Japan Academy Prize to:

Yoshihiro KAWAOKA
Professor, Institute of Medical Science,
The University of Tokyo
Professor, School of Veterinary Medicine,
The University of Wisconsin, USA
Director, International Research Center for Infectious Diseases, Institute of Medical Science, The University of Tokyo
Visiting Professor, Kyoto University

for "Molecular Basis of Pathogenicity and Control of Influenza Viruses"

Outline of the Work:

Influenza viruses infect not only humans but also other animals including poultry, pigs, and horses. The H5N1 highly pathogenic avian influenza virus that emerged in 1997 continues to circulate, causing devastating outbreaks in poultry and wild birds and humans with a fatality rate of approximately 60%. The H7N9 avian virus, which emerged in 2013, continues to infect humans with a fatality rate of approximately 30%. If these avian viruses acquire the ability to efficiently transmit among humans, this can result in a pandemic（worldwide epidemic）. Thus, avian influenza viruses can be devastating to the poultry industry and simultaneously pose a serious threat to public health. To effectively control these viruses, a thorough understanding of their pathogenicity is required.

I. Pathogenesis of highly pathogenic avian influenza viruses

Prof. Yoshihiro Kawaoka demonstrated that a single amino acid change in the viral protein hemagglutinin（HA）was responsible for the high virulence of an avian influenza virus that caused an outbreak in the US state of Pennsylvania in 1983[1,2]. In addition, he showed that the sequences of the cleavage site and nearby N-glycosylation sites of the HA determine avian influenza virus pathogenicity[3–6]. This discovery, now considered to be a fundamental principle in influenza virology, is applied to attenuate H5N1 virus strains for the production of pre-pandemic vaccine and to rapidly identify highly pathogenic avian influenza viruses by the US Department of Agriculture and the World Organization for Animal Health.

II. Establishment of a method to generate "designed" influenza viruses and its use for understanding viral pathogenesis

Prof. Kawaoka developed a novel technology, termed reverse genetics, which can be used to generate an influenza virus entirely from plasmids[7,8]. With this technology, Prof. Kawaoka demonstrated that mutations in the polymerase complex of avian influenza viruses increase pathogenicity in mammals and that only a few additional amino acid changes are required for these viruses to be transmissible among mammals[9–13]. Prof. Kawaoka found that receptors for avian influenza viruses are present in human lungs, but not in the upper airways; therefore, these viruses cause severe pneumonia when they infect humans[14,15]. Prof. Kawaoka used reverse genetics to generate the Spanish influenza virus（H1N1）and found that aberrant immune responses cause severe outcomes associated with this virus[16–18]. Moreover, he found that avian influenza viruses similar

to the Spanish influenza virus continue to circulate in nature and require only few minor changes to be transmissible in mammals[19]. In addition, Prof. Kawaoka reported that the 2009 pandemic virus（H1N1）and the H7N9 avian virus that emerged in China in 2013 can cause pneumonia in animal models[20-23]. These findings are essential for determining the pandemic potential of viruses in nature and preparing for future pandemics.

III. Influenza control

Live attenuated influenza vaccines generated by reverse genetics are used worldwide. Vaccines based on attenuated H5N1 avian influenza viruses, generated using reverse genetics, are effective in human clinical trials and are stockpiled worldwide, attesting to Prof. Kawaoka's contribution to pandemic preparedness.

Prof. Kawaoka discovered oseltamivir-resistance in seasonal and H5N1 viruses, and warned of the implications of this resistance with regard to public health[24-27]. Furthermore, he reported regarding a new influenza drug that is effective against the 2009 pandemic and H7N9 avian influenza viruses[20,23,28-30]. In addition, he has elucidated a mechanism of influenza virus genome packaging[31-35] and identified host gene products important for influenza virus replication[36-38]. These findings form the basis for developing novel antiviral compounds.

IV. Conclusion

In summary, Prof. Kawaoka established a method to artificially generate influenza viruses and used this method to further our understanding of influenza pathogenesis, resulting in a global contribution to the control of influenza. The technology developed by Prof. Kawaoka is used to generate proper virus strains for pandemic and seasonal influenza vaccines, and contribute to worldwide public health. Prof. Kawaoka is at the forefront of influenza research and development, with seminal discoveries in basic and applied influenza research. Therefore, Prof. Kawaoka's research has made major contributions to the control and prevention of avian influenza outbreak and pandemics.

References

1）Kawaoka Y, Naeve CW, Webster RG. Is virulence of H5N2 influenza viruses in chickens associated with loss of carbohydrate from the hemagglutinin? **Virology** 139: 303–316, 1984.

2）Kawaoka Y, Webster RG. Evolution of the A/Chicken/Pennsylvania/83（H5N2）influenza virus. **Virology** 146: 130–137, 1985.

3）Kawaoka Y, Webster RG. Sequence requirements for cleavage activation of influenza virus hemagglutinin expressed in mammalian cells. **Proc Natl Acad Sci USA** 85: 324–328, 1988.

4）Kawaoka Y, Webster RG. Interplay between carbohydrate in the stalk and the length of the connecting peptide determines the cleavability of influenza virus hemagglutinin. **J Virol** 63: 3296–3300, 1989.

5）Horimoto T, Kawaoka Y. Reverse genetics provides direct evidence for a correlation of hemagglutinin cleavability and virulence of an avian influenza A virus. **J Virol** 68: 3120–3128, 1994.

6）Horimoto T, Nakayama K, Smeekens SP, Kawaoka Y. Proprotein-processing endoproteases PC6 and furin both activate hemagglutinin of virulent avian influenza viruses. **J Virol** 68: 6074–6078, 1994.

7）Neumann G, Watanabe T, Ito H, Watanabe S, Goto H, Gao P, Hughes M, Perez DR, Donis R, Hoffmann E, Hobom G, Kawaoka Y. Generation of influenza A viruses entirely from cloned cDNAs. **Proc Natl Acad Sci USA** 96: 9345–9350, 1999.

8）Neumann G, Fujii K, Kino Y, Kawaoka Y. An improved reverse genetics system for influenza A virus

generation and its implications for vaccine production. **Proc Natl Acad Sci** 102: 16825–16829, 2005.
9) Hatta M, Gao P, Halfmann P, Kawaoka Y. Molecular basis for high virulence of Hong Kong H5N1 influenza A viruses. **Science** 298: 1840–1842, 2001.
10) Hatta M, Hatta Y, Kim JH, Watanabe S, Shinya K, Kawaoka Y. Growth of H5N1 influenza A viruses in the upper respiratory tracts of mice. **PLoS Pathog** 3: 1374–1379, 2007.
11) Horimoto T, Kawaoka Y. Influenza: Lessons from past pandemics, warnings from current incidents. **Nature Rev Microbiol** 8: 591–600, 2005.
12) Imai M, Watanabe H, Hatta M, Das SC, Ozawa M, Shinya K, Zhong G, Hanson A, Katsura H, Watanabe S, Li C, Kawakami E, Yamada S, Kiso M, Suzuki Y, Maher EA, Neumann G, Kawaoka Y. Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets. **Nature** 486: 420–428, 2012.
13) Neumann G, Macken CA, Karasin AI, Fouchier RA, Kawaoka Y. Egyptian H5N1 Influenza Viruses –Cause for Concern? **PLoS Pathog** 8: e1002932, 2012.
14) Shinya K, Ebina M, Yamada S, Ono M, Kasai N, Kawaoka Y. Avian flu: influenza virus receptors in the human airway. **Nature** 440: 435–436, 2006.
15) Yamada S, Suzuki Y, Suzuki T, Le MQ, Nidom CA, Sakai-Tagawa Y, Muramoto Y, Ito M, Kiso M, Horimoto T, Shinya K, Sawada T, Kiso K, Usui T, Murata T, Lin Y, Hay A, Haire LF, Stevens DJ, Russell RJ, Gamblin SJ, Skehel JJ, Kawaoka Y. Hemagglutinin mutations responsible for the binding of H5N1 influenza A viruses to human-type receptors. **Nature** 444: 378–382, 2006.
16) Kobasa D, Takada A, Shinya K, Hatta M, Halfmann P, Theriault S, Suzuki H, Nishimura H, Mitamura K, Sugaya N, Usui T, Murata T, Maeda Y, Watanabe S, Suresh M, Suzuki T, Suzuki Y, Feldmann H, Kawaoka Y. Enhanced virulence of influenza A viruses with the haemagglutinin of the 1918 pandemic virus. **Nature** 431: 703–707, 2004.
17) Kobasa D, Jones SM, Shinya K, Kash JC, Copps J, Ebihara H, Hatta Y, Kim JH, Halfmann P, Hatta M, Feldmann F, Alimonti JB, Fernando L, Li Y, Katze MG, Feldmann H, Kawaoka Y. Aberrant innate immune response in lethal infection of macaques with the 1918 influenza virus. **Nature** 445: 319–323, 2007.
18) Watanabe T, Watanabe S, Shinya K, Kim JH, Hatta M, Kawaoka Y. Viral RNA polymerase complex promotes optimal growth of 1918 virus in the lower respiratory tract of ferrets. **Proc Natl Acad Sci USA** 106: 588–592, 2009.
19) Watanabe T, Zhong G, Russell CA, Nakajima N, Hatta M, Hanson A, McBride R, Burke DF, Takahashi K, Fukuyama S, Tomita Y, Maher EA, Watanabe S, Imai M, Neumann G, Hasegawa H, Paulson JC, Smith DJ, Kawaoka Y. Circulating avian influenza viruses closely related to the 1918 virus have pandemic potential. **Cell Host Microbe** 15: 692–705, 2014.
20) Itoh Y, Shinya K, Kiso M, Watanabe T, Sakoda Y, Hatta M, Muramoto Y, Tamura D, Sakai-Tagawa Y, Noda T, Sakabe S, Imai M, Hatta Y, Watanabe S, Li C, Yamada S, Fujii K, Murakami S, Imai H, Kakugawa S, Ito M, Takano R, Iwatsuki-Horimoto K, Shimojima M, Horimoto T, Goto H, Takahashi K, Makino A, Ishigaki H, Nakayama M, Okamatsu M, Takahashi K, Warshauer D, Shult PA, Saito R, Suzuki H, Furuta Y, Yamashita M, Mitamura K, Nakano K, Nakamura M, Brockman-Schneider R, Mitamura H, Yamazaki M, Sugaya N, Suresh M, Ozawa M, Neumann G, Gern J, Kida H, Ogasawara K, Kawaoka Y. *In vitro* and *in vivo* characterization of new swine-origin H1N1 influenza viruses. **Nature** 460: 1021–1025, 2009.
21) Yamada S, Hatta M, Staker B, Watanabe A, Imai M, Shinya K, Sakai-Tagawa Y, Ito M, Ozawa M, Watanabe T, Li C, Kim JH, Myler PJ, Phan I, Raymond A, Smith E, Stacy R, Nidom CA, Lank SM, Wiseman RW, Bimber BN, O'Connor DH, Neumann G, Stewart LJ, Kawaoka Y. Biological and structural characterization of a host-adapting amino acid in influenza virus. **PLoS Pathog** 6: e1001034, 2010.
22) Neumann G, Noda T, Kawaoka Y. Emergence and pandemic potential of H1N1 influenza virus. **Nature**

459: 931–939, 2009.
23) Watanabe T, Kiso M, Fukuyama S, Nakajima N, Imai M, Yamada S, Murakami S, Yamayoshi S, Iwatsuki-Horimoto K, Sakoda Y, Takashita E, McBride R, Noda T, Hatta M, Imai H, Zhao D, Kishida N, Shirakura M, de Vries RP, Shichinohe S, Okamatsu M, Tamura T, Tomita Y, Fujimoto N, Goto K, Katsura H, Kawakami E, Ishikawa I, Watanabe S, Ito M, Sakai-Tagawa Y, Sugita Y, Uraki R, Yamaji R, Eisfeld AJ, Zhong G, Fan S, Ping J, Maher EA, Hanson A, Uchida Y, Saito T, Ozawa M, Neumann G, Kida H, Odagiri T, Paulson JC, Hasegawa H, Tashiro M, Kawaoka Y. Characterization of H7N9 influenza A viruses isolated from humans. **Nature** 501: 551–555, 2013.
24) Kiso M, Mitamura K, Sakai-Tagawa Y, Shiraishi K, Kawakami C, Kimura K, Hayden FG, Sugaya N, Kawaoka Y. Resistant influenza A viruses in children treated with oseltamivir: descriptive study. **Lancet** 364: 759–765, 2004.
25) Le QM, Kiso M, Someya K, Sakai YT, Nguyen TH, Nguyen KY, Pham ND, Nguyen HH, Yamada S, Muramoto Y, Takada A, Goto H, Suzuki T, Suzuki Y, Kawaoka Y. Avian flu: isolation of drug-resistant H5N1 virus. **Nature** 437: 1108, 2005.
26) Hatakeyama S, Sugaya N, Ito M, Yamazaki M, Ichikawa M, Kimura K, Kiso M, Shimizu H, Kawakami C, Koike K, Mitamura K, Kawaoka Y. Emergence of influenza B viruses with reduced sensitivity to neuraminidase inhibitors. **JAMA** 297: 1435–1442, 2007.
27) Gambotto A, Barratt-Boyes SM, de Jong MD, Neumann G, Kawaoka Y. Highly pathogenic H5N1 influenza virus infection in humans. **Lancet** 371: 1464–1475, 2008.
28) Kiso M, Takahashi K, Sakai-Tagawa Y, Shinya K, Sakabe S, Le QM, Ozawa M, Furuta Y, Kawaoka Y. T-705（favipiravir）activity against lethal H5N1 influenza A viruses. **Proc Natl Acad Sci USA** 107: 882–887, 2010.
29) Kiso M, Kubo S, Ozawa M, Le QM, Nidom CA, Yamashita M, Kawaoka Y. Efficacy of the new neuraminidase inhibitor CS-8958 against H5N1 influenza viruses. **PLoS Pathog** 6: 21000786, 2010.
30) Kiso M, Shinya K, Shimojima M, Takano R, Takahashi K, Katsura H, Kakugawa S, Le MQ, Yamashita M, Furuta Y, Ozawa M, Kawaoka Y. Characterization of oseltamivir-resistant 2009 H1N1 pandemic influenza A viruses. **PLoS Pathog** 6: e1001079, 2010.
31) Fujii Y, Goto H, Watanabe T, Yoshida T, Kawaoka Y. Selective incorporation of influenza virus RNA segments into virions. **Proc Natl Acad Sci USA** 100: 2002–2007, 2003.
32) Noda T, Sagara H, Yen A, Takada A, Kida H, Cheng RH, Kawaoka Y. Architecture of ribonucleoprotein complexes in influenza A viruses particles. **Nature** 439: 490–492, 2006.
33) Li C, Hatta M, Nidom CA, Muramoto Y, Watanabe S, Neumann G, Kawaoka Y. Reassortment between avian H5N1 and human H3N2 influenza viruses creates hybrid viruses with substantial virulence. **Proc Natl Acad Sci USA** 107: 4678–4683, 2010.
34) Noda T, Sugita Y, Aoyama K, Hirase A, Kawakami E, Miyazawa A, Sagara H, Kawaoka Y. Three-dimensional analysis of ribonucleoprotein complexes in influenza A virus. **Nat Commun** 3: 639, 2012.
35) Noda T, Kawaoka Y. Packaging of influenza virus genome: Robustness of selection. **Proc Natl Acad Sci USA** 109: 8797–8798, 2012.
36) Hao L, Sakurai A, Watanabe T, Sorensen E, Nidom CA, Newton MA, Ahlquist P, Kawaoka Y. Drosophila RNAi screen identifies host genes important for influenza virus replication. **Nature** 454: 890–893, 2008.
37) Gorai T, Goto H, Noda T, Watanabe T, Kozuka-Hata H, Oyama M, Takano R, Neumann G, Watanabe S, Kawaoka Y. The F1Fo-ATPase, F-type proton-translocating ATPase, at the plasma membrane is critical for efficient influenza virus budding. **Proc Natl Acad Sci USA** 109: 4615–4620, 2012.
38) Watanabe T, Watanabe S, Kawaoka Y. Cellular networks involved in the influenza virus life cycle. **Cell Host Microbe** 7: 427–439, 2010.