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Research Article

MxA interacts with and is modified by the SUMOylation machinery

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ABSTRACT

Mx proteins are evolutionarily conserved dynamin-like large GTPases involved in viral resistance triggered by types I and III interferons. The human MxA is a cytoplasmic protein that confers resistance to a large number of viruses. The MxA protein is also known to self-assembly into high molecular weight homo-oligomers. Using a yeast two-hybrid screen, we identified 27 MxA binding partners, some of which are related to the SUMOylation machinery. The interaction of MxA with Small-Ubiquitin MOdifier 1 (SUMO1) and Ubiquitin conjugating enzyme 9 (Ubc9) was confirmed by co-immunoprecipitation and co-localization by confocal microscopy. We identified one SUMO conjugation site at lysine 48 and two putative SUMO interacting motifs (SIMa and SIMb). We showed that MxA interacts with the EIL loop of SUMO1 in a SIM-independent manner *via* its CID–GED domain. The yeast two-hybrid mapping also revealed that Ubc9 binds to the MxA GTPase domain. Mutation in the putative SIMa and SIMb, which are located in the GTPase binding domain, reduced MxA antiviral activity. In addition, we showed that MxA can be conjugated to SUMO2 or SUMO3 at lysine 48 and that the SUMOylation-deficient mutant of MxA (MxA_{K48R}) retained its capacity to oligomerize and to inhibit Vesicular Stomatitis Virus (VSV) and Influenza A Virus replication, suggesting that MxA SUMOylation is not essential for its antiviral activity.

Introduction

Viral resistance can be induced by treatment of cells with interferons (IFNs) [1]. This resistance is caused by the expression of a large number of IFN-stimulated genes including *MX1* [2]. *MX1* was first discovered due to the resistant phenotype of some inbred mice to the infection by Influenza A Virus (FLUAV) [3,4]. These mice were described to carry a functional copy of the *MX1* gene, which was named *Myxovirus resistance gene 1* due to its antiviral activity against viruses of the *Orthomyxoviridae* family. Most of the vertebrates, including humans, rodents, fish and birds, and some invertebrates are known to have one or more copies of the *MX* gene [5]. Humans harbor two *MX* (*MX1* and *MX2*) genes in the chromosome 21 [6], which are induced only in response to type I or type III IFNs *via* activation of the JAK/STAT pathway [7–10].

MX1 and *MX2* gene products are MxA and MxB, respectively. MxA is a cytoplasmic protein [11] while MxB can be found in the cytoplasm and in the nucleus [12]. Mx proteins are classified as

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large dynamin-like GTPases due to their similarities to dynamins [5]. MxA and dynamins share structural and functional aspects including domain organization, GTPase activity and homo-oligomerization capacity [13].

Based on sequence alignment with dynamins, MxA was initially considered to be organized in three domains: a N-terminal GTP-binding domain (GTPase), a central interactive domain (CID) and a C-terminal GTPase effector domain (GED) [13]. Recently, crystallography studies have revealed that MxA is in fact composed of two domains connected to each other by a central region. The first domain still corresponds to the GTPase domain, which binds and hydrolyzes GTP. However, according to this new nomenclature, the two domains previously called CID and GED are now involved in the formation of a single tertiary structure named stalk, which coordinates the formation of MxA homo-oligomers. The connective central region, called Bundle-Signaling Element (BSE), stands between the GTPase and stalk domains. The BSE is generated by sequences present in the N-terminal, C-terminal and the middle portion of MxA, between the GTPase and stalk domains. The BSE exerts an important function in the formation of MxA homo-oligomers [14]. The integrity of both GTPase and stalk domains is necessary for the correct assembly of MxA homo-oligomers [14–17]. MxA homo-oligomerization is required for its GTPase activity [18,19]. However, it is not clear whether GTPase activity is required or if GTP-binding alone is sufficient to confer antiviral activity [5,20]. Accordingly, the L612K monomeric MxA mutant lacks detectable GTPase activity but retains its GTP-binding capacity and antiviral activity against Thogoto virus (THOV) and Vesicular Stomatitis Virus (VSV) [21].

MxA acts as a mediator of the IFN-induced antiviral state, indeed its expression confers resistance to a large number of RNA viruses including species belonging to the *Orthomyxoviridae* and *Rhabdoviridae* families [5,11,22,23].

The SUMOylation machinery modifies many cellular proteins involved in the antiviral response as well as viral proteins. Some viruses have developed mechanisms to evade host response by targeting components of the SUMOylation machinery [24–26].

SUMO (Small-Ubiquitin MOdifier) proteins are members of the ubiquitin-like family of modifiers [27]. Mammals have four SUMO proteins named SUMO1 through SUMO4. SUMO1, SUMO2 and SUMO3 have a widespread expression, whereas SUMO4 expression seems to be restricted to kidney, lymph nodes and spleen [28]. SUMO interacts with proteins by two mechanisms: (1) it can be covalently attached to proteins that contain a consensus motif of SUMOylation (ΨKxE/D), where a glycine residue of SUMO is linked to the lysine residue of the target protein and/or (2) it can interact in a non-covalent manner to proteins containing a SUMO-interacting motif (SIM), composed of an hydrophobic core (ΨΨxΨ). While SUMO1–3 paralogs are covalently attached to proteins under normal conditions, SUMO4 seems to be attached to its targets only under stress conditions [27,29]. SUMO2–3, but not SUMO1, can lead to the formation of poly-SUMO2/3 chains [32–33]. SUMOylated proteins are found mainly in the nucleus, but they can also be found in the cytoplasm [30,31].

Protein SUMOylation is mainly involved in the modification of protein activities, localization or stability [27,32,33]. Moreover, SUMOylation contributes to the regulation of gene transcription, cell apoptosis, intracellular stress response and cell cycle progression [33]. These processes can be associated with the presence of SUMOylated proteins in PML NBs (ProMyelocytic Leukemia protein Nuclear Bodies), subnuclear structures where SUMO and SUMOylated proteins are found [34]. Almost 40% of PML partners have been confirmed to be SUMOylated, suggesting that PML NBs are enriched sites for SUMOylated proteins and may function as nuclear SUMOylation hotspots [34].

Here we found by a yeast two-hybrid system that MxA interacts with 27 nuclear and cytoplasmic putative partners, including SUMO1, Ubc9, PML NB-associated proteins and proteins implicated in cell cycle control. MxA interaction with SUMO1 and Ubc9 has been validated by co-immunoprecipitation and co-localization. In addition, we showed that MxA contains one site of SUMOylation (K48) used for the covalent conjugation to SUMO2 or SUMO3. We identified two putative SIM sequences, SIMa ($V_{260}V_{261}D_{262}V_{263}$) and SIMb ($V_{171}P_{172}D_{173}L_{174}T_{175}$), on MxA primary structure that are not involved in the interaction with SUMO1.

We have determined that the MxA CID–GED (stalk) domain is involved in the interaction with SUMO1 in a SIM-independent manner. Furthermore, the interaction between SUMO1 and MxA required the E67-interacting loop (EIL) but not the SUMO-interacting groove (SIG). In addition, the MxA oligomerization capacity was important for its interaction with SUMO1 and Ubc9.

Finally, the MxA SUMOylation-deficient mutant (MxA_{K48R}) still oligomerizes and inhibits VSV and Influenza A Virus replication, suggesting that MxA covalent modification by SUMO is not essential for its antiviral activity. The SIMa ($MxA_{V260A–V261A–D262A–V263A}$) and SIMb ($MxA_{V171A–P172A–L174A}$) mutants, which are located in the GTPase domain, have a reduced antiviral activity.

Materials and methods

Strains, plasmids, chemicals and antibodies

Plasmids and primers used in this study are listed in Supplementary Tables 1 and 2. All chemicals were obtained from Sigma, United States Biological or Thermo Scientific unless otherwise indicated.

The rabbit anti-MxA polyclonal antibody was produced for this study. The efficacy of the polyclonal anti-MxA (α-pMxA) was determined by immunofluorescence and Western blot as shown in Supplementary Fig. S1. The mouse monoclonal anti-MxA antibody (α-mMxA) was provided by Dr. Otto Haller. The rabbit anti-VSV polyclonal antibody was provided by Dr. Danielle Blondel. The Rabbit anti-PML (sc-5621), rabbit anti-SUMO1 (Sc9060), rabbit anti-LexA and rabbit anti-Actin clone C-11 (sc-1615) antibodies were from Santa Cruz Biotechnology. The mouse anti-6xHis, the mouse anti-Ubc9 (AM1261a) and the rabbit anti-SUMO2/3 antibodies were from Clontech, Abgent and Invitrogen, respectively.

Cell culture

Human HeLa cells and murine NIH3T3 cells were grown at 37 °C in DMEM supplemented with 10% FCS. NIH3T3 harboring the empty vector or the plasmid coding for MxA, kindly provided by Dr. J. Pavlovic, were kept in medium supplemented with 0.5 mg/mL of neomycin [11].