AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Virology

GEM

To Conquer the Host, Influenza Virus Is Packing It In: Interferon-Antagonistic Strategies beyond NS1

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The nonstructural protein NS1 is well established as a virulence factor of influenza A virus counteracting induction of the antiviral type I interferon system. Recent studies now show that viral structural proteins, their derivatives, and even the genome itself also contribute to keeping the host defense under control. Here, we summarize the current knowledge on these NS1-independent interferon escape strategies.

Influenza A virus (FLUAV; family *Orthomyxoviridae*) is a truly global threat. From the virus reservoirs in aquatic birds, new strains are constantly spilling over into poultry, swine, and humans, causing regular epidemics and pandemics, with serious illness and substantial economic losses (1). FLUAV particles have a lipid envelope with the transmembrane proteins hemagglutinin (HA), neuraminidase (NA), and M2 inserted. The inner leaflet of the lipid bilayer is covered by the M1 protein that connects to the genome-containing ribonucleoproteins (RNPs) inside. The FLUAV genome is divided into 8 segments of negative-strand RNA. In the RNPs, each RNA segment is packaged along its length by viral nucleoprotein (NP), while the partially complementary 5′ and 3′ RNA ends are held together by the viral-RNA-dependent RNA polymerase (RdRP, consisting of subunits PB1, PB2, and PA) (2). The structural proteins HA, NA, M1, M2, NP, PB1, PB2, and PA (and the nuclear export protein [NEP]) drive the basic viral replication cycle. In infected cells, additional nonstructural proteins are produced to support viral propagation. Of these, the nonstructural protein NS1 (of which low levels are also present in virions [3]) is a well-known antagonist of the antiviral type I interferon (IFN-α/β) system (4, 5). However, it is becoming increasingly clear that escaping innate immunity is a task that requires more than one factor. Here, we summarize the current knowledge of the function of structural virus components in counteracting the IFN system.

INNATE IMMUNITY AT A GLANCE

Antiviral responses are stimulated by conserved molecular features of pathogens. Specific pathogen recognition receptors (PRRs) of the host recognize so-called pathogen-associated molecular patterns (PAMPs) as nonself. Typical viral PAMPs are conserved nucleic acid structures, most prominently double-stranded RNA (dsRNA) (6). PRR-triggered signaling eventually results in the synthesis of IFN-α/β, cytokines which establish an antiviral state in the cell by docking onto their cognate receptor (IFNAR) and upregulating IFN-stimulated genes (ISGs) via the so-called JAK/STAT pathway (Fig. 1). Many products of ISGs are able to either inhibit specific stages of infection or generally hamper viral propagation by destroying viral RNA, blocking translation, or inducing cell death (7).

INFLUENZA A VIRUS AND RIG-I

The dominant PRR recognizing FLUAV infection is cytoplasmic retinoic acid-inducible gene I (RIG-I) (8). RIG-I possesses two N-terminal caspase recruitment domains (CARDs), a central RNA helicase domain of the DExD/H box type, and a C-terminal domain (CTD) that is important for RNA ligand binding (9). RIG-I responds strongly to 5′-end-triphosphorylated dsRNA structures (5′-ppp-dsRNA), like the "panhandle," which can be formed by complementary sequences of the 5′ and 3′ termini of the FLUAV genome (10–12). The binding of RIG-I to the FLUAV panhandle occurs immediately after the RNPs enter the host cell and can impose a direct antiviral effect via the disassembling of the RdRP complex (13). Also, for other viral systems, it was shown that the binding of RIG-I to regulatory RNA structures can restrict viral functions and result in such a signaling-independent inhibitory activity (14, 15). RIG-I activators of FLUAV besides the panhandle structure are erroneous RNA replication products (16) and U/A-rich sequences in the 3′ untranslated region (UTR) of the genome segments (17). In all cases, upon detection of the RNA ligand, RIG-I exposes the CARDs and interacts with the mitochondrial antiviral signaling (MAVS) adapter molecule to assemble a signaling platform that activates IFN regulatory factor 3 (IRF-3) and other transcription factors of the IFN system (6, 9). Thus, the binding of FLUAV PAMPs by RIG-I results in antiviral signaling and the expression of IFNs and ISGs. Moreover, RIG-I can slow down viral propagation in a direct manner.

RIG-I ESCAPE MECHANISMS BY STRUCTURAL COMPONENTS OF FLUAV

FLUAV exhibits a wide variety of evasion strategies (Fig. 2). First of all, it is conceivable that replication in the nucleus, which is quite unusual for an RNA virus, has evolved to minimize exposure of the 8 genomic RNAs to cytoplasmic RIG-I. The well-known and major anti-IFN factor not covered here, the nonstructural protein NS1, targets dsRNA, RIG-I cofactors, antiviral ISGs, and host cell mRNA synthesis (4, 5). However, structural proteins, like the components of the RNPs, i.e., NP, PB1, PB2, PA, and even the

Accepted manuscript posted online 20 July 2016

Citation Weber-Gerlach M, Weber F. 2016. To conquer the host, influenza virus is packing it in: interferon-antagonistic strategies beyond NS1. J Virol 90:8389–8394. doi:10.1128/JVI.00041-16.

Editor: M. Gack, Harvard Medical School

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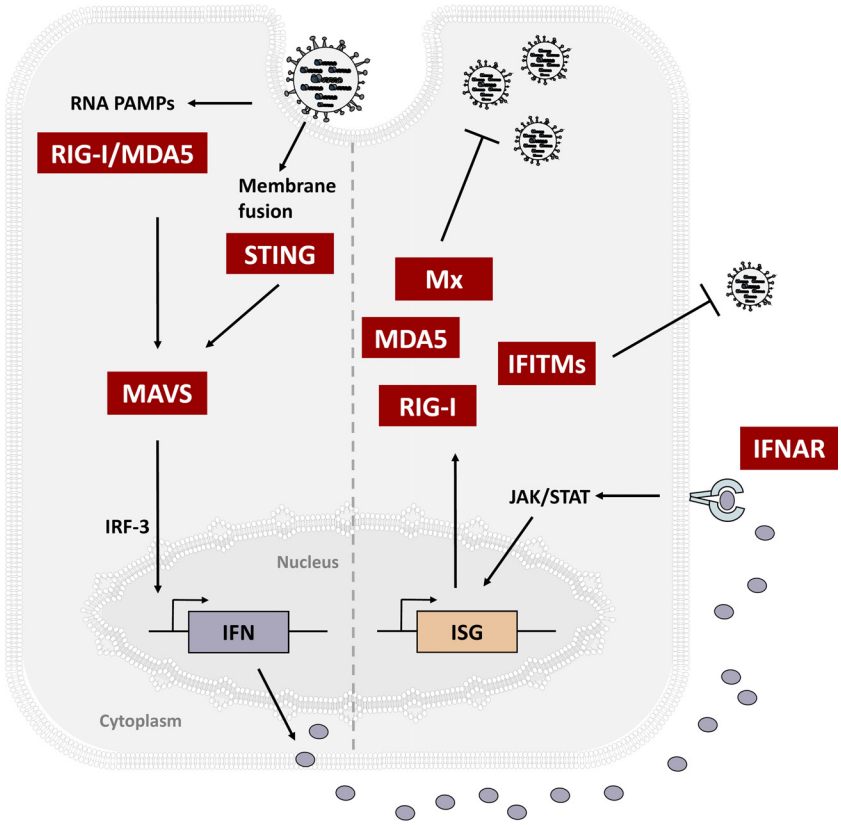

FIG 1 Innate immunity pathways that are targeted by structural proteins of FLUAV. RNA PAMPs of FLUAV activate RIG-I and MDA5 (and therefore the MAVS adaptor molecule), whereas membrane fusion activates STING. These PRR signaling pathways converge on the transcription factor IRF-3 for IFN induction. Secreted IFNs dock onto their receptor, IFNAR, and mediate expression of antiviral ISGs via JAK/STAT signaling. The ISG products Mx and IFN-induced transmembrane (IFITM) protein are involved in IFN-mediated inhibition of FLUAV.

genomic RNA itself, also indirectly or directly contribute to impairing RIG-I-mediated antiviral responses. Access of RIG-I to the 5′-ppp-dsRNA panhandle and the U/A-rich sequences in the 3′ UTR is hindered by different means. NP covers the viral RNA, thereby preventing the formation of extensive dsRNA structures (18, 19), and the viral RdRP complex binds the 5′ and 3′ termini of the panhandle (2). Thus, encapsidation by NP and RdRP limits the availability of the viral PAMPs to RIG-I and other PRRs. In line with this, measures that affect the stability of the viral RdRP have repercussions on RIG-I activation. A mammal-adapted mutation (from bird-adapted PB2-627E to PB2-627K) that increases the binding of PB2 to NP (20) strongly reduces RIG-I activation by RNPs, whereas artificial RdRP disruption by a PB1-derived inhibitor peptide boosts RIG-I activation (13). The viral RNA also contributes to RIG-I escape. First, the 5′-ppp and 3′-OH ends do not form a perfect dsRNA stretch but rather fold into a hook-like structure (2), and second, there are nucleotide mismatches that further reduce dsRNA formation and, hence, RIG-I interaction (21). Besides its structural role, NP also diminishes PRR activation by recruiting the cellular RNA helicases UAP56 and URH49, supposedly by unwinding any dsRNA that arises during genome replication (19). Moreover, two studies found that RNPs can interact with RIG-I or sequester it to the nucleus (22, 23). PB1, PB2, and PA also interact with the host cell RNA polymerase II repressor DR1, which downregulates the expression of FLUAV-relevant ISGs, like *RIG-I*, *MDA5*, *MX1*, and *IFITM* (24). DR1 was originally identified as a positive regulator of FLUAV replication (25), and it is likely that DR1 recruitment by FLUAV proteins contributes to suppression of the RIG-I pathway.

Several RNPs or their derivatives also target the RIG-I signaling MAVS adaptor molecule. The accessory proteins PA-X and PB1-F2 are frameshift products of the PA and PB1 genes, respectively, that have been linked to innate immune response inhibition (26–28). PA-X is an endonuclease that cleaves host cell mRNAs (26). PB1-F2 associates via a C-terminal portion with the MAVS adaptor, and this interaction can be enhanced by an asparagine-to-serine exchange at position 66 (N66S) that is present in virulent strains (27). PB1-F2–MAVS adaptor interaction decreases the mitochondrial membrane potential required for MAVS adaptor-mediated antiviral signaling and thus robust IFN induction (28). Also, the full-length RdRP subunits, especially PB2, can target the MAVS adaptor (29, 30). An amino acid change at amino acid residue 9 from bird-adapted aspartic acid to mammal-adapted asparagine (N9D) results in PB2 translocation to mitochondria and reduced MAVS adaptor-mediated IFN induction by FLUAV (29). Although the exact mechanism of how PB2-9D affects MAVS has not been resolved so far, the facts that this key amino acid is close to the MAVS adaptor interaction domain of PB2 (31), that the polymorphism is maintained in most of the seasonal FLUAV strains, and that the polymorphism is associated with increased virulence in mice (29, 32) highlight the importance of this residue in mammalian-host adaptation. Curiously, a 10-kDa frag-

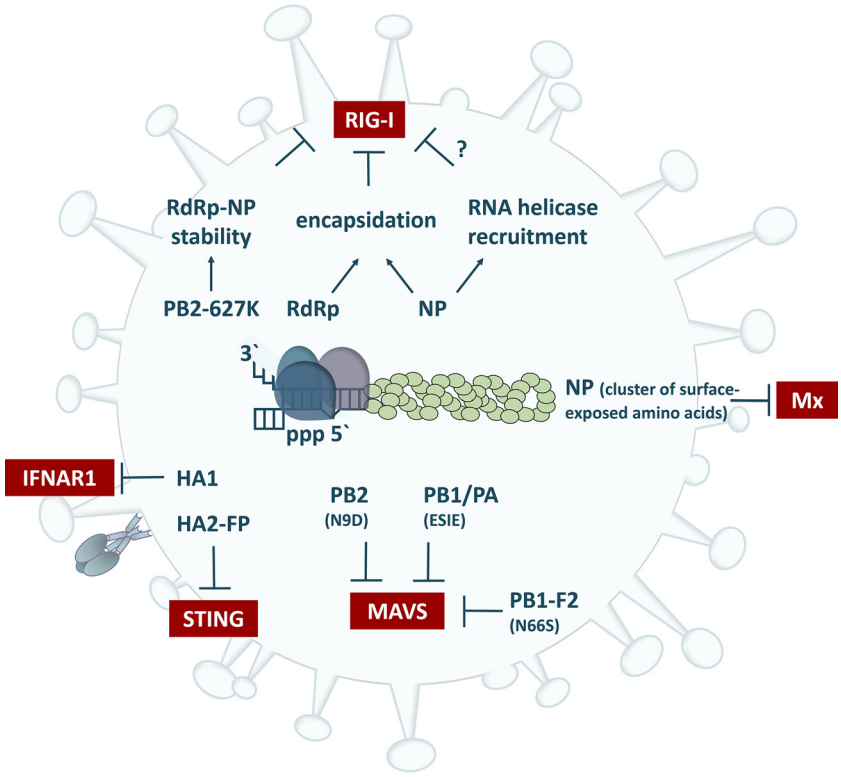

FIG 2 Influenza virus structural proteins and their derivatives restrict innate immune responses. Encapsidation of the viral genome and enhanced RdRP-NP interaction provided by PB2-627K interfere with RNP recognition by RIG-I. Additionally, NP recruits host cell RNA helicases to avoid dsRNA production, most likely also impairing RIG-I signaling. Adaptive mutations in influenza virus PB1-F2, PB1, PB2, PA, and NP counteract MAVS adaptor-mediated downstream signaling or provide MxA escape, as indicated. The fusion peptide of HA2 (HA2-FP) blocks STING activation, and HA1 degrades the IFN receptor subunit IFNAR1.

ment of PB2 (PB2Δ) that is produced by defective interfering FLUAV particles directly interacts with MAVS and activates antiviral signaling rather than inhibiting it like the full-length PB2 (33). In addition to full-length PB2-9D, both PB1 and PA contain the amino acid motif ESIE, which interferes with the recruitment of RNPs to mitochondria, thus contributing to the impairment of RIG-I–MAVS adaptor signaling and an increase in virulence (23).

RIG-I-INDEPENDENT CYTOPLASMIC RESPONSES AND FLUAV COUNTERMEASURES

Recently, the stimulator of IFN genes (STING) and the RIG-I-like PRR, melanoma differentiation-associated protein-5 (MDA5), signaling factors were identified as contributors to the antiviral response against FLUAV (34–36). STING is known as a downstream signaling adapter of the DNA PRR cyclic GMP-AMP synthase (cGAS) (37). Holm et al. reported that a STING-dependent, but cGAS-independent, pathway is activated upon FLUAV entry (35). Fusion of the viral envelope with the host endosome membrane can stimulate STING and hence IFN induction; however, FLUAV counteracts this via the fusion peptide of HA subunit 2 (HA2-FP), which associates with STING and prevents its activation (35). Interestingly, subunit 1 of HA (HA1) was recently shown to drive the degradation of the IFN receptor chain IFNAR1, thereby suppressing IFN-triggered JAK/STAT signaling (38). Thus, even the viral envelope proteins are involved in innate immune escape.

In mammalian cells, STING interacts with RIG-I and MAVS and supports early IFN induction (39, 40). Chickens lack the RIG-I gene *ddx58* (41), and IFN induction is mediated by the related RNA helicase and PRR, MDA5 (36). In contrast to mammalian STING, chicken STING forms a complex with MDA5 (and the MAVS adaptor) to induce IFN at later stages of FLUAV infection (42). Moreover, chicken MDA5 was recently identified to sense short dsRNAs, just like mammalian RIG-I (but unlike mammalian MDA5) does (43). Thus, chicken MDA5 can at least partially compensate for the lack of RIG-I in these animals, but FLUAV can counteract this by dsRNA sequestering and the anti-MAVS activities of NS1 and PB2 (36, 44).

EVASION FROM RESTRICTION BY Mx, THE KEY ISG AGAINST FLUAV

RIG-I-, MDA5-, and STING-mediated host responses to FLUAV result in the expression of numerous ISGs, which elicit a broad variety of antiviral effects (7, 9). Among the ISG products, the Mx family of large GTPases is key to the antiviral effect of IFN against FLUAV (45, 46). The human MxA protein interacts with orthomyxovirus NP, particularly if it is part of the RNPs (47–51). MxA acts together with the NP interactors UAP56 and URH49 (see above) (52) and possibly other IFN-induced cofactors, and it restricts the access of RNPs to the nucleus, thus impairing viral primary transcription (53). Mx proteins also interfere with viral genome replication, most likely by the sequestration of NP and PB2 (51, 54–56).

In line with the documented MxA-RNP interaction, it was

shown that MxA sensitivity is determined by a cluster of surface-exposed amino acids on the NP of human pandemic FLUAV strains from 1918 and 2009 (57–59). Interestingly, however, these MxA escape adaptations impair at the same time the trafficking of RNPs into the nucleus, resulting in genetic instability and loss of viral fitness (58, 60). Therefore, when comparable adaptive NP mutations were introduced into avian H5N1, compensatory mutations appeared that rescued viral fitness (60). However, these compensatory mutations (with one exception) again increased MxA sensitivity. Notably, the recently emerged FLUAV strain H7N9 contains another MxA escape mutation in NP (52N) that does not hamper viral fitness too much (61). These observations nicely illustrate the evolutionary trade-off involved in host adaptations and indicate that human MxA poses a barrier to avian FLUAV strains that is difficult but not impossible to overcome by changes in the structural protein NP.

CONCLUSIONS

As the FLUAV RdRP has a high error rate, it constantly generates an extensive pool of viral quasispecies (1). Recently, FLUAV was passaged onto IFN-deficient cells, and the resulting virus mutants were characterized by deep sequencing and IFN induction assays (62). Surprisingly, only few amino acid substitutions occurred in the well-established IFN antagonist NS1. Rather, in the absence of IFN pressure, mutations arose in all structural proteins except NP. Thus, although NS1 appears to be the most potent IFN antagonist of FLUAV (27, 29, 30, 62), more than one factor and strategy are required to efficiently suppress activation of the powerful IFN response.

With every newly emerging strain, FLUAV provides further proof that the barriers that are imposed by the cellular antiviral defense systems might be more of a challenge for virologists to understand than for the virus to overcome.

ACKNOWLEDGMENTS

We thank Georg Kochs, Martin Schwemmle, and Jacob Michael Cabrejas for critically reading the manuscript.

FUNDING INFORMATION

This work, including the efforts of Friedemann Weber, was funded by Deutsche Forschungsgemeinschaft (DFG) (SFB 1021 and SPP 1596). This work, including the efforts of Michaela Weber-Gerlach, was funded by Japan Society for the Promotion of Science (JSPS).

REFERENCES

1. **Klenk HD.** 2014. Influenza viruses en route from birds to man. Cell Host Microbe **15:**653–654. http://dx.doi.org/10.1016/j.chom.2014.05.019.
2. **Pflug A, Guilligay D, Reich S, Cusack S.** 2014. Structure of influenza A polymerase bound to the viral RNA promoter. Nature **516:**355–360. http://dx.doi.org/10.1038/nature14008.
3. **Hutchinson EC, Charles PD, Hester SS, Thomas B, Trudgian D, Martinez-Alonso M, Fodor E.** 2014. Conserved and host-specific features of influenza virion architecture. Nat Commun **5:**4816. http://dx.doi.org/10.1038/ncomms5816.
4. **Ayllon J, Garcia-Sastre A.** 2015. The NS1 protein: a multitasking virulence factor. Curr Top Microbiol Immunol **386:**73–107.
5. **Krug RM.** 2015. Functions of the influenza A virus NS1 protein in antiviral defense. Curr Opin Virol **12:**1–6. http://dx.doi.org/10.1016/j.coviro.2015.01.007.
6. **Chan YK, Gack MU.** 2016. Viral evasion of intracellular DNA and RNA sensing. Nat Rev Microbiol **14:**360–373. http://dx.doi.org/10.1038/nrmicro.2016.45.
7. **Schneider WM, Chevillotte MD, Rice CM.** 2014. Interferon-stimulated genes: a complex web of host defenses. Annu Rev Immunol **32:**513–545. http://dx.doi.org/10.1146/annurev-immunol-032713-120231.
8. **Kato H, Takeuchi O, Sato S, Yoneyama M, Yamamoto M, Matsui K, Uematsu S, Jung A, Kawai T, Ishii KJ, Yamaguchi O, Otsu K, Tsujimura T, Koh CS, Reis e Sousa C, Matsuura Y, Fujita T, Akira S.** 2006. Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. Nature **441:**101–105. http://dx.doi.org/10.1038/nature04734.
9. **Kato H, Takahasi K, Fujita T.** 2011. RIG-I-like receptors: cytoplasmic sensors for non-self RNA. Immunol Rev **243:**91–98. http://dx.doi.org/10.1111/j.1600-065X.2011.01052.x.
10. **Liu G, Park HS, Pyo HM, Liu Q, Zhou Y.** 2015. Influenza A virus panhandle structure is directly involved in RIG-I activation and interferon induction. J Virol **89:**6067–6079. http://dx.doi.org/10.1128/JVI.00232-15.
11. **Pichlmair A, Schulz O, Tan CP, Naslund TI, Liljestrom P, Weber F, Reis e Sousa C.** 2006. RIG-I-mediated antiviral responses to single-stranded RNA bearing 5′-phosphates. Science **314:**997–1001. http://dx.doi.org/10.1126/science.1132998.
12. **Rehwinkel J, Tan CP, Goubau D, Schulz O, Pichlmair A, Bier K, Robb N, Vreede F, Barclay W, Fodor E, Reis e Sousa C.** 2010. RIG-I detects viral genomic RNA during negative-strand RNA virus infection. Cell **140:**397–408. http://dx.doi.org/10.1016/j.cell.2010.01.020.
13. **Weber M, Sediri H, Felgenhauer U, Binzen I, Banfer S, Jacob R, Brunotte L, Garcia-Sastre A, Schmid-Burgk JL, Schmidt T, Hornung V, Kochs G, Schwemmle M, Klenk HD, Weber F.** 2015. Influenza virus adaptation PB2-627K modulates nucleocapsid inhibition by the pathogen sensor RIG-I. Cell Host Microbe **17:**309–319.
14. **Sato S, Li K, Kameyama T, Hayashi T, Ishida Y, Murakami S, Watanabe T, Iijima S, Sakurai Y, Watashi K, Tsutsumi S, Sato Y, Akita H, Wakita T, Rice CM, Harashima H, Kohara M, Tanaka Y, Takaoka A.** 2015. The RNA sensor RIG-I dually functions as an innate sensor and direct antiviral factor for hepatitis B virus. Immunity **42:**123–132. http://dx.doi.org/10.1016/j.immuni.2014.12.016.
15. **Yao H, Dittmann M, Peisley A, Hoffmann HH, Gilmore RH, Schmidt T, Schmid-Burgk JL, Hornung V, Rice CM, Hur S.** 2015. ATP-dependent effector-like functions of RIG-I-like receptors. Mol Cell **58:**541–548. http://dx.doi.org/10.1016/j.molcel.2015.03.014.
16. **Baum A, Sachidanandam R, Garcia-Sastre A.** 2010. Preference of RIG-I for short viral RNA molecules in infected cells revealed by next-generation sequencing. Proc Natl Acad Sci U S A **107:**16303–16308. http://dx.doi.org/10.1073/pnas.1005077107.
17. **Davis WG, Bowzard JB, Sharma SD, Wiens ME, Ranjan P, Gangappa S, Stuchlik O, Pohl J, Donis RO, Katz JM, Cameron CE, Fujita T, Sambhara S.** 2012. The 3′ untranslated regions of influenza genomic sequences are 5′PPP-independent ligands for RIG-I. PLoS One **7:**e32661. http://dx.doi.org/10.1371/journal.pone.0032661.
18. **Weber F, Wagner V, Rasmussen SB, Hartmann R, Paludan SR.** 2006. Double-stranded RNA is produced by positive-strand RNA viruses and DNA viruses but not in detectable amounts by negative-strand RNA viruses. J Virol **80:**5059–5064. http://dx.doi.org/10.1128/JVI.80.10.5059-5064.2006.
19. **Wisskirchen C, Ludersdorfer TH, Muller DA, Moritz E, Pavlovic J.** 2011. The cellular RNA helicase UAP56 is required for prevention of double-stranded RNA formation during influenza A virus infection. J Virol **85:**8646–8655. http://dx.doi.org/10.1128/JVI.02559-10.
20. **Labadie K, Dos Santos Afonso E, Rameix-Welti MA, van der Werf S, Naffakh N.** 2007. Host-range determinants on the PB2 protein of influenza A viruses control the interaction between the viral polymerase and nucleoprotein in human cells. Virology **362:**271–282. http://dx.doi.org/10.1016/j.virol.2006.12.027.
21. **Anchisi S, Guerra J, Mottet-Osman G, Garcin D.** 2015. Mismatches in the influenza A virus RNA panhandle prevent retinoic acid-inducible gene I (RIG-I) sensing by impairing RNA/RIG-I complex formation. J Virol **90:**586–590.
22. **Li W, Chen H, Sutton T, Obadan A, Perez DR.** 2014. Interactions between the influenza A virus RNA polymerase components and retinoic acid-inducible gene I. J Virol **88:**10432–10447. http://dx.doi.org/10.1128/JVI.01383-14.
23. **Liedmann S, Hrincius ER, Guy C, Anhlan D, Dierkes R, Carter R, Wu G, Staeheli P, Green DR, Wolff T, McCullers JA, Ludwig S, Ehrhardt C.** 2014. Viral suppressors of the RIG-I-mediated interferon response are pre-packaged in influenza virions. Nat Commun **5:**5645. http://dx.doi.org/10.1038/ncomms6645.

24. **Hsu SF, Su WC, Jeng KS, Lai MM.** 2015. A host susceptibility gene, DR1, facilitates influenza A virus replication by suppressing host innate immunity and enhancing viral RNA replication. J Virol **89:**3671–3682. http://dx.doi.org/10.1128/JVI.03610-14.
25. **Su WC, Chen YC, Tseng CH, Hsu PW, Tung KF, Jeng KS, Lai MM.** 2013. Pooled RNAi screen identifies ubiquitin ligase Itch as crucial for influenza A virus release from the endosome during virus entry. Proc Natl Acad Sci U S A **110:**17516–17521. http://dx.doi.org/10.1073/pnas.1312374110.
26. **Jagger BW, Wise HM, Kash JC, Walters KA, Wills NM, Xiao YL, Dunfee RL, Schwartzman LM, Ozinsky A, Bell GL, Dalton RM, Lo A, Efstathiou S, Atkins JF, Firth AE, Taubenberger JK, Digard P.** 2012. An overlapping protein-coding region in influenza A virus segment 3 modulates the host response. Science **337:**199–204. http://dx.doi.org/10.1126/science.1222213.
27. **Varga ZT, Ramos I, Hai R, Schmolke M, Garcia-Sastre A, Fernandez-Sesma A, Palese P.** 2011. The influenza virus protein PB1-F2 inhibits the induction of type I interferon at the level of the MAVS adaptor protein. PLoS Pathog **7:**e1002067. http://dx.doi.org/10.1371/journal.ppat.1002067.
28. **Varga ZT, Grant A, Manicassamy B, Palese P.** 2012. Influenza virus protein PB1-F2 inhibits the induction of type I interferon by binding to MAVS and decreasing mitochondrial membrane potential. J Virol **86:**8359–8366. http://dx.doi.org/10.1128/JVI.01122-12.
29. **Graef KM, Vreede FT, Lau YF, McCall AW, Carr SM, Subbarao K, Fodor E.** 2010. The PB2 subunit of the influenza virus RNA polymerase affects virulence by interacting with the mitochondrial antiviral signaling protein and inhibiting expression of beta interferon. J Virol **84:**8433–8445. http://dx.doi.org/10.1128/JVI.00879-10.
30. **Iwai A, Shiozaki T, Kawai T, Akira S, Kawaoka Y, Takada A, Kida H, Miyazaki T.** 2010. Influenza A virus polymerase inhibits type I interferon induction by binding to interferon beta promoter stimulator 1. J Biol Chem **285:**32064–32074. http://dx.doi.org/10.1074/jbc.M110.112458.
31. **Patel D, Schultz LW, Umland TC.** 2013. Influenza A polymerase subunit PB2 possesses overlapping binding sites for polymerase subunit PB1 and human MAVS proteins. Virus Res **172:**75–80. http://dx.doi.org/10.1016/j.virusres.2012.12.003.
32. **Kim JH, Hatta M, Watanabe S, Neumann G, Watanabe T, Kawaoka Y.** 2010. Role of host-specific amino acids in the pathogenicity of avian H5N1 influenza viruses in mice. J Gen Virol **91:**1284–1289. http://dx.doi.org/10.1099/vir.0.018143-0.
33. **Boergeling Y, Rozhdestvensky TS, Schmolke M, Resa-Infante P, Robeck T, Randau G, Wolff T, Gabriel G, Brosius J, Ludwig S.** 2015. Evidence for a novel mechanism of influenza virus-induced type I interferon expression by a defective RNA-encoded protein. PLoS Pathog **11:**e1004924. http://dx.doi.org/10.1371/journal.ppat.1004924.
34. **Benitez AA, Panis M, Xue J, Varble A, Shim JV, Frick AL, Lopez CB, Sachs D, tenOever BR.** 2015. *In vivo* RNAi screening identifies MDA5 as a significant contributor to the cellular defense against influenza A virus. Cell Rep **11:**1714–1726. http://dx.doi.org/10.1016/j.celrep.2015.05.032.
35. **Holm CK, Rahbek SH, Gad HH, Bak RO, Jakobsen MR, Jiang Z, Hansen AL, Jensen SK, Sun C, Thomsen MK, Laustsen A, Nielsen CG, Severinsen K, Xiong Y, Burdette DL, Hornung V, Lebbink RJ, Duch M, Fitzgerald KA, Bahrami S, Mikkelsen JG, Hartmann R, Paludan SR.** 2016. Influenza A virus targets a cGAS-independent STING pathway that controls enveloped RNA viruses. Nat Commun **7:**10680. http://dx.doi.org/10.1038/ncomms10680.
36. **Liniger M, Summerfield A, Zimmer G, McCullough KC, Ruggli N.** 2012. Chicken cells sense influenza A virus infection through MDA5 and CARDIF signaling involving LGP2. J Virol **86:**705–717. http://dx.doi.org/10.1128/JVI.00742-11.
37. **Ablasser A, Goldeck M, Cavlar T, Deimling T, Witte G, Rohl I, Hopfner KP, Ludwig J, Hornung V.** 2013. cGAS produces a 2′-5′-linked cyclic dinucleotide second messenger that activates STING. Nature **498:**380–384. http://dx.doi.org/10.1038/nature12306.
38. **Xia C, Vijayan M, Pritzl CJ, Fuchs SY, McDermott AB, Hahm B.** 2016. Hemagglutinin of influenza A virus antagonizes type I interferon (IFN) responses by inducing degradation of type I IFN receptor 1. J Virol **90:**2403–2417. http://dx.doi.org/10.1128/JVI.02749-15.
39. **Ishikawa H, Barber GN.** 2008. STING is an endoplasmic reticulum adaptor that facilitates innate immune signalling. Nature **455:**674–678. http://dx.doi.org/10.1038/nature07317.
40. **Tanaka Y, Chen ZJ.** 2012. STING specifies IRF3 phosphorylation by TBK1 in the cytosolic DNA signaling pathway. Sci Signal **5:**ra20. http://dx.doi.org/10.1126/scisignal.2002521.
41. **Barber MR, Aldridge JR, Jr, Webster RG, Magor KE.** 2010. Association of RIG-I with innate immunity of ducks to influenza. Proc Natl Acad Sci U S A **107:**5913–5918. http://dx.doi.org/10.1073/pnas.1001755107.
42. **Cheng Y, Sun Y, Wang H, Yan Y, Ding C, Sun J.** 2015. Chicken STING mediates activation of the IFN gene independently of the RIG-I gene. J Immunol **195:**3922–3936. http://dx.doi.org/10.4049/jimmunol.1500638.
43. **Hayashi T, Watanabe C, Suzuki Y, Tanikawa T, Uchida Y, Saito T.** 2014. Chicken MDA5 senses short double-stranded RNA with implications for antiviral response against avian influenza viruses in chicken. J Innate Immun **6:**58–71. http://dx.doi.org/10.1159/000351583.
44. **Liniger M, Moulin HR, Sakoda Y, Ruggli N, Summerfield A.** 2012. Highly pathogenic avian influenza virus H5N1 controls type I IFN induction in chicken macrophage HD-11 cells: a polygenic trait that involves NS1 and the polymerase complex. Virol J **9:**7. http://dx.doi.org/10.1186/1743-422X-9-7.
45. **Haller O, Staeheli P, Schwemmle M, Kochs G.** 2015. Mx GTPases: dynamin-like antiviral machines of innate immunity. Trends Microbiol **23:**154–163. http://dx.doi.org/10.1016/j.tim.2014.12.003.
46. **Ciancanelli MJ, Abel L, Zhang SY, Casanova JL.** 2016. Host genetics of severe influenza: from mouse Mx1 to human IRF7. Curr Opin Immunol **38:**109–120. http://dx.doi.org/10.1016/j.coi.2015.12.002.
47. **Gao S, von der Malsburg A, Dick A, Faelber K, Schroder GF, Haller O, Kochs G, Daumke O.** 2011. Structure of myxovirus resistance protein A reveals intra- and intermolecular domain interactions required for the antiviral function. Immunity **35:**514–525. http://dx.doi.org/10.1016/j.immuni.2011.07.012.
48. **Kochs G, Haller O.** 1999. GTP-bound human MxA protein interacts with the nucleocapsids of Thogoto virus (*Orthomyxoviridae*). J Biol Chem **274:**4370–4376. http://dx.doi.org/10.1074/jbc.274.7.4370.
49. **Nigg PE, Pavlovic J.** 2015. Oligomerization and GTP-binding requirements of MxA for viral target recognition and antiviral activity against influenza A virus. J Biol Chem **290:**29893–29906. http://dx.doi.org/10.1074/jbc.M115.681494.
50. **Turan K, Mibayashi M, Sugiyama K, Saito S, Numajiri A, Nagata K.** 2004. Nuclear MxA proteins form a complex with influenza virus NP and inhibit the transcription of the engineered influenza virus genome. Nucleic Acids Res **32:**643–652. http://dx.doi.org/10.1093/nar/gkh192.
51. **Verhelst J, Parthoens E, Schepens B, Fiers W, Saelens X.** 2012. Interferon-inducible protein Mx1 inhibits influenza virus by interfering with functional viral ribonucleoprotein complex assembly. J Virol **86:**13445–13455. http://dx.doi.org/10.1128/JVI.01682-12.
52. **Wisskirchen C, Ludersdorfer TH, Muller DA, Moritz E, Pavlovic J.** 2011. Interferon-induced antiviral protein MxA interacts with the cellular RNA helicases UAP56 and URH49. J Biol Chem **286:**34743–34751. http://dx.doi.org/10.1074/jbc.M111.251843.
53. **Xiao H, Killip MJ, Staeheli P, Randall RE, Jackson D.** 2013. The human interferon-induced MxA protein inhibits early stages of influenza A virus infection by retaining the incoming viral genome in the cytoplasm. J Virol **87:**13053–13058. http://dx.doi.org/10.1128/JVI.02220-13.
54. **Huang T, Pavlovic J, Staeheli P, Krystal M.** 1992. Overexpression of the influenza virus polymerase can titrate out inhibition by the murine Mx1 protein. J Virol **66:**4154–4160.
55. **Pavlovic J, Zurcher T, Haller O, Staeheli P.** 1990. Resistance to influenza virus and vesicular stomatitis virus conferred by expression of human MxA protein. J Virol **64:**3370–3375.
56. **Stranden AM, Staeheli P, Pavlovic J.** 1993. Function of the mouse Mx1 protein is inhibited by overexpression of the Pb2 protein of influenza virus. Virology **197:**642–651. http://dx.doi.org/10.1006/viro.1993.1639.
57. **Dittmann J, Stertz S, Grimm D, Steel J, Garcia-Sastre A, Haller O, Kochs G.** 2008. Influenza A virus strains differ in sensitivity to the antiviral action of Mx-GTPase. J Virol **82:**3624–3631. http://dx.doi.org/10.1128/JVI.01753-07.
58. **Mänz B, Dornfeld D, Gotz V, Zell R, Zimmermann P, Haller O, Kochs G, Schwemmle M.** 2013. Pandemic influenza A viruses escape from restriction by human MxA through adaptive mutations in the nucleoprotein. PLoS Pathog **9:**e1003279. http://dx.doi.org/10.1371/journal.ppat.1003279.
59. **Zimmermann P, Manz B, Haller O, Schwemmle M, Kochs G.** 2011. The viral nucleoprotein determines Mx sensitivity of influenza A viruses. J Virol **85:**8133–8140. http://dx.doi.org/10.1128/JVI.00712-11.
60. **Götz V, Magar L, Dornfeld D, Giese S, Pohlmann A, Hoper D, Kong BW, Jans DA, Beer M, Haller O, Schwemmle M.** 2016. Influenza A

viruses escape from MxA restriction at the expense of efficient nuclear vRNP import. Sci Rep **6:**23138. http://dx.doi.org/10.1038/srep23138.

61. **Riegger D, Hai R, Dornfeld D, Manz B, Leyva-Grado V, Sanchez-Aparicio MT, Albrecht RA, Palese P, Haller O, Schwemmle M, Garcia-Sastre A, Kochs G, Schmolke M.** 2015. The nucleoprotein of newly emerged H7N9 influenza A virus harbors a unique motif conferring resistance to antiviral human MxA. J Virol **89:**2241–2252. http://dx.doi.org/10.1128/JVI.02406-14.
62. **Pérez-Cidoncha M, Killip MJ, Oliveros JC, Asensio VJ, Fernandez Y, Bengoechea JA, Randall RE, Ortin J.** 2014. An unbiased genetic screen reveals the polygenic nature of the influenza virus anti-interferon response. J Virol **88:**4632–4646. http://dx.doi.org/10.1128/JVI.00014-14.