Pro-515 of the dynamin-like GTPase MxB contributes to HIV-1 inhibition by regulating MxB oligomerization and binding to HIV-1 capsid

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ABSTRACT

Interferon-regulated myxovirus resistance protein B (MxB) is an interferon-induced GTPase belonging to the dynamin superfamily. It inhibits infection with a wide range of different viruses, including HIV-1, by impairing viral DNA entry into the nucleus. Unlike the related antiviral GTPase MxA, MxB possesses an N-terminal region that contains a nuclear localization signal (NLS) and is crucial for inhibiting HIV-1. Because MxB previously has been shown to reside in both the nuclear envelope and the cytoplasm, here we used bioinformatics and biochemical approaches to identify a nuclear export signal (NES) responsible for MxB’s cytoplasmic location. Using the online computational tool, Locating Nuclear Export Signals or NESs (LocNES), we identified five putative NES candidates in MxB and investigated whether their deletion caused nuclear localization of MxB. Our results revealed that none of the five deletion variants re-locates to the nucleus,
Pro515 of MxB contributes to its antiviral activity

suggesting that these five predicted NES sequences do not confer NES activity. Interestingly, deletion of one sequence, encompassing amino acids 505-527, abrogated the anti-HIV-1 activity of MxB. Further mutation experiments disclosed that amino acids 515-519, and Pro-515 in particular, regulate MxB oligomerization and its binding to HIV-1 capsid, thereby playing an important role in MxB-mediated restriction of HIV-1 infection. In summary, our results indicate that none of five predicted NES sequences in MxB appears to be required for its nuclear export. Our findings also reveal several residues in MxB, including Pro-515, critical for its oligomerization and anti-HIV-1 function.

Introduction

A group of cellular proteins have been discovered to possess potent anti-HIV-1 activity. These include APOBEC3G (apolipoprotein B mRNA-editing catalytic polypeptide 3G) (1), TRIM5α (tripartite motif protein 5-alpha) (2,3), ZAP (zinc-finger antiviral protein) (4), tetherin (5,6), SAMHD1 (SAM domain- and HD domain-containing protein1) (7-11), IFITM (interferon- induced transmembrane protein) (12-14), MxB (15-17), SERINC5 (serine incorporator 5) (18-20), and others. MxB is one of the interferon induced myxovirus resistance proteins that restrict a wide range of different viruses. Infection of some RNA and DNA viruses, such as influenza viruses, vesicular stomatitis virus, Thogoto virus, and hepatitis B virus, has been reported to be restricted by human MxA (21-25). The antiviral function of MxB was only reported recently, inhibiting HIV-1, herpesviruses, and hepatitis C virus (15-17,26-31).

Both MxA and MxB are GTPases (guanosine triphosphatases), belonging to the dynamin superfamily. They share 63% sequence identity and a similar structure, containing a GTPase domain, BSE (bundle signaling elements) domain and a stalk domain (32-34). Different from MxA, MxB has an extra N-terminal region which has a nuclear localization signal (NLS), contribute to binding to HIV-1 capsid, and is indispensable for inhibiting HIV-1 infection (35-38). Several nucleoporins, components of the nuclear pore complex (NPC), were recently shown to assist MxB to localize at the NPC and facilitate MxB inhibition of HIV-1 (39,40). In addition to the NLS, MxB oligomerization is also central to inhibit HIV-1 (36,38,41-43). The cryo-EM structures of MxB reveal that MxB assembles into helical tubes consisting of MxB dimer units (43). Six dimers interlock with each other to form one rung, and the sixth dimer interacts with the first dimer to help forming the helical tube. The crystal and cryo-EM structures of MxB have the dimer interface (interface 2), oligomer interfaces (interface 1 and 3) and helical assembly interface (interface 4) (43,44). Interface 2 mutations M574D, Y651D, and M567D/L570D disrupt MxB dimerization, thus are unable to inhibit HIV-1 (38,41-44). Mutations F495D and R449D in interface 3 prevent oligomerization, also diminish its anti-HIV-1 activity (43,44).

In this study, given that MxB is found in both the nuclear envelope and the cytoplasm (36,38,44-47), we used online software to predict the putative nuclear export signal (NES) in MxB which might have led to the cytoplasmic location of MxB, and further tested their nuclear export functions by deleting each of the 5 candidates. All of these five deleted MxB mutants were still found in...
of MxB contributes to its antiviral activity

the cytoplasm, suggesting that none of these five sequences bears NES activity. To our surprise, we found that the MxBdel505-527 mutant lost both its ability to impair HIV-1 infection and the subcellular localization pattern of wild type MxB. Further study showed that amino acids 515 to 519 (especially Pro515) were critical for oligomerization of MxB and capsid binding ability, thus essential for MxB antiviral activity.

Results

Deletion of amino acids 505 to 527 diminishes antiviral ability of MxB

Although it was reported that MxB had a NLS at its N terminus, MxB was found in the cytoplasm (36,38,44-47). It is unclear whether MxB has a nuclear export signal (NES) to assist its translocation from nucleus to cytoplasm. Using online software to predict the putative NES in MxB (LocNES, http://prodata.swmed.edu/LocNES/LocNES.php), several high score regions with overlapping sequences were suggested (Table 1). Thus we created five DNA constructs, each deleting one of these potential regions, named (51-68), (98-123), (199-223), (374-389), (505-527) deletions. Schematic representation of these five deletions was shown in Fig. 1A. These five mutants, wild type MxB, or N terminal (1-25) deletion were transfected into HeLa cells. As shown in Fig. 1B, MxB was localized at the nuclear envelope and in the form of cytoplasmic puncta/granules, while the N terminal (1-25) deletion, lacking the NLS, was dispersed in the cytoplasm. All of the five NES deletion candidates were seen in the cytoplasm, suggesting that these five sequences do not bear NES function.

In the meantime, we measured the antiviral activity of these MxB deletions. Cells were transfected with these DNA constructs, and then infected with VSV-G pseudotyped HIV-1_{NL4-3-ΔE-YFP}. Infected cells were scored by flow cytometry. The results showed that the MxBdel(505-527) mutant exhibited dramatically decreased antiviral activity, similar to the MxBdel(1-25) mutant (Fig. 1C, upper panel). The expression levels of these deletions were comparable with wild type MxB (Fig. 1C, lower panel). Previous reports showed that, the stalk domain of MxB contains leucine zipper repeats, contributes to MxB dimerization, and is critical for restricting HIV-1 (36,38,41,42,45). To test whether the loss of antiviral activity of MxBdel(505-527) was due to the lack of dimer formation, the wild type or MxB mutants tagged with a Flag epitope were tested for association with Myc-MxB by immunoprecipitation. Only the dimer interface mutant 574/651D lost the ability to oligomerize, and the five deletions did not disrupt MxB homodimer formation (Fig. 1D).

Deletion of amino acids 515 to 519 impairs the antiviral ability of MxB

In order to determine which amino acids in the region of (505-527) are required for the anti-HIV-1 activity of MxB, we used alanine to replace every five amino acids and generated mutants (505-509)A, (510-514)A, (515-519)A, (520-524)A, and (525-527)A (Fig. 2A). Subcellular localization of these MxB mutants was analyzed by indirect immunofluorescence microscopy. As shown in Fig. 2B, mutants (505-509)A, (510-514)A, (520-524)A, and (525-527)A, and wild type MxB all accumulated at the nuclear envelope with large cytoplasmic puncta/granules, while (515-519)A exhibited dispersed distribution in...
Pro515 of MxB contributes to its antiviral activity

the cytoplasm. Then cells were transfected with wild type or these mutated MxB DNA, and infected with VSV-G pseudotyped HIV-1\textsubscript{NL4-3-ΔE-YFP}. Mutants (505-509)A, (510-514)A, (520-524)A, and (525-527)A showed anti-HIV-1 activity similar to wild type MxB; whereas MxB(515-519)A was as impaired as MxB\textsubscript{del}(1-25) and MxB\textsubscript{del}(505-527) in inhibiting HIV-1 (Fig. 2C, upper panel). Expression levels of these mutations were comparable with wild type MxB (Fig. 2C, lower panel). Results of co-immunoprecipitation assays showed that all MxB mutants including (515-519)A interacted with Myc-MxB as efficiently as the wild type except MxB\textsubscript{574/651D} (Fig. 2D), which suggests that MxB(515-519)A is able to form homodimer yet is defective in inhibiting HIV-1.

**Pro515 is important for the antiviral activity of MxB**

To understand the role of the individual amino acids within region 515 to 519 in MxB inhibiting HIV-1, we mutated each amino acid to alanine (Fig. 3A). Results of immunofluorescence experiments showed that mutants MxB\textsubscript{515A}, MxB\textsubscript{517A}, MxB\textsubscript{518A}, MxB\textsubscript{519A} exhibited similar patterns of distribution at nuclear envelope with cytoplasmic puncta (Fig. 3B). These MxB mutants expressed HEK293 cells were infected with VSV-G pseudotyped HIV-1\textsubscript{NL4-3-ΔE-YFP}. Results of flow cytometry showed that, the antiviral activity of MxB decreased when Pro515 was mutated, while their expression levels were similar to that of MxB (Fig. 3C). Co-immunoprecipitation assays showed that MxB mutants MxB\textsubscript{515A}, MxB\textsubscript{517A}, MxB\textsubscript{518A}, and MxB\textsubscript{519A} interacted with Myc-MxB as efficiently as the wild type (Fig. 3D), which suggests that these mutants did not disrupt MxB homodimer formation.

**Mutations (515-519)A and MxB\textsubscript{515A} inhibit the formation of oligomer**

The 3D density map (PDB ID code 5UOT) of the MxB helical assembly has been determined by Frances J. D. Alvarez, et al. at 4.6Å resolution, through cryo-EM and real-space helical reconstruction (43). MxB was shown to assemble into highly ordered long helical tubes like other dynamin family members. Oligomerization of MxB has been shown essential for binding to HIV-1 core and inhibiting HIV-1 infection (36,38,41-43). The cryo-EM structure of the MxB assembly reveals that amino acids 515 to 519 are located on the $\alpha_2$ helix of MxB, which is adjacent to oligomer interface 3 (Fig. 4A and B). It was reported that interface 3 mutations (R449D and F495D) abrogate MxB oligomerization and diminish its anti-HIV-1 activity (43), which suggests that mutating the sequence of 515 to 519 may affect the hydrophobic interactions at interface 3, and thus alter rung stacking and oligomer formation. We therefore measured the antiviral activity of interface 2 mutant (MxB\textsubscript{574/651D}), interface 3 mutant (MxB\textsubscript{449/495D}), together with MxB(515-519)A and MxB\textsubscript{515A}. As shown in Fig. 4C, interface 2 mutant (MxB\textsubscript{574/651D}) and interface 3 mutant (MxB\textsubscript{449/495D}) lost their antiviral activity, consistent with previous reports (36,38,41-44). MxB(515-519)A and MxB\textsubscript{515A} were as impaired as MxB\textsubscript{449/495D} in inhibition of HIV-1.

To determine the oligomerization ability of MxB(515-519)A and MxB\textsubscript{515A}, chemical cross-linking assay and gel filtration
chromatography were performed. As determined by cross-linking with disuccinimidyl suberate (DSS), the interface 2 mutant-MxB574/651D lost its oligomerization ability completely (Fig. 5A). Similar to interface 3 mutant-MxB449/495D, MxB(515-519)A was defective in oligomer formation, and oligomerization of MxB515A decreased as well. Furthermore, results of gel filtration chromatography showed that, the higher-molecular oligomerization of MxB449/495D, MxB574/651D, MxB(515-519)A, MxB515A decreased with concomitant increase in lower-molecular-mass complexes (Fig. 5B), which correlates with their incapability to inhibit HIV-1 infection. These data suggest that loss of oligomerization has led to the deficiency of MxB(515-519)A and MxB515A in inhibiting HIV-1.

**The 515-519A and 515A mutants lose the ability to bind HIV-1 capsid**

It was previously reported that MxB dimerization and binding to HIV-1 capsid are necessary for inhibiting HIV-1 infection (36,38,41-44). To measure the binding of (515-519)A to HIV-1 capsid, we performed the step gradient analyses to detect the interaction between p24 and MxB (wild type, MxB449/495D and MxB(515-519)A). Wild type MxB showed strong interaction with p24. Interface 3 mutant-MxB449/495D reduced MxB binding to HIV-1 capsid, the capsid binding ability of (515-519)A was also dramatically reduced (Fig. 6A-C). These data suggest that the lack of binding to HIV-1 capsid underlies the loss of HIV-1 inhibition by (515-519)A. Association of 515A mutant with HIV-1 capsid was also disrupted, as opposed to moderate or no adverse effect by other mutants MxB517A, MxB518A, and MxB519A (Fig. 6D and E).

**Discussion**

The N-terminal 25 amino acids are essential for the anti-HIV-1 activity of MxB by regulating MxB subcellular localization and binding to HIV-1 capsid(15-17,35,36,38,45,46). The amino acids R11R12R13 are involved in association with HIV-1 capsid; mutation of R11R12R13 impairs the anti-HIV-1 function (35,36). Amino acids K20Y21 contribute to the NLS function, yet mutation of K20 does not affect MxB inhibition of HIV-1 infection, casting doubt on the role of NLS in MxB anti-HIV-1 activity (35). Since MxB is localized to both the nucleus and the cytoplasm (36,38,44-47), it is speculated that MxB has a NES to assist its translocation. In this study, using online software to predict the putative NES of MxB, we identified five regions and deleted each of these five sequences to test their potential NES function. Unfortunately, these five sequences do not bear NES function.

MxB mutants del(98-123), del(199-223), del(374-389), del(505-527) and (515-519)A showed dispersed distribution in the cytoplasm, yet del(98-123), del(199-223) and del(374-389) preserved certain levels of anti-HIV-1 activity. This may reflect the possibility that these MxB mutants retain the ability of binding HIV-1 capsid, thus inhibiting HIV-1 infection. However, the del(505-527), (515-519)A, and 515A mutations abrogate the ability to inhibit HIV-1. Different from del(505-527), (515-519)A, the 515A single mutation still exhibits punctate cytoplasmic distribution. Future study is needed to determine whether the punctate localization of MxB is important for the antiviral function.

MxB is able to assemble into highly
ordered helical tubes. Using molecular dynamics flexible fitting, the cryo-EM structure of MxB assembly reveals that six MxB dimers are interconnected one by one to form a rung, which further form a one-start helical tube (43). The ability to dimerize has been shown to be critical for its antiviral activity, as the dimer interface mutations ablate the ability to inhibit HIV-1 (36,38,41-44). Mutations (F495D, R449D, and E484K) in one of the oligomerization interfaces, interface 3, lose both the ability to form oligomers and the ability to restrict HIV-1(43). Here we observed that, the (515-519)A mutation disrupts the anti-HIV-1 activity of MxB. Based on the cryo-EM structure, amino acids 515 to 519 are located adjacent to oligomer interface 3. The (515-519)A and 515A mutations may thus affect the hydrophobic interactions at interface 3, and affect rung stacking and oligomer formation. Indeed, results of chemical cross-linking and gel filtration chromatography showed that (515-519)A and 515A mutations diminish MxB oligomerization. We have also tested the capsid binding ability of these mutations, and observed that (515-519)A and 515A did not bind to HIV-1 capsid. It indicates possible association between MxB oligomerization and its binding to HIV-1 capsid. Oligomerization of MxB has been reported to play a pivotal role in inhibiting HIV-1 and herpesviruses, likely through a coordinated binding to viral capsid and thus blockade of viral DNA from entering the nucleus (26-28,36,38,41-44). Through investigating a series of MxB mutants, we have identified new amino acids in MxB, including amino acids 515-519 which participate in MxB oligomerization and contribute to the antiviral function of MxB.

Our data further strengthen the importance of MxB oligomerization in viral restriction.

**Experimental procedures**

**Plasmids and cells**

The Flag-tagged MxB in the pQCXIP expression vector was described previously (15). The Myc-tagged MxB, MxB deletions and mutations were generated with PCR or KOD-Plus-Mutagenesis Kit (TOYOBO). Plasmid DNA was transfected into cells using polyethylenimine (PEI, Sigma-Aldrich) in accordance with the manufacturer’s instruction. HEK293T cells, HeLa cells and HEK293 cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum (Gibco), 1% penicillin and streptomycin (100×, Solarbio). MxB stably expressed HEK293 cells were constructed by pQCXIP plasmids expressing wild type or mutant MxB proteins transfection and puromycin (0.6μg/ml) selection.

**HIV-1 pseudovirus stock preparation and infection**

VSV-G pseudotyped HIV-1NL4-3ΔE-YFP was prepared by transfecting HEK293T cells with HIV-1NL4-3ΔE-YFP DNA and vesicular stomatitis virus glycoprotein (VSV-G) DNA. The pseudoviruses in supernatants were harvested at 48h after transfection. MxA or MxB transfected HEK293T cells were incubated with VSV-G pseudotyped HIV-1NL4-3ΔE-YFP for 48h. Cells were harvested and fixed in 1% paraformaldehyde, permeabilized by 0.1% Triton X-100 for 10 min, and then stained with Alexa Fluor® 647-conjugated Flag antibody (Cell Signaling Biotechnology). Flow cytometry was used to quantitate the infection of HIV-1.
**Western blotting**

Cells were lysed in RIPA buffer comprised of 25 mM Tris/HCl (pH 7.4), 150 mM NaCl, 1% NP-40, 0.25% sodium deoxycholate, 1 mM EDTA, and a proteinase inhibitor cocktail (Sigma-Aldrich). Lysates were cleared by centrifugation. Proteins were separated by SDS-PAGE, and then transferred onto the nitrocellulose membranes (Millipore). After blocking in 5% (w/v) non-fat skim milk for 1h, membranes were incubated with anti-Flag antibody (Sigma-Aldrich), anti-Myc antibody (Sigma-Aldrich), anti-actin antibody (Sigma-Aldrich), or anti-p24 antibody (Sino Biological) at 4 °C for overnight. The corresponding IRDye™ secondary antibodies (Odyssey) were then applied to the membranes. After extensive washing, the membranes were scanned by Odyssey Infrared Imaging System (Li-Cor). Protein signals were quantified using Image J automated digitizing program (NIH).

**Immunoprecipitation assay**

Immunoprecipitation was conducted as described previously (48). Briefly, cells were lysed in RIPA buffer 48h post-transfection. After centrifugation cell lysates were incubated with anti-Flag Ab conjugated agarose (Sigma-Aldrich) at 4 °C for overnight. After extensive washing, agarose was boiled in SDS-PAGE sample buffer. Then western blotting was performed as described above.

**Immunofluorescence staining and confocal microscopy**

For immunostaining, transfected HeLa cells were fixed in 4% paraformaldehyde for 10 min, and then permeabilized by 0.1% Triton X-100 for 10 min. After incubation in blocking buffer, staining was performed with anti-Flag antibody (Sigma-Aldrich) for one hour, followed by anti-mouse Alexa Fluor® 488-conjugated antibody (Thermo Scientific) incubation. DAPI was used for nuclei staining. Images were acquired with a Leica TCS SP5, DMI6000 confocal microscope (Leica Microsystems).

**Cross-linking assay**

As previously described (42), Flag-tagged MxB or mutants transfected HEK293T cells were harvested and lysed with 0.5% NP-40/PBS. Cell lysates were cleared by centrifugation at 16,000 ×g for 10 min. Supernatants were incubated with DSS (disuccinimidyl suberate; Thermo Scientific) at different final concentrations for 1h at room temperature. The mixtures were then incubated with SDS-PAGE sample buffer for 30 min at 37°C. Samples were resolved by 6% SDS-PAGE and detected by western blotting.

**Gel filtration chromatography**

For gel filtration chromatography, 1×10^7 HEK293T cells expressing MxB or mutants were collected and lysed in buffer containing 20 mM Tris/HCl (pH 7.4), 150 mM NaCl, and 14 mM CHAPS. Cell lysates were separated using a Superdex™ 200 column (GE Healthcare, Life Sciences) at an elution rate of 0.5 mL/min. Samples from each fraction was analyzed by western blotting.

**Step gradient analyses**

1×10^7 HEK293T cells expressing MxB or mutants were lysed in buffer comprised of 10 mM Tris/HCl (pH 7.4), 10 mM KCl, 1.5 mM MgCl_2_, and 0.5 mM DTT. Purified virus treated with 0.1% Triton X-100 and cell lysates underwent step sucrose gradient analyses as reported (49,50). The soluble
Pro515 of MxB contributes to its antiviral activity

proteins containing fraction (F1) and virus-core containing fraction (F3) were analyzed by western blotting as described above.  

Data availability statement
All data are contained within the article.
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Pro515 of MxB contributes to its antiviral activity

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FOOTNOTES
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The abbreviations used are: IFN, interferon; NLS, nuclear localization signal; NES, nuclear export signal; APOBEC3G, apolipoprotein B mRNA-editing catalytic polypeptide 3G; TRIM5α, tripartite motif protein 5-alpha; ZAP, zinc-finger antiviral protein; SAMHD1, SAM domain- and HD domain-containing protein1; IFITM, interferon-induced transmembrane protein; SERINC5, serine incorporator 5; GTPases, guanosine triphosphatases; BSE, bundle signaling elements; NPC, nuclear pore complex; DSS, disuccinimidyl suberate; PEI, polyethyleneimine; PAGE, polyacrylamide gel electrophoresis
Table 1. The putative NES in MxB. Online software (Loc NES) was used to predict the putative NES in MxB. Putative NES with high scores and overlapping sequences are shown.
Figure 1. Deletion of amino acids 505 to 527 impairs the anti-HIV-1 activity of MxB. (A) Schematic representation of putative NES deletions. (B) Indirect immunofluorescence analysis of HeLa cells that were transfected with wild type MxB DNA or truncations (anti-Flag, green). The nuclei were stained with DAPI (blue). Scale bar: 10 μm. (C) Cells were transfected with wild type MxB DNA or truncations, and then infected with VSV-G pseudotyped HIV-1NL4-3ΔE-YFP for 48h. Flow cytometry was performed to measure HIV-1 infection. Cells expressing MxA were challenged with HIV-1NL4-3ΔE-YFP as a control. Error bars represent SD (***P<0.001, **P<0.01, *P<0.05, t test). The expression of MxA, MxB and truncations was shown in lower panel. (D) Co-immunoprecipitation of Flag-tagged wild type MxB or truncations together with Myc-MxB.
HEK293T cells were transfected with plasmids expressing Myc-tagged MxB together with Flag-MxB or truncations. Flag-tagged proteins were immunoprecipitated with anti-Flag antibody, followed by western blotting with the indicated antibodies.
Figure 2. The (515-519)A mutation loses the ability to inhibit HIV-1. (A) Schematic representation of the substitution mutations. (B) Indirect immunofluorescence analysis of HeLa cells transfected with wild type or mutated MxB DNA (anti-Flag, green). The nuclei were stained with DAPI (blue). Scale bar: 10 μm. (C) Cells were transfected with wild type or mutated MxB DNA, and then infected with VSV-G pseudotyped HIV-1\textsubscript{NL4-3,ΔE,YFP} for 48h. Flow cytometry was performed to score HIV-1 infected cells. Cells expressing MxA were infected with HIV-1\textsubscript{NL4-3,ΔE,YFP} as a control. Error bars represent SD (\(**P<0.001, **P<0.01, *P<0.05\), t-test). The expression of MxA, MxB and mutants was shown in lower panel. (D) Co-immunoprecipitation of Flag-tagged wild type MxB or mutants together with Myc-MxB. HEK293T cells were transfected with plasmids expressing Myc-tagged MxB together with...
Flag-MxB or mutants. Flag-tag proteins were immunoprecipitated with anti-Flag antibody, followed by western blotting with the indicated antibodies.
Pro515 of MxB contributes to its antiviral activity

Figure 3. Mutation of P515 decreases MxB anti-HIV-1 activity. (A) Schematic representation of substitution mutations. (B) Indirect immunofluorescence analysis of HeLa cells transfected with wild type or mutated MxB DNA (anti-Flag, green). The nuclei were stained with DAPI (blue). Scale bar: 10 μm. (C) HEK293 cells expressing wild type or mutated MxB were infected with VSV-G pseudotyped HIV-1NL4-3-ΔE-YFP for 48h. Flow cytometry was performed to measure HIV-1 infection efficiency. Cells expressing MxA were challenged with VSV-G pseudotyped HIV-1NL4-3-ΔE-YFP as a control. Error bars represent SD (**P<0.01, *P<0.05, t test).
The expression of MxA, MxB and mutants was shown in the lower panel. (D) Co-immunoprecipitation of Flag-tagged wild type MxB or mutants together with Myc-MxB. HEK293T cells were transfected with plasmids expressing Myc-tagged MxB together with Flag-MxB or mutants. Flag-tag proteins were immunoprecipitated with anti-Flag antibody, followed by western blotting with the indicated antibodies.
Pro515 of MxB contributes to its antiviral activity

Figure 4. Amino acids 515 to 519 close to interface 3 affect MxB oligomerization. (A) The cryo-EM structure of an MxB oligomer reveals that amino residues 515 to 519 (yellow) are located on the Sα2 helix, which is adjacent to interface 3 (magenta and black circle). (B) Expanded views of the interface 3. (C) Cells expressing wild type or mutated MxB were infected with VSV-G pseudotyped HIV-1NL4-3-ΔE-YFP for 48h. Flow cytometry was performed to measure HIV-1 infection efficiency. Cells expressing MxA were challenged with VSV-G pseudotyped HIV-1NL4-3-ΔE-YFP as a control. Error bars represent SD (***$P<0.001$, **$P<0.01$, *$P<0.05$, t test). The expression of MxA, MxB and mutants was shown in the right panel.
Pro515 of MxB contributes to its antiviral activity

Figure 5. MxB(515-519)A and MxB515A are defective in oligomerization. (A) Cells expressing Flag-tagged wild type MxB or MxB mutants (MxB449/495D, MxB574/651D, MxB515-519A, or MxB515A) were harvested and lysed. Disuccinimidyl suberate (DSS) was then added into lysates at an increasing concentration of 0 to 10 μg/ml (0 indicates DMSO only). After 1 h, the reaction was quenched, and Flag-tagged protein was resolved by 6% SDS-PAGE and detected by western blotting. (B) Cell lysates expressing wild type or mutated MxB were fractionated on a Superdex 200 gel filtration column. Different fractions were analyzed by western blotting.
Figure 6. MxB mutants (515-519)A and 515A are deficient in binding to HIV-1 capsid. (A) Illustration of MxB-HIV-1 capsid binding assay. VSV-G pseudotyped HIV-1\textsubscript{NL4-3-ΔENF} viruses were mixed with MxB (wild type or mutants) containing cell lysates, treated with 0.1% Triton X-100, and loaded onto step sucrose gradients. (B and D) The individual gradient fractions representing soluble MxB (F1) and core-associated MxB (F3) were subjected to immunoblot analysis. Antibody against HIV-1 CAp24 was used as control. (C and E) Intensity of Flag-tagged protein band was quantified using Image J (NIH). Error bars represent SD (**P<0.001, *P<0.01, *P<0.05, t test).
Pro-515 of the dynamin-like GTPase MxB contributes to HIV-1 inhibition by regulating MxB oligomerization and binding to HIV-1 capsid

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