



MxA: a broadly acting effector of interferon-induced human innate immunity

Shuang Liao* and Song Gao

State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Sun Yat-sen University Cancer Center, 510060 Guangzhou, China

Received 9 March 2022, Accepted 9 March 2022, Published online 6 April 2022

Abstract – The Human MxA protein belongs to the dynamin superfamily of large GTPases and plays a vital role in human immunity against a broad spectrum of viruses. Evasion from MxA restriction accounts for the zoonotic transmission of many pathogenic viruses. In addition to its antiviral activity, MxA has also been implicated as an inhibitor against tumor cell motility and invasion. Over the past few decades, many advances have been made in elucidating the molecular mechanisms of MxA-mediated autoimmunity, including the determination of MxA structures at high resolutions. Together, they provide exciting insights into the antiviral function of MxA, laying a solid foundation for antiviral drug development and pandemic virus infection control, and also shed light on the development of novel approaches for the prevention and treatment against cancer metastasis.

Key words: MxA, Antiviral activity.

Introduction

Induced by type I and type III interferons (IFN), Mx proteins are important mediators of innate immunity against a broad spectrum of RNA and DNA viruses. The discovery of the first Mx protein, Mx1, dates back to 1962 when an inbred mouse strain showed resistance against lethal doses of influenza A virus (IVA) [1]. The resistance was caused by the gene *MX1*. Most of the other inbred strains of mice which carried nonfunctional *MX1* alleles with large deletions or nonsense mutations were susceptible to FLUAV infection [2]. Human Mx proteins were discovered later via coincidence. A monoclonal antibody against mouse Mx1 exhibited cross-reaction against an interferon-induced protein in human cells. Two cytoplasmic Mx proteins, MxA and MxB, which are encoded by human *MX1* and *MX2* genes, respectively, were then identified [3]. Both genes are located on the distal part of the long arm of the human chromosome 21 [4]. Mx proteins are vertebrate-specific proteins found in zebrafish to humans.

Human MxA is a 78 kDa cytoplasmic protein closely associated with the smooth endoplasmic reticulum (ER) [5]. The importance of MxA as part of innate immunity has been underlined in extensive cell cultures and animal model studies. MxA was first discovered as an antiviral factor against negative-stranded RNA viruses, including the *Orthomyxoviridae* [6–8], *Paramyxoviridae* [9], *Rhabdoviridae* [6], and *Bunyaviridae* families [10, 11]. Further studies revealed that the antiviral activity of MxA stretches far beyond negative-stranded RNA

viruses, including a striking diversity of positive-stranded RNA viruses [12], double-stranded RNA viruses [13], and some DNA viruses [14–17]. To avoid antibiotic abuse, clinical studies have shown that the combined interpretation of MxA with C-reactive protein (CRP) and/or procalcitonin dramatically improves the clinical sensitivity and specificity in discriminating between viral and bacterial infections (reviewed in [18]). However, the detailed molecular mechanisms underlying the antiviral activity of MxA remained elusive for a long time.

In addition to its antiviral activity, recent studies revealed the functions of MxA in different cancer types. An international multicenter study indicated that robust MxA expression is a positive indicator for breast cancer patients who might benefit from adjuvant chemotherapy [19]. In another retrospective study, a positive correlation between MxA and tumor-infiltrating lymphocytes (TIL) levels was revealed in triple-negative breast cancer (TNBC) cases [20]. Mushinski et al. demonstrated that stable exogenous MxA expression inhibited the motility and invasiveness of prostate carcinoma cells, both in vitro and in vivo [21]. Therefore, MxA may serve as a potential target for novel anti-cancer drug development.

Viral targets of MxA

Extensive efforts have been made among various viruses to identify the viral targets of MxA, as well as the molecular mechanism of MxA-mediated autoimmunity against virus infections.

The influenza virus belongs to the family of *Orthomyxoviridae*, which is the most well-studied myxovirus family.

*Corresponding author: liaoshuang@sysucc.org.cn

Accumulating evidence has demonstrated that the nucleoprotein (NP) component of Orthomyxoviruses is the main determinant of MxA susceptibility. Sequestration of NP by MxA in the cytoplasm leads to the block of viral amplification and infection [22].

As the key effector protein against IVAs, MxA has been shown to block viral replication at an early stage of the infection [7]. Upon IVA infection, viral mRNA synthesis in MxA-positive cells was reported to be within a normal level, while viral protein synthesis and genome amplification was strongly inhibited, suggesting that MxA interferes with either viral protein translation in the cytoplasm or translocation of newly synthesized viral proteins to the cell nucleus [7]. Growing evidence suggests that MxA confers protection against IVA by targeting the viral NP protein [23–26]. The genome of the IVA is segmented into eight RNA molecules, each compacted as a rod-shaped ribonucleoprotein complex (RNP) with the NP proteins. The RNP structure is crucial for the viral infection cycle. Upon infection, RNPs are delivered to the cytoplasm of host cells through endocytosis, followed by active transportation into the nucleus. After transcription of the vRNAs in the nucleus, mRNAs are exported to the cytoplasm for translation of the viral proteins NP, PB1, PB2, and PA. Newly synthesized NP is then imported back into the nucleus for the production of nascent virions (reviewed in [27]). Therefore, sequestration of the viral RNP in the cytoplasm by MxA upon infection can impair the IVA replication process at an early stage, providing protection against further infection.

There are several lines of evidence supporting the importance of MxA-NP interaction against IVA infection. Utilizing a transient influenza virus transcription reporter assay system, Turan et al. revealed that the nuclear-localized MxA strongly inhibits the influenza virus transcription, which may be neutralized by over-expression of NP, but not (or only slightly) by other RNA polymerase subunits PB1, PB2, and PA [24]. Furthermore, studies using influenza virus-infected cells proved that apart from the nuclear-localized MxA, cytosolic MxA also interacts with NP *in vitro*, which may confer resistance upon infection of the influenza virus.

Besides blocking the infection of human viruses, human MxA also acts as an efficient protective barrier against the zoonotic introduction of pathogenic viruses into the human population. However, some viruses have successfully evaded MxA restriction through evolution, such as the IVA H1N1 strain, which caused the 2009 pandemic, and a thogotovirus isolate, another member of the *Orthomyxoviridae* [28]. Individual substitution of each H1N1 component involved in vRNA replication with the H5N1 counterpart in a polymerase reconstitution assay proved that NP was the main determinant of MxA sensitivity [25]. An analysis of the adaptive mutations in the NPs of two MxA resistant IVA strains revealed that the resistance-related mutations were clustered in a surface-exposed region in the body domain of NP. The introduction of this cluster into the MxA-sensitive strain resulted in a gain of resistance, although viral replication fitness was affected [26]. Similarly, two amino acid substitutions (G327R and R328V) in the viral nucleoprotein of the MxA-resistant thogotovirus isolate were shown to be responsible for its resistance. Unlike in the cases of IVAs [26], when the R328V mutation was introduced into the MxA-sensitive thogotovirus isolate SiAr126, the resulting

recombinant virus gained complete MxA resistance without any viral fitness loss, indicating even greater zoonotic potential than previously expected. These studies demonstrate that NP is the essential viral component underlying MxA susceptibility and that NP alone is sufficient to determine MxA susceptibility in *Orthomyxoviruses*. Experimental evidence with the La Crosse Virus (LACV), which belongs to the *Bunyaviridae* family, demonstrated a direct interaction between MxA and the virus nucleocapsid (N) protein, suggesting a similar working mode of MxA with the *Orthomyxovirus* NP. Replication of LACV takes place in the cytoplasm of infected cells through the budding of nucleocapsids into the Golgi apparatus. Electron microscopy (EM) studies have shown that MxA binds to the viral N protein and forms cytoplasmic complexes which accumulate in the perinuclear area, forming highly ordered fibrillary structures [29]. Further studies have revealed that MxA and the viral N protein both localize to a specific sub-compartment of the smooth ER, resulting in efficient sequestration of N, which leads to the depletion of this important viral component from viral replication sites, thereby preventing the assembly of new virus particles [30]. Interestingly, tubular structures of MxA entangled with a viral structural protein were also observed in another study with the West Nile virus (WNV), which belongs to the *Flaviviridae* family. While MxA was reported to be less important in restricting HCV and WNV_{kun} replication [31, 32], one study has shown that in an expression-replication system, in which MxA is retargeted from cytoplasmic inclusions to the ER during WNV_{kun} replication, the MxA protein can sequester the capsid (C) protein of WNV in tubular structures in the cytoplasm and reduce the titers of secreted viral particles [33]. Using a rapid and compartmentalized replication and assembly strategy, WNV can hinder the recognition of the capsid protein by MxA *in vivo*, resulting in evasion from MxA-mediated host surveillance. More importantly, this evasion can actually be reversed by the expression of recombinant ER-targeting MxA, which is translocated to intracellular viral assembly sites upon infection.

Although the formation of tubular bundles indicates an interaction between MxA and the C protein of the WNV_{kun}, there is no direct evidence showing the physical interaction between these two proteins, probably due to lack of other cellular factors [33]. In hepatitis B virus (HBV) infected cells, physical interaction between MxA and the HBV core antigen protein (HBcAg) has been elucidated, which results in the immobilization of HBcAg in the cytoplasm and hampers viral capsid formation [15]. Interestingly, MxA seems to restrict the positive-sense RNA virus Semliki Forest virus (SFV) by targeting a component of the replicase but not the structural protein [12].

In summary, MxA targets highly divergent proteins in different types of viruses. And the molecular mechanisms for MxA to recognize distinct viral proteins, which evolve rapidly to evade recognition by the host immune system, are of vital importance for containing the epidemic caused by these viruses.

Crystal structure and the ring-like model of MxA depicting its antiviral mechanism

The MxA protein belongs to the dynamin superfamily of multi-domain GTPases, which are characterized by the self-assembly ability and assembly-stimulated GTPase activity.

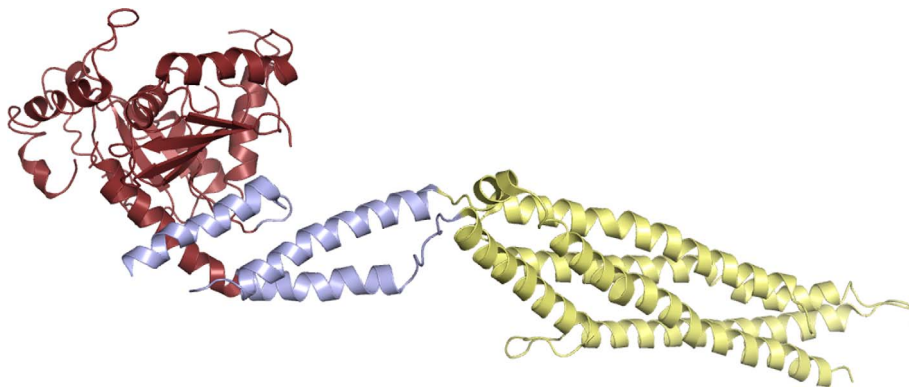


Figure 1. Structure of the MxA monomer. Ribbon-type representation of an MxA monomer. The G domain, BSE, and stalk domain are colored ruby, light blue and pale yellow, respectively.

Like dynamin, MxA can form highly-ordered oligomers and self-assemble into ring-like or helical structures *in vitro* [34]. Both MxA and dynamin can tubulate liposomes [35], but stimulated GTPase activity upon this liposome-induced assembly is observed only for dynamin, not MxA [36], suggesting different cellular functions of these two mechano-chemical GTPases upon activation. GTP binding allows for the dimerization of MxA via the GTPase (G) domain and facilitates ER membrane-associated assembly of MxA, whereas GTP hydrolysis promotes the redistribution of MxA from cellular membranes to viral targets [37].

In order to elucidate the molecular mechanisms of MxA action as a broadly-acting antiviral effector, high-resolution crystal structures of the MxA stalk region and the full-length MxA protein were determined successively. Together with the results of biochemical studies and EM studies, a more detailed picture of MxA's antiviral function has now been elucidated.

Back in 2010, we solved the crystal structure of the stalk region of MxA (PDB: 3LJB) [38], followed by the near-full-length MxA crystal structure in 2011 (PDB: 3SZR) [39]. MxA is an elongated molecule composed of three domains, an N-terminal GTPase domain, a bundle-signaling-element (BSE), and a C-terminal stalk responsible for self-assembly (Figure 1). The unstructured L4 loop has been proposed as the lipid-binding moiety of MxA based on biochemical analysis [36]. The most variable region of Mx proteins is L4, which accounts for the antiviral specificity probably by direct interaction with different target viral proteins [40]. Biochemical studies using single-molecule fluorescence resonance energy transfer (smFRET) revealed that while the G domain-BSE region prefers to adopt an "open" state in nucleotide-free conditions, loading of GTP changed the conformational preference to the "closed" state. The frequent relative movement was also observed between the BSE and the stalk via hinge 1, which may be key elements for mechano-chemical coupling of MxA molecules [41]. Unlike the linear MxA filaments in our crystal structure, MxA oligomerizes into ring-like structures around tubulated liposomes were observed in EM studies [36]. As the experimental data supporting the role of MxA-NP interaction during MxA-mediated influenza virus restriction was accumulating, a theory was put forward that the oligomeric rings represent antiviral molecular machines that oligomerize around the tubular structure of viral ribonucleocapsids (RNPs) [39].

Based on the evidence from previous EM studies, this theory was subsequently depicted by a ring-like MxA oligomer model, which was constructed using the structure of near-full-length MxA as the building block (Figure 2) [39, 42]. This MxA-ring has a diameter of 24 nm, and a 23° rotation around the hydrophobic interface-1 was introduced between the building block dimers to facilitate the formation of the ring. The inner layer of the MxA-ring is composed of the stalk domains and the outer layer of the G domains, allowing for transient dimerization with the G domains from adjacent rings (Video 1). The predicted substrate-binding loop L4 points towards the center of the ring and allowed for interactions with viral components, like nucleocapsids. The architecture of this MxA-ring model was later substantiated by a subsequent cryo-EM analysis on MxA-decorated lipid tubules [36, 43]. Upon GTP binding, the G domains from neighboring rings come into contact and confer stimulated GTP hydrolysis. These actions trigger conformational changes of MxA molecules, which collectively produce a synchronized power stroke, resulting in the sequestration and disintegration of enwrapped essential viral components and eventually the disruption of viral replication [39, 44].

Remarkably, MxA and the WNV C protein form tightly packed bundles built from hollow tubes with a diameter of ~30 nm [33]. A similar ultrastructure was also reported for the complex of MxA LACV NP [29]. In both cases, the viral-component sequestration manner of MxA resembles what was found for the influenza virus. The flexibility of interface-1, to a certain extent, provides freedom of rotation between the building block MxA dimers, which facilitates the formation of MxA-rings with proper diameters to contain target viral structures with various sizes, thereby explaining the broad antiviral spectrum of MxA (reviewed in [45]).

Future prospects

Substantial efforts and achievements have been made by researchers over the past few decades in studying the highly diverse viral targets of MxA, as well as the evolutionary strategies of viruses in evading MxA-mediated host surveillance. Further studies, especially the high-resolution interpretation of MxA-viral component complexes, are required to elucidate this issue, which will contribute to the development of novel

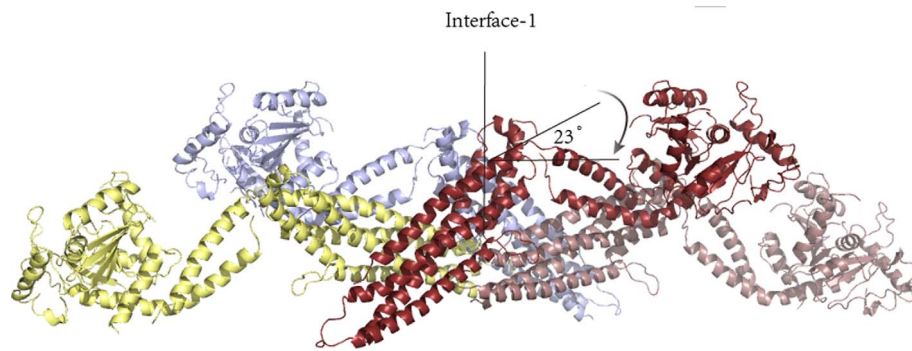
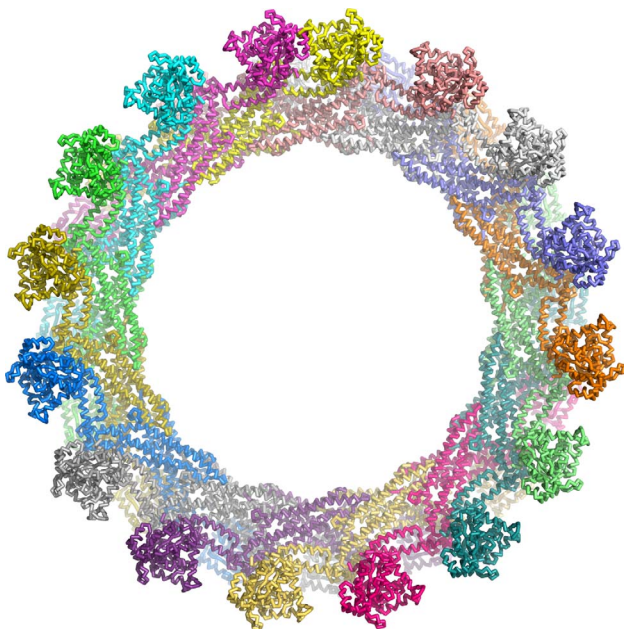


Figure 2. Model of Ring-like MxA Oligomer. A. Structural model of the MxA tetramer. The indicated 23° rotation around the hydrophobic interface-1 leads to the formation of the ring-like MxA oligomer model. B. A model of the ring-like MxA oligomer. The MxA-ring is composed of 16 constitutive dimers, each represented by a distinct color.



Video 1. To better present the 3D structure of the ring-like MxA oligomer, this movie was made using PyMOL. The first and second appearing MxA monomers are colored cyan and green, respectively. The other 15 dimers are differently colored and gradually emerge to form the ring-like structure. <https://vcm.edpsciences.org/10.1051/vcm/2022002#V1>

and timely antiviral means against corresponding infectious diseases.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements. We would like to thank Mr. Christopher Laven-der for his kind revision of the English language.

References

- Lindenmann J. Resistance of mice to mouse-adapted influenza A virus. *Virology*. 1962;16:203–204.
- Staeli P, Grob R, Meier E, et al. Influenza virus-susceptible mice carry Mx genes with a large deletion or a nonsense mutation. *Molecular and Cellular Biology*. 1988;8(10):4518–4523.
- Aebi M, Fah J, Hurt N, et al. cDNA structures and regulation of two interferon-induced human Mx proteins. *Molecular and Cellular Biology*. 1989;9(11):5062–5072.
- Horisberger MA, Wathelet M, Szpirer J, et al. cDNA cloning and assignment to chromosome 21 of IFI-78K gene, the human equivalent of murine Mx gene. *Somatic Cell and Molecular Genetics*. 1988;14(2):123–131.
- Stertz S, Reichelt M, Krijnse-Locker J, et al. Interferon-induced, antiviral human MxA protein localizes to a distinct subcompartment of the smooth endoplasmic reticulum. *Journal of Interferon & Cytokine Research: The Official Journal of the International Society for Interferon and Cytokine Research*. 2006;26(9):650–660.
- Pavlovic J, Zurcher T, Haller O, et al. Resistance to influenza virus and vesicular stomatitis virus conferred by expression of human MxA protein. *Journal of Virology*. 1990;64(7):3370–3375.
- Pavlovic J, Haller O, Staeli P. Human and mouse Mx proteins inhibit different steps of the influenza virus multiplication cycle. *Journal of Virology*. 1992;66(4):2564–2569.
- Frese M, Kochs G, Meier-Dieter U, et al. Human MxA protein inhibits tick-borne Thogoto virus but not Dhori virus. *Journal of Virology*. 1995;69(6):3904–3909.
- Schnorr JJ, Schneider-Schaulies S, Simon-Jodicke A, et al. MxA-dependent inhibition of measles virus glycoprotein synthesis in a stably transfected human monocytic cell line. *Journal of Virology*. 1993;67(8):4760–4768.
- Frese M, Kochs G, Feldmann H, et al. Inhibition of bunyaviruses, phleboviruses, and hantaviruses by human MxA protein. *Journal of Virology*. 1996;70(2):915–923.
- Hefti HP, Frese M, Landis H, et al. Human MxA protein protects mice lacking a functional alpha/beta interferon system against La crosse virus and other lethal viral infections. *Journal of Virology*. 1999;73(8):6984–6991.
- Landis H, Simon-Jodicke A, Kloti A, et al. Human MxA protein confers resistance to Semliki Forest virus and inhibits the amplification of a Semliki Forest virus-based replicon in the absence of viral structural proteins. *Journal of Virology*. 1998;72(2):1516–1522.
- Mundt E. Human MxA protein confers resistance to double-stranded RNA viruses of two virus families. *The Journal of General Virology*. 2007;88(Pt 4):1319–1323.

14. Netherton CL, Simpson J, Haller O, et al. Inhibition of a large double-stranded DNA virus by MxA protein. *Journal of Virology*. 2009;83(5):2310–2320.
15. Li N, Zhang L, Chen L, et al. MxA inhibits hepatitis B virus replication by interaction with hepatitis B core antigen. *Hepatology*. 2012;56(3):803–811.
16. Gordien E, Rosmorduc O, Peltekian C, et al. Inhibition of hepatitis B virus replication by the interferon-inducible MxA protein. *Journal of Virology*. 2001;75(6):2684–2691.
17. Mitchell PS, Emerman M, Malik HS. An evolutionary perspective on the broad antiviral specificity of MxA. *Current Opinion in Microbiology*. 2013;16(4):493–499.
18. Zav'yalov VP, Hamalainen-Laanaya H, Korpela TK, et al. Interferon-inducible myxovirus resistance proteins: potential biomarkers for differentiating viral from bacterial infections. *Clinical Chemistry*. 2019;65(6):739–750.
19. Sistigu A, Yamazaki T, Vacchelli E, et al. Cancer cell-autonomous contribution of type I interferon signaling to the efficacy of chemotherapy. *Nature Medicine*. 2014;20(11):1301–1309.
20. Kim YA, Lee HJ, Heo SH, et al. MxA expression is associated with tumor-infiltrating lymphocytes and is a prognostic factor in triple-negative breast cancer. *Breast Cancer Research and Treatment*. 2016;156(3):597–606.
21. Mushinski JF, Nguyen P, Stevens LM, et al. Inhibition of tumor cell motility by the interferon-inducible GTPase MxA. *The Journal of Biological Chemistry*. 2009;284(22):15206–15214.
22. Kochs G, Haller O. GTP-bound human MxA protein interacts with the nucleocapsids of Thogoto virus (Orthomyxoviridae). *The Journal of Biological Chemistry*. 1999;274(7):4370–4376.
23. Deeg CM, Hassan E, Mutz P, et al. In vivo evasion of MxA by avian influenza viruses requires human signature in the viral nucleoprotein. *The Journal of Experimental Medicine*. 2017; 214(5):1239–1248.
24. Turan K, Mibayashi M, Sugiyama K, et al. Nuclear MxA proteins form a complex with influenza virus NP and inhibit the transcription of the engineered influenza virus genome. *Nucleic Acids Research*. 2004;32(2):643–652.
25. Zimmermann P, Manz B, Haller O, et al. The viral nucleoprotein determines Mx sensitivity of influenza A viruses. *Journal of Virology*. 2011;85(16):8133–8140.
26. Manz B, Dornfeld D, Gotz V, et al. Pandemic influenza A viruses escape from restriction by human MxA through adaptive mutations in the nucleoprotein. *PLoS Pathogens*. 2013;9(3): e1003279.
27. Zheng W, Tao YJ. Structure and assembly of the influenza A virus ribonucleoprotein complex. *FEBS Letters*. 2013;587(8): 1206–1214.
28. Fuchs J, Oswald A, Graf L, et al. Tick-transmitted thogotovirus gains high virulence by a single MxA escape mutation in the viral nucleoprotein. *PLoS Pathogens*. 2020;16(11):e1009038.
29. Kochs G, Janzen C, Hohenberg H, et al. Antivirally active MxA protein sequesters La Crosse virus nucleocapsid protein into perinuclear complexes. *Proceedings of the National Academy of Sciences of the United States of America*. 2002;99(5):3153–3158.
30. Reichelt M, Stertz S, Krijnse-Locker J, et al. Missorting of LaCrosse virus nucleocapsid protein by the interferon-induced MxA GTPase involves smooth ER membranes. *Traffic*. 2004; 5(10): 772–784.
31. Frese M, Pietschmann T, Moradpour D, et al. Interferon-alpha inhibits hepatitis C virus subgenomic RNA replication by an MxA-independent pathway. *The Journal of General Virology*. 2001;82(Pt 4):723–733.
32. Hoenen A, Liu W, Kochs G, et al. West Nile virus-induced cytoplasmic membrane structures provide partial protection against the interferon-induced antiviral MxA protein. *The Journal of General Virology*. 2007;88(Pt 11):3013–3017.
33. Hoenen A, Gillespie L, Morgan G, et al. The West Nile virus assembly process evades the conserved antiviral mechanism of the interferon-induced MxA protein. *Virology*. 2014;448:104–116.
34. Kochs G, Haener M, Aebi U, et al. Self-assembly of human MxA GTPase into highly ordered dynamin-like oligomers. *The Journal of Biological Chemistry*. 2002;277(16):14172–14176.
35. Accola MA, Huang B, Al Masri A, et al. The antiviral dynamin family member, MxA, tubulates lipids and localizes to the smooth endoplasmic reticulum. *The Journal of biological chemistry*. 2002;277(24):21829–21835.
36. von der Malsburg A, Abutbul-Ionita I, Haller O, et al. Stalk domain of the dynamin-like MxA GTPase protein mediates membrane binding and liposome tubulation via the unstructured L4 loop. *The Journal of Biological Chemistry*. 2011;286(43): 37858–37865.
37. Dick A, Graf L, Olal D, et al. Role of nucleotide binding and GTPase domain dimerization in dynamin-like myxovirus resistance protein A for GTPase activation and antiviral activity. *The Journal of Biological Chemistry*. 2015;290(20):12779–12792.
38. Gao S, von der Malsburg A, Paeschke S, et al. Structural basis of oligomerization in the stalk region of dynamin-like MxA. *Nature*. 2010;465(7297):502–506.
39. Gao S, von der Malsburg A, Dick A, et al. Structure of myxovirus resistance protein a reveals intra- and intermolecular domain interactions required for the antiviral function. *Immunity*. 2011;35(4):514–525.
40. Patzina C, Haller O, Kochs G. Structural requirements for the antiviral activity of the human MxA protein against Thogoto and influenza A virus. *The Journal of Biological Chemistry*. 2014;289(9):6020–6027.
41. Chen Y, Zhang L, Graf L, et al. Conformational dynamics of dynamin-like MxA revealed by single-molecule FRET. *Nature Communications*. 2017;8:15744.
42. Daumke O, Gao S, von der Malsburg A, et al. Structure of the MxA stalk elucidates the assembly of ring-like units of an antiviral module. *Small GTPases*. 2010;1(1):62–64.
43. Alvarez FJD, He S, Perilla JR, et al. CryoEM structure of MxB reveals a novel oligomerization interface critical for HIV restriction. *Science Advances*. 2017;3(9):e1701264.
44. Rennie ML, McKelvie SA, Bulloch EM, et al. Transient dimerization of human MxA promotes GTP hydrolysis, resulting in a mechanical power stroke. *Structure*. 2014;22(10):1433–1445.
45. Haller O, Kochs G. Human MxA protein: an interferon-induced dynamin-like GTPase with broad antiviral activity. *Journal of Interferon & Cytokine Research: The Official Journal of the International Society for Interferon and Cytokine Research*. 2011;31(1):79–87.