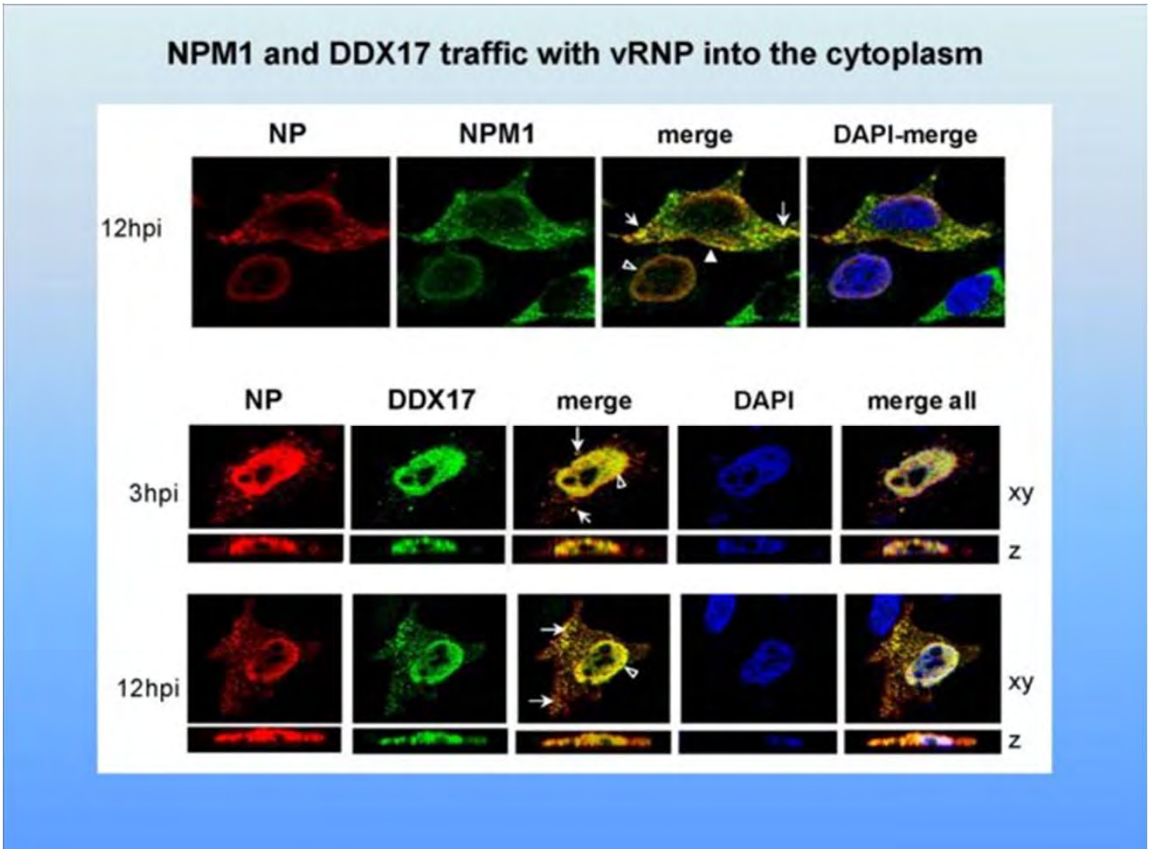
And one of the factors which was most prominent and had the biggest difference in phenotype was this RNA helo case DDX17. It's not a transport protein, it's not something to do with temperature sensitivity, these have all been proposed as reasons for this difference in avian influenza polymerase but I think it's actually this RNA binding protein. And exactly how is still at this point a very good question. But 627K, if you knock down EDX17, you get little polymerase activity, it's required. If you knock down DDX17 and you look in the avian virus, you actually get more preliminary. So this factor is differential between the 627K, the human or wild HPI and the avian flu. And it's a conserved RNA binding protein. And now I'm looking at molecular mechanisms, ATPAs for example to see if ATPA's function is required for this process. The enzymatic activity, the RNA binding activity of this protein required or is just doing something else? Maybe it's a scaffold for other processes. And that maybe can give us an idea how this RNA binding protein is actually controlling adaptation of the H5N1 PD2 to human cells.



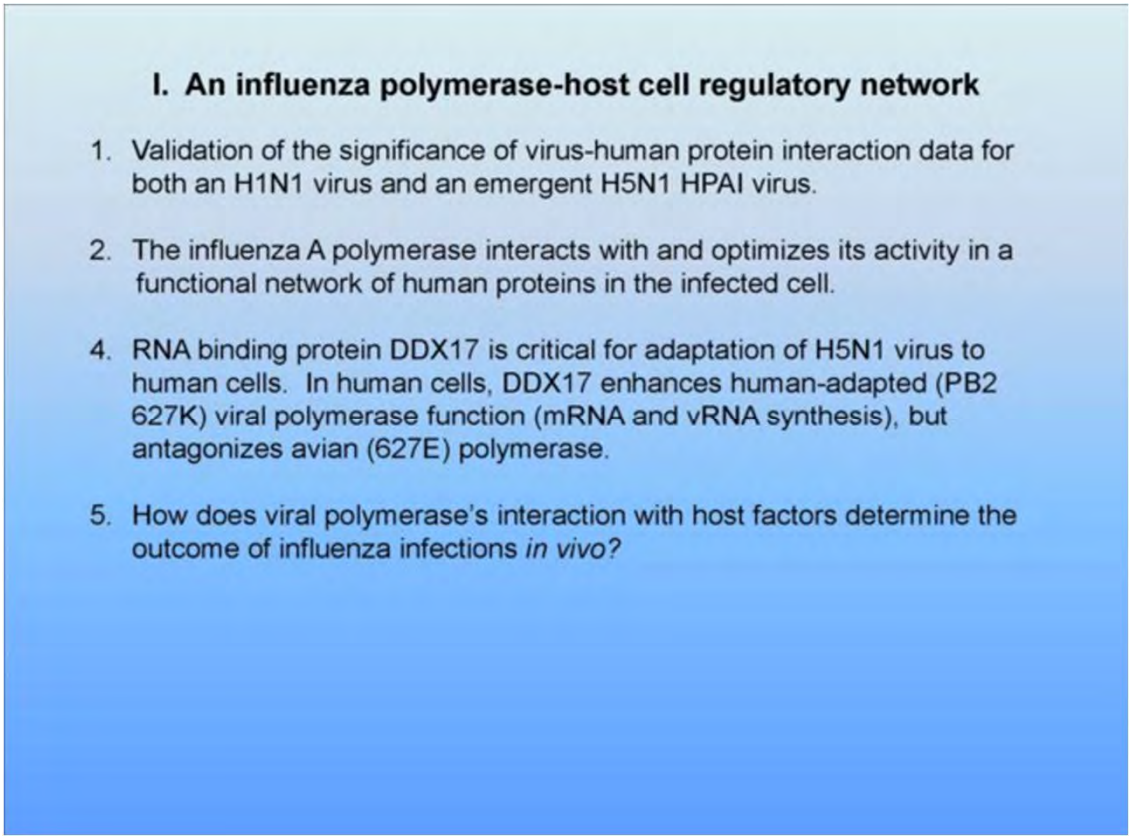
So one of the things to look at was that the virus if you knock it down, you get less viral protein. Theirs is less DDX17, you've got less viral protein NP for example, less viral N1 protein, you also get a little bit less. This is a primer extension it looks at the viral RNA, VRNA and MRNA species, you get a little bit less viral VRNA and MRNA too, and for another segment of the viral genome you get less VRNA but the MRNA's not significant. So it's having some direct effect on RNA synthesis activity. And this is where the mechanism is right now except for the idea of testing about the actual helo case function of DDX17. But somehow it's affecting RNA synthesis directly.



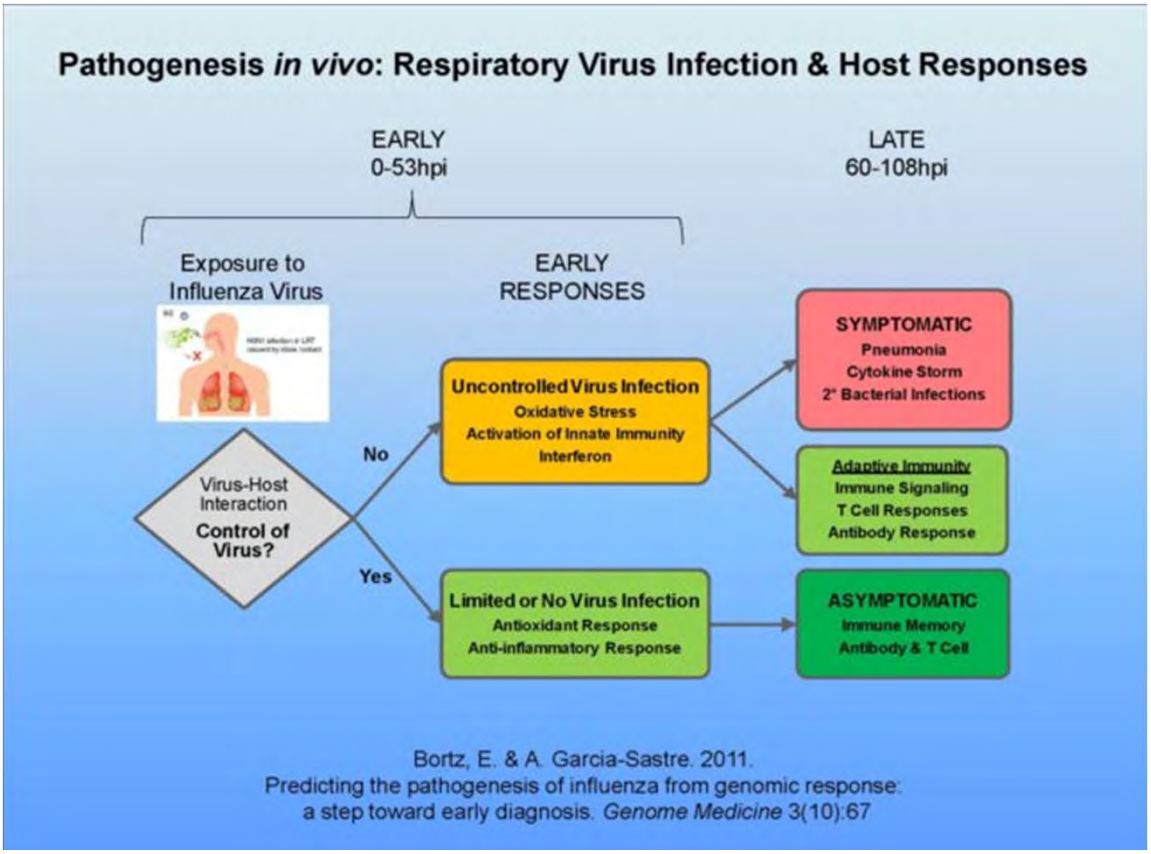
That is not to say though that this protein does not have the capability of affecting other parts of the virus lifecycle in the nucleus of infected cells, this is a nuclear protein, it highly co localizes, it's yellow here, it's with NP and infected cells three hours in.



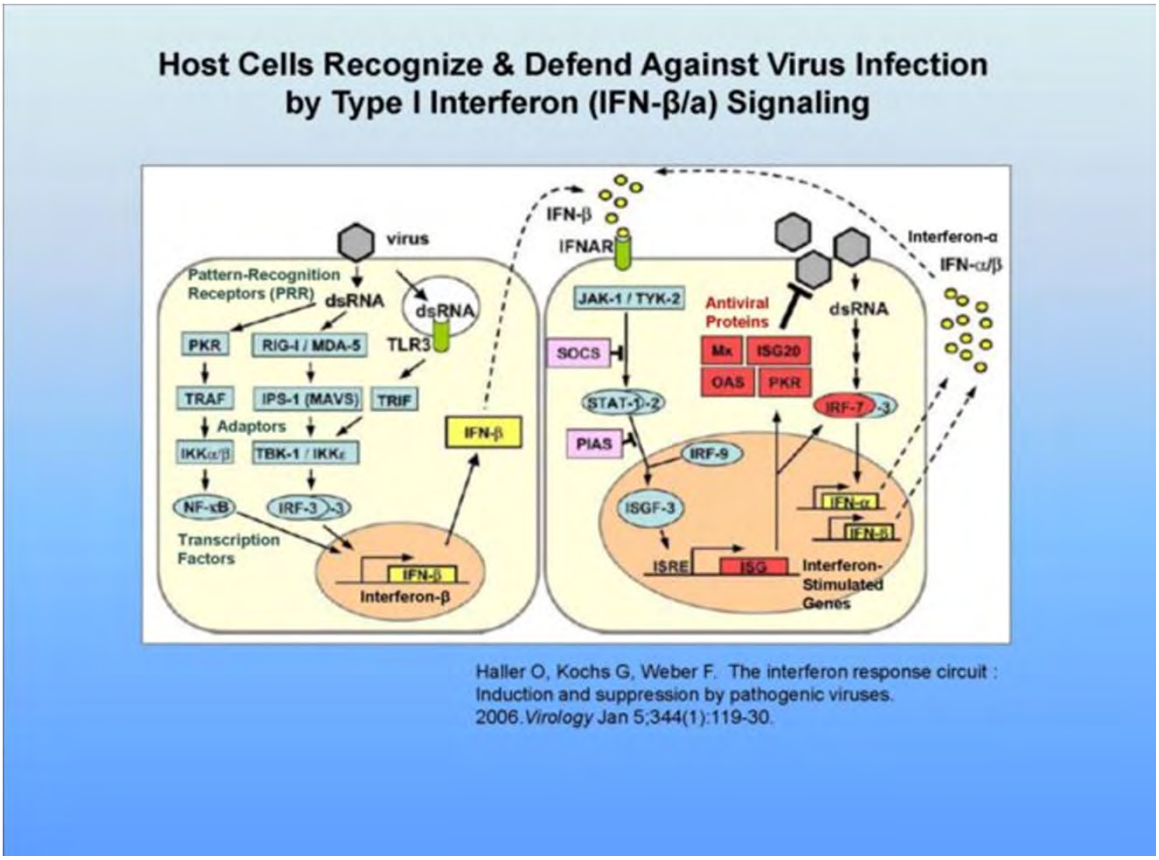
On the other hand in infected cells 12 hours in you see a redistribution of DDX17 to the cytoplasm of the cell. So you see yellow spots on the cytoplasm, maybe it has something to do with the trafficking of the RNP also from the nucleus into the cytoplasm. And this is also true for another RNA binding protein NPM1 though to a lesser extent. So this is also something we're actively investigating and take some good microscopy to do.



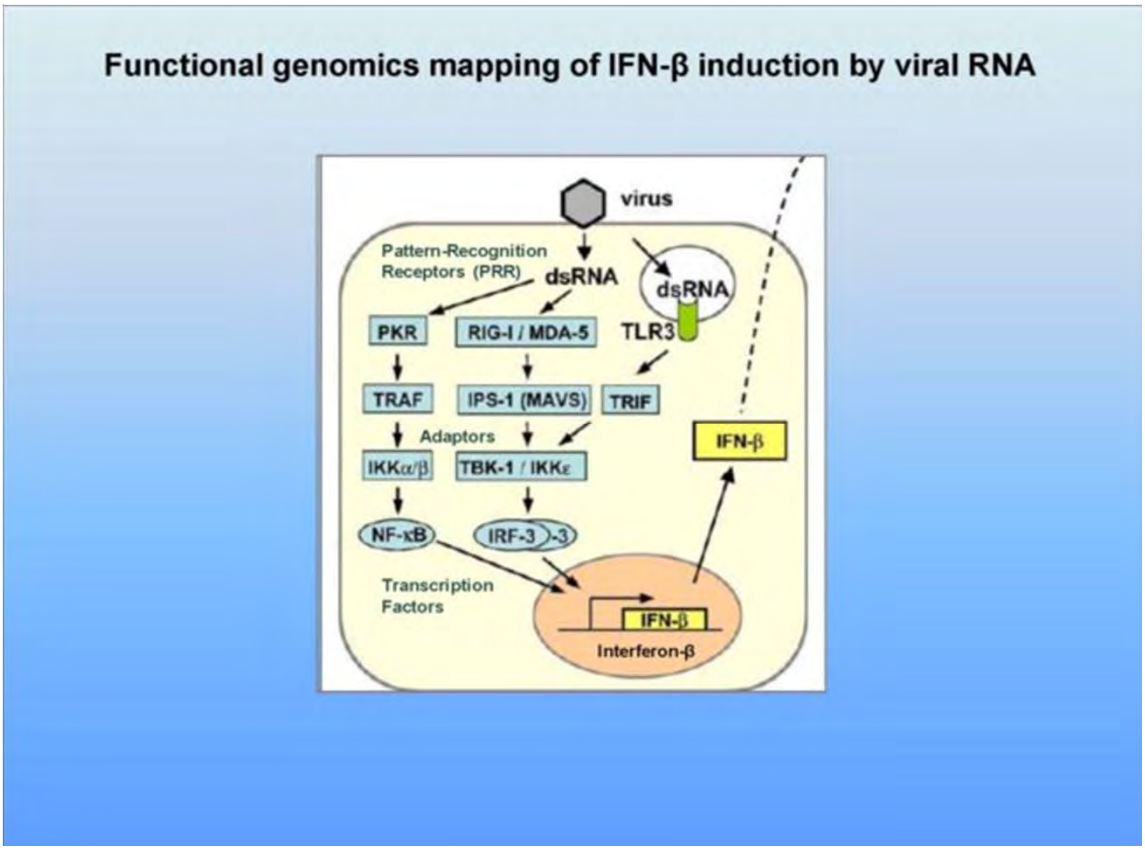
So this host cell regulatory network validates the significance of the protein interaction data from the mass spect, the polymerase optimizes activity with dysfunctional regulatory network. The protein DDX17 is critical for a adaptation of H5N1 to human cells and for the adaptation of the avianized polymerase 627E to RNA synthesis in human cells with the 627K mutation which is the humanized isolates and then the question is, how does this affect interactions in [inaudible] And I guess number three got lost there. So as we said, we'll go back to this.



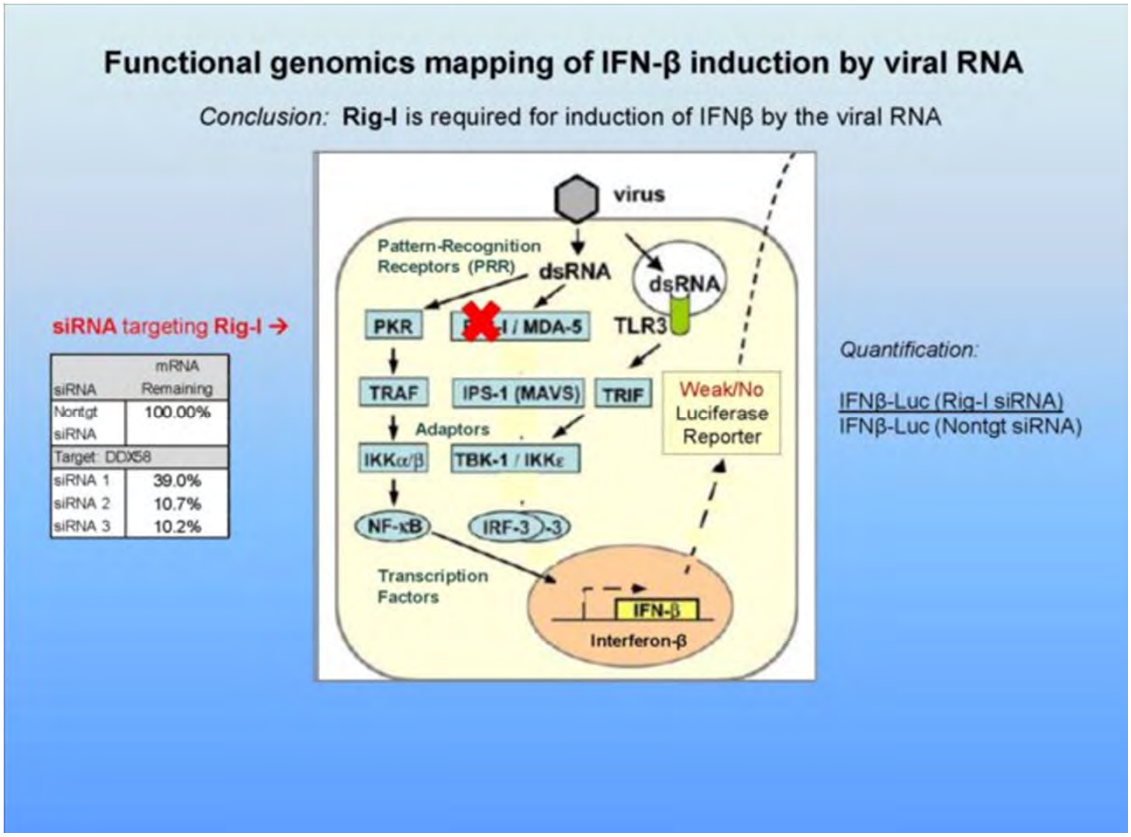
There's a couple other experiments I'm not really going to have time to go into too much detail, but I'll tell you quickly. I'm doing a number of other experiments looking at the next step here. If you have lots of RNA virus, you have infection, you have RNA synthesis, how does this turn on oxidative stress? What does this do to innate immunity, what does this do to interferon responses?



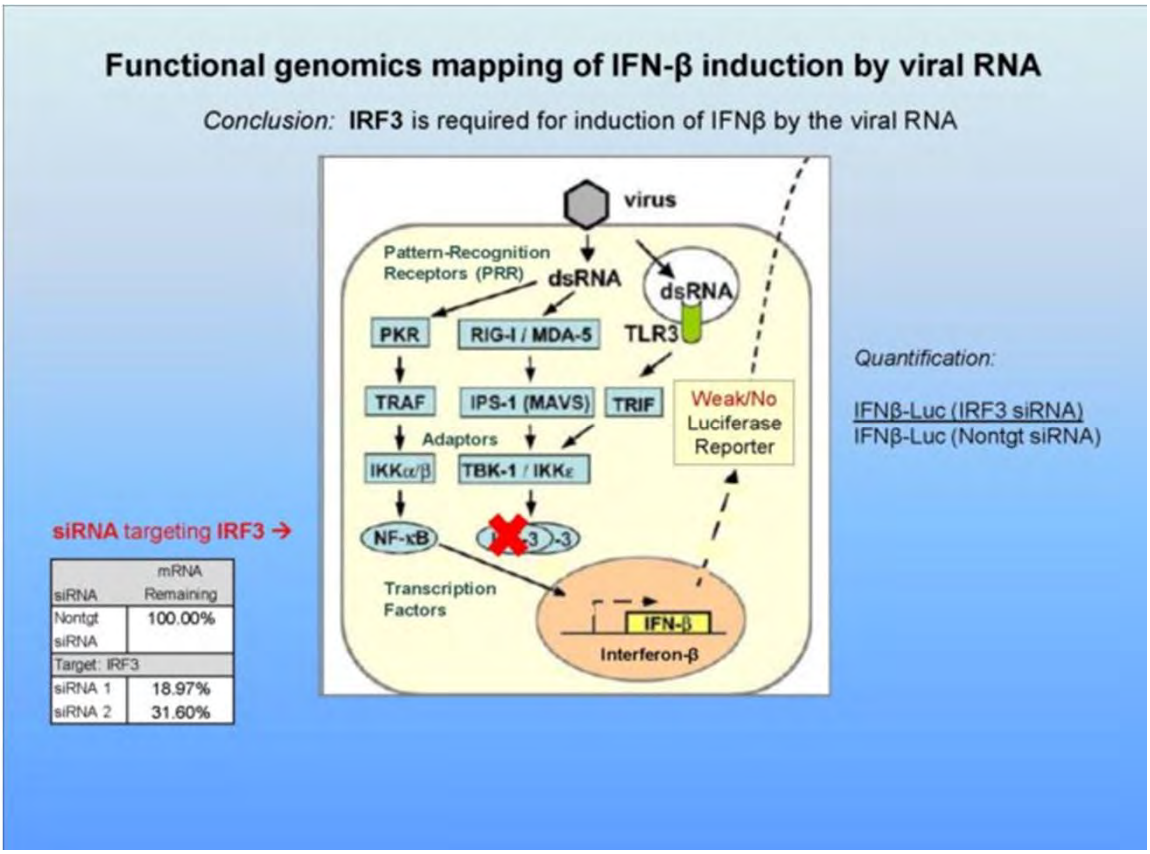
So one of the ways of doing this is to look at bronchial aviolar lavage fluid from a mouse. You can infect mice with a respiratory virus, squirt PBS into their lungs, take it out, go through a purification process, run it on a 2D gel, all the proteins, and then identify the proteins that are up regulated in infection. I used a different virus for this which is a gamma herpes virus of mice, called MHV68, but it infects mouse lung and causes an acute infection in the lung followed by kind of fibrotic phenotype and we see, at least in this model a C-up regulation of oxidative stress proteins, for example peroxiredoxin and this has a pretty strong P value as well, and this method can be applied to influenza and actually to any respiratory virus and has recently been published for RSV, respiratory syncytial virus. RSV actually down regulates oxidative stress. MHV68 up regulates and we think influenza probably up regulates MHV -- oxidative stress but we're not quite sure because there's few studies on it and they're not molecular in nature. And also we see up regulation of oxidative and stress genes in the lungs of these mice by RTPCR and these oxidative stress proteins apparently there's a lot of oxidator stress pathways that have been described in the lung and may have something to do with replication of the virus. And this is the network of proteins to look at.



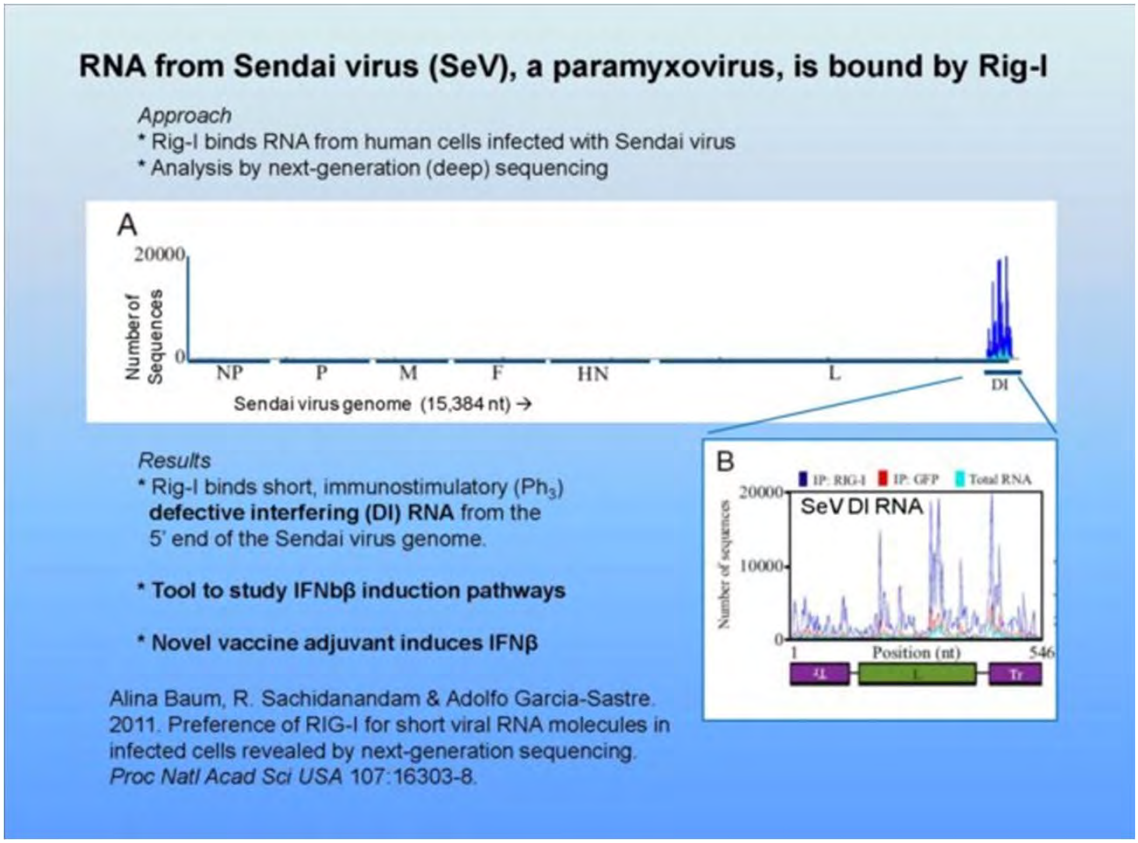
So I'll go briefly into how host cells defend against virus infection through innate immune responses particularly, for example, in viruses the interferon beta and alpha beta type one interferon response. What happens is viruses and code pathogen associated molecular patterns like RNAs that are unique to them and these are recognized by pathogen recognition receptors like rigi which are RNA helo cases in the cell, they transmit a signal down through adapter proteins to IRF three, interferon regulatory factors are turning on interferon beta interferon beta interactive interferon receptor on neighboring cells and fashion, turning on interferon, interferon stimulated genes and these produce antiviral protein that will directly inhibit virus infection, and up regulate the immune response and signal to be adaptive immune response. So this process is where viral RNA is recognized and cells are activated. Right?



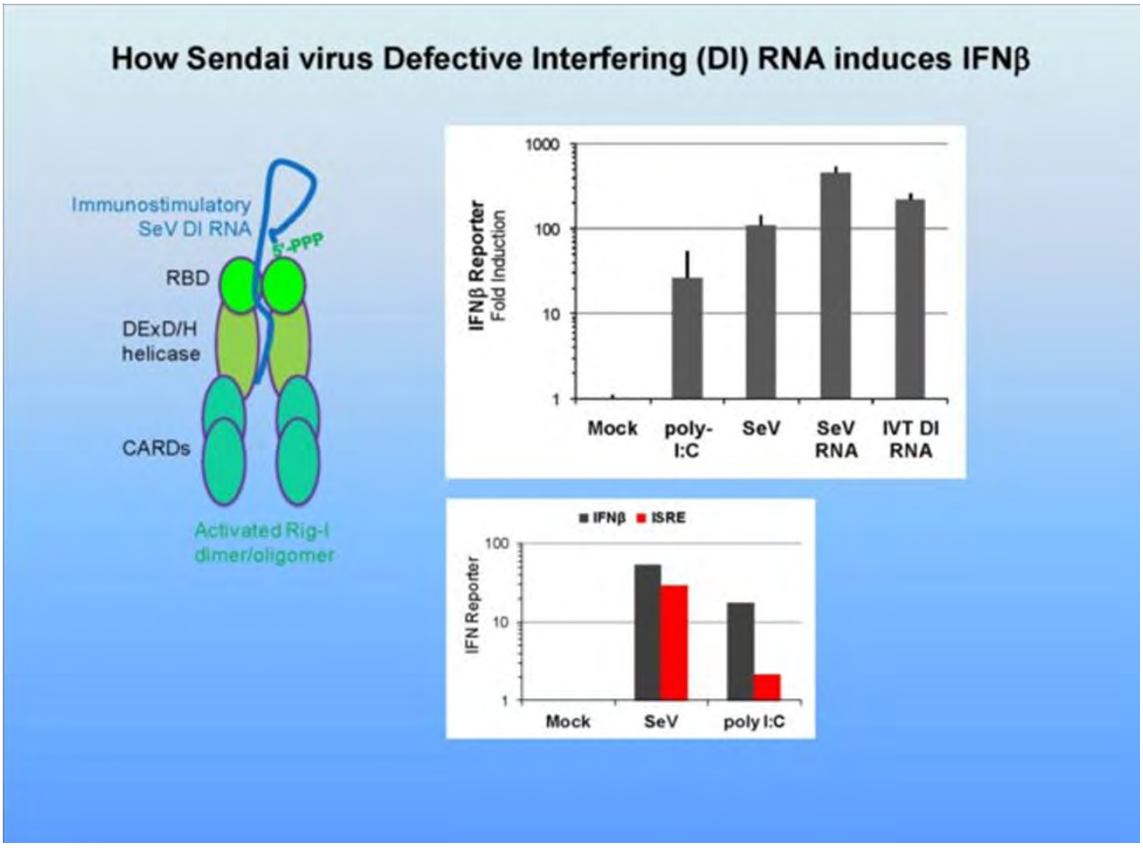
And the idea was can we use functional genomics RNA interference to query what is happening in this process and to do that instead of a mini reporter he's a luciferase reporter cell line that refuses luciferase in response to interferon beta agonist, you throw in viral RNA, another agonist that signals a pathway turns on luciferase.



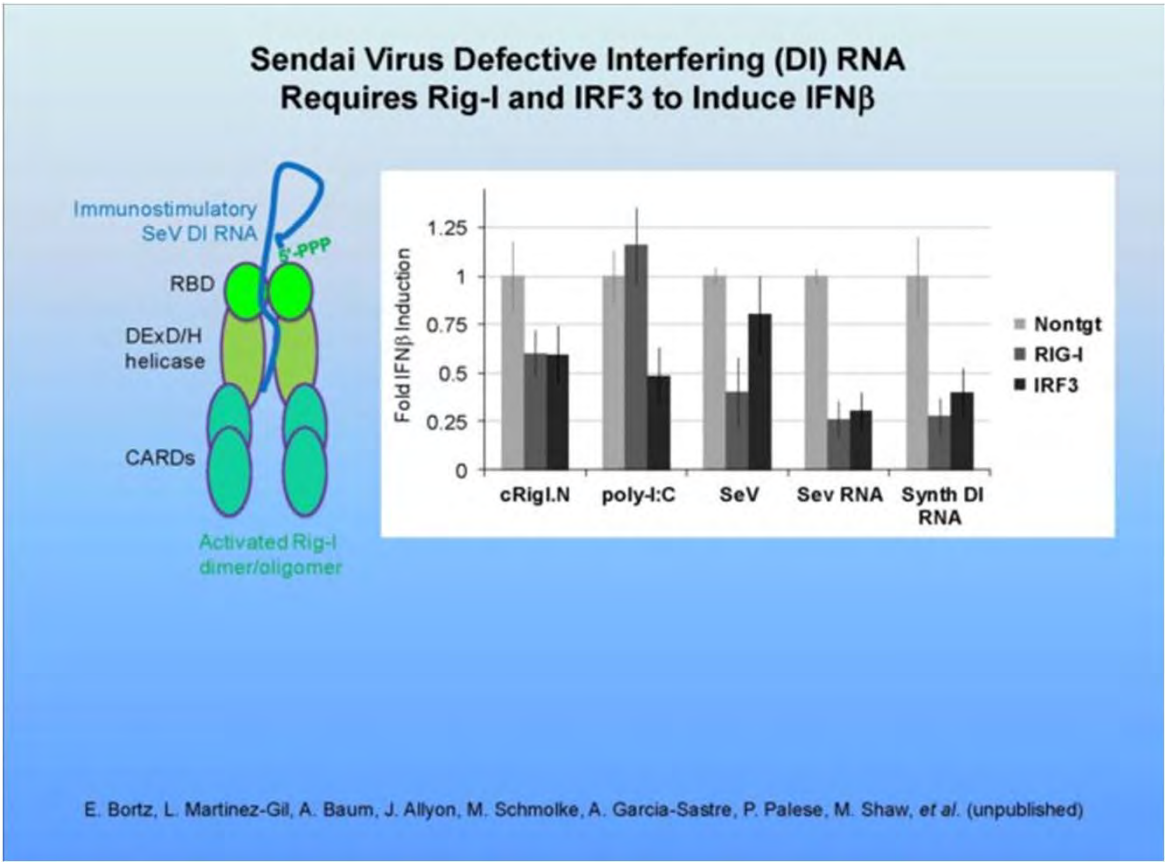
And if you knock down components of this pathway for example rigi it doesn't recognize the viral RNA, you don't very much signaling and you get weak luciferase and you can quantify what the response is if you knock down another factor like the RIF3 you get a similar response.



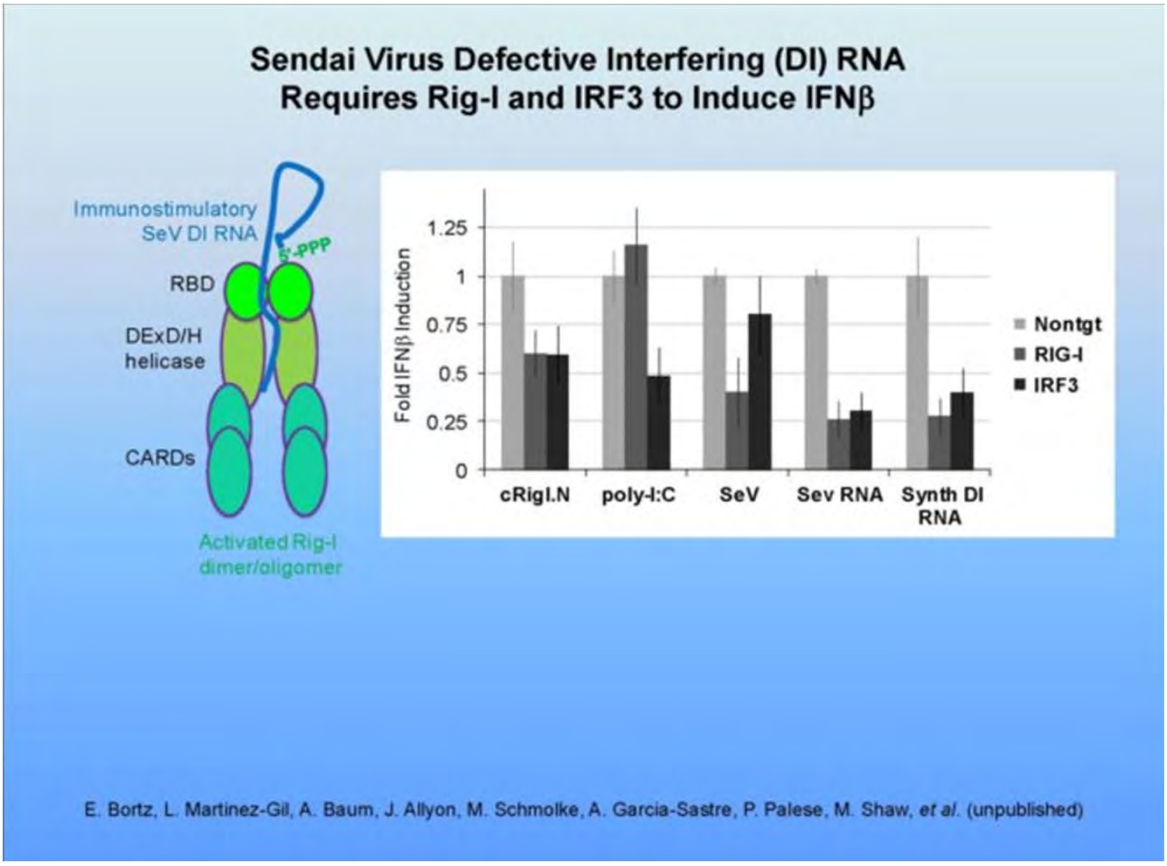
So the model virus for this instead of going straight to flu you've got eight gnome segments to deal with, Aldofo's lab with a talented graduate student, Alena, did a study with deep sequencing and found that in a paramyxovirus like which are also human viruses like paramyxovirus that will affect the lungs, will produce a defective interfering or small RNA particle, small RNA copy back RNA species at the end of the gnome and that rigi loves this. Rigi sees this and it will turn on the interferon response pathway and this is an idea that you could actually use this as a vaccine, for example, to turn on. So I've been doing a project in collaboration looking at this possibility of a vaccine, but also to look at using this RNA as a tool to study interferon beta induction pathways by virus. This is something we'll be able to apply to influenza.



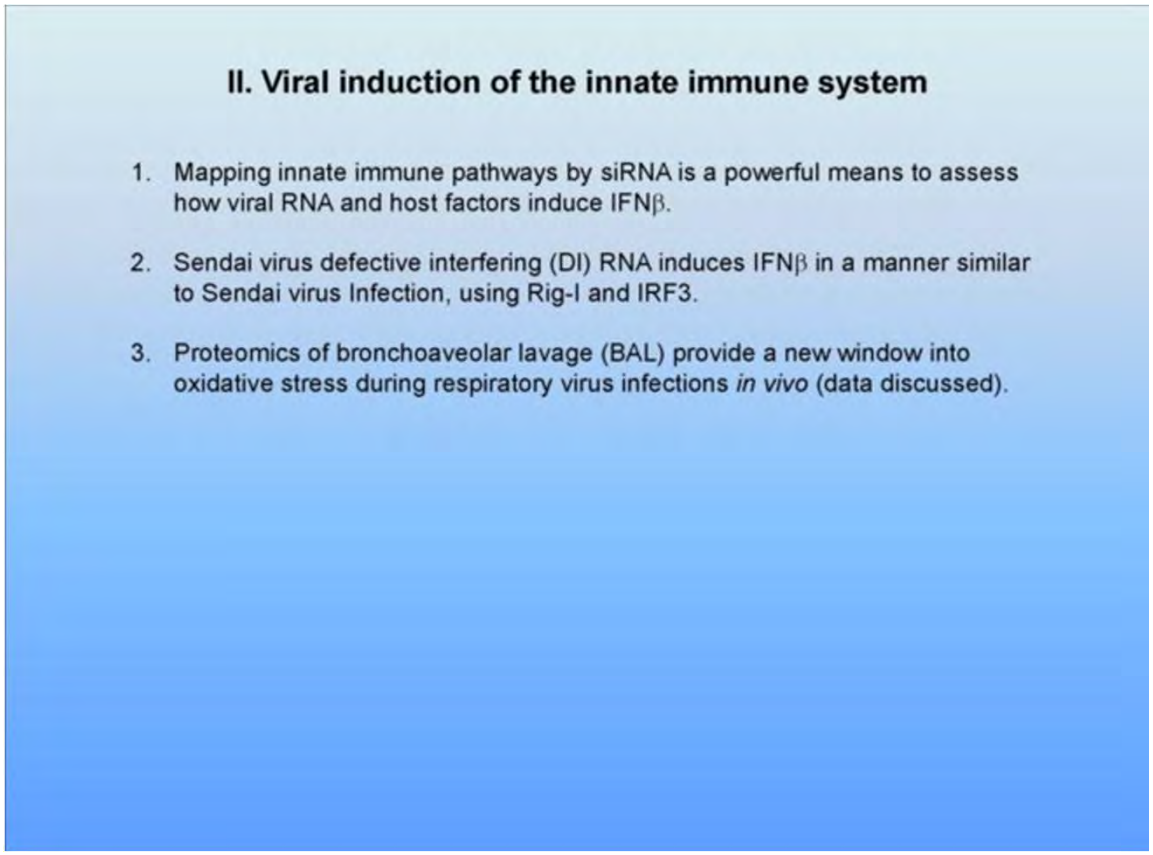
So rigi actually is an RNA helo case as well, like the DDX17 it recognizes immuno stimulatory viral defective interfering RNA or the small RNA and it will activate signaling through these card domains which will cast base recruitment domains they're called and they'll activate downstream signaling. RNA purified providers or invitro transcribed DIU all get interferon production, you also get interferon alpha production in the next step.



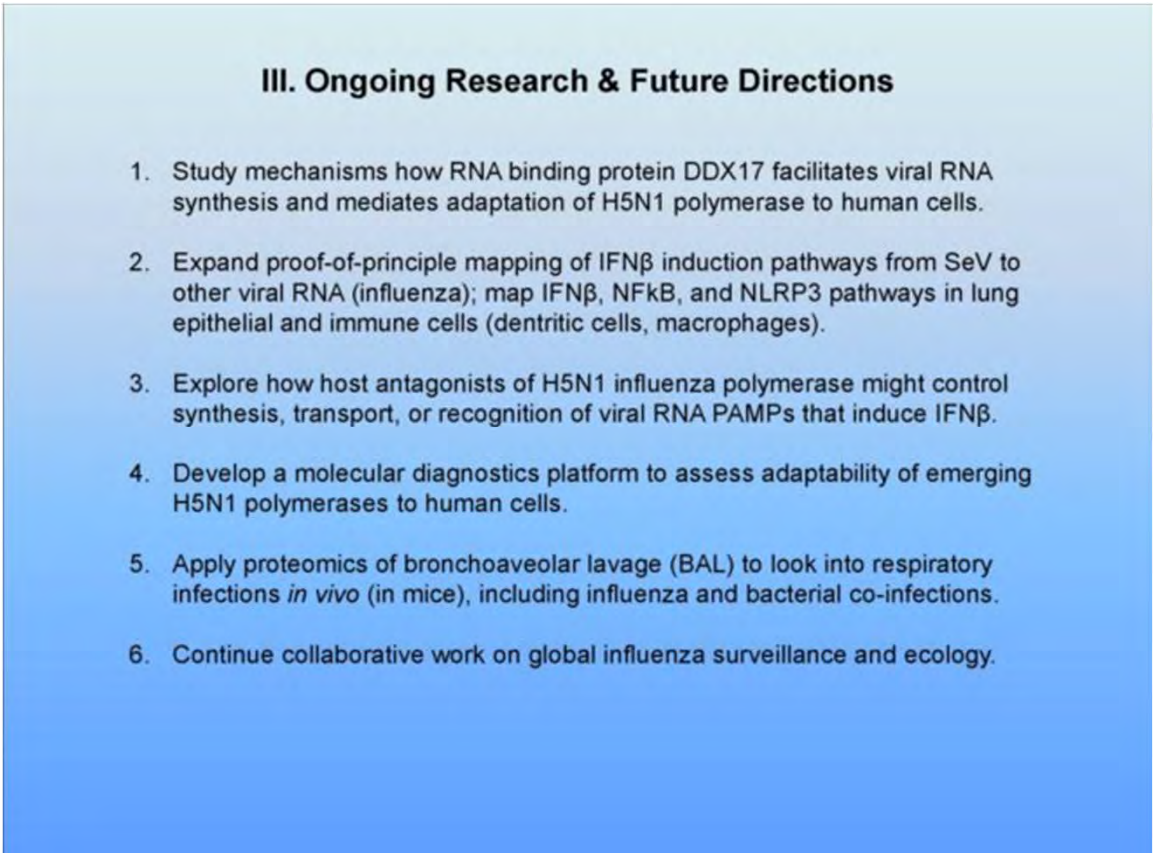
And if you knock down these targets with RNA interference, you see for example we knock down rigi, we use these RNA we get significantly diminished interferon induction, the same thing with RIF three. And this is similar synthetic DI, RNA and virus have similar profiles, but the virus seems to be also possibly activating other pathogen recognition receptors or activate interferon through. For example, through like receptors. The virus itself may also activate other receptors so you don't get a complete phenotype like you do for the RNA just by itself. And the RNA mimic poly IC doesn't seem to work for very much, the rigi it actually goes to another pathogen recognition receptor called the MVA5, mostly in this cell system. So this is a way to query how different viral RNA species which we can do for influenza which will activate interferon and moreover can look at this network. Do proteins in this network since they modulate the amount of RNA and the types of RNA that the viral polymerase are producing, are they also going to modulate the polymerases these RNA's activation of interferon responses and if they do, then these host factors have something to do with not only controlling the RNA synthesis and the early level of RNA the early production of RNA or inhibiting it in the case of several but also of stimulation to the innate immune response and possibly leading to things. In immunopathology you see NH5N1 infection. So I think the polymerase is going to be a very key molecular complex for HPAI to understand this.



And for Sendai virus, some of these same factors are known if you knock them down you get less interferon in response to virus infection, for example DDX3 KPNA2 that are from the same network and that's not that surprising because viruses tend to use some of the same RNA processes, especially for cytoplasmic protein viruses cytoplasmic so you expect the cytoplasmic ones to have a phenotype but for example the literature has a little bit of information about this already. RNA stress complex is HNRNP NF90 for example, we knock that down we get more interferon signaling and it may be involved in antagonizing influenza HIV but activating hepatitis C for example. So these are molecular targets to look at in terms of not only polymerase RNA synthesis but activation of interferon beta pathways.



So in summary for that the innate immune pathways, mapping them by SNRA, the powerful need to assess how viral RNA and host factors induce interferon beta virus defective interferon RNA induces interferon beta in a similar manner to infection using rigi and RIF3, or I should say purified RNA and aviolar lavage is the new way of looking into respiratory viruses, infections including of course influenza.



And some of the future directions I've mentioned as I've gone along but it's the study, the mechanisms of the DVX17 protein that's to expand the proof of principle with the interferon beta induction pathways from [inaudible] to influenza. It's to explore host antagonist factors and other factors in the network that might control induction of interferon for H5N1 viruses and thus the possibility of things like storm in the outcome of these infections. And these ideas, we know what host factors to look at and we have purified polymerase which we can clone from newly emerging strains, we can create a molecular diagnostic platform to look at new H5N1 viruses and assess their polymerases' ability to active -- its action in human cells so it looks like it's a polymerase that's going to induce lots of RNA -- lots of RNA dependent interferon pathways, is it going to produce high levels or RNA, is it going to be something to worry about, is this a genotype of influenza we should be worried about. And that information can be used along with other information with HA to maybe give a guess for any influenza H5N1, this is a strain really to watch. And then using mix to look at and as I said for bacteria for influenza and also the idea of doing a spector of co infections which I mentioned before and which was brought up this morning being something that's important in respiratory viruses. And I didn't really talk much about the work in surveillance on doing wild birds, but most of it's computation.

So this is the group I'm part of. Aldopho is here, he has been my adviser for -- as a post-doc and as a research assistant professor and still affiliated with his group and he's a really great mentor, he knows everything there is to know about influenza. He's quite a genius in the subject and Peter the department chairman. He is of the original generation of influenza virologists and he's really pushed this field forward to designing new kinds of vaccines, more effective and more timely vaccines against influenza and all of these people have contributed a lot to this project biology help, cloning DDX17, giving advice about RNA, about innate immunity. Alena did those experiments you saw with the deep sequencing. Randy knows everything there is to know about culturing every kind of influenza virus and any type of high path conditions he's really great with that. And did the very beautiful microscopy that you saw. John Steel he's made the halo virus, that attenuated virus and a number of other people have contributed. The mix of lavage experiment was done with my graduate school mentor at UCLA in collaboration with Julian at UCLA Mass Spectrometry Center. And I didn't mention the work done on viral structures with the Chinese Academy of Science now. They've taken over some of this with [inaudible] but that's something else so that's interesting. And there's a lot of other people that have contributed.

And then finally, this is New York City, this is where I work, it's Mt. Sinai, this building right by the reservoir. This is the reservoir here, this building's from Ghostbusters right here, that's the one -- it's right up the street from the Guggenheim Museum, people play baseball here. This new building right here, right next to Mt. Sinai, see, it's not even in this picture, the gleaming new glass building, it's part of Mt. Sinai campus but it's actually going to be residences, it's not labs unfortunately. So this is the work. Thank you.