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NVL2, a nucleolar AAA-ATPase, is associated with the nuclear exosome and is involved in pre-rRNA processing

Yuki Yoshikatsu [a], Yo-ichi Ishida [b], Haruka Sudo [b], Keizo Yuasa [a], Akihiko Tsuji [a], Masami Nagahama [b, *]

[a] Department of Life Systems, Institute of Technology and Science, The University of Tokushima Graduate School, Tokushima 770-8506, Japan
[b] Department of Molecular and Cellular Biochemistry, Meiji Pharmaceutical University, Kiyose, Tokyo 204-8588, Japan

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ABSTRACT

Nuclear VCP-like 2 (NVL2) is a member of the chaperone-like AAA-ATPase family and is involved in the biosynthesis of 60S ribosomal subunits in mammalian cells. We previously showed the interaction of NVL2 with a DExD/H-box RNA helicase MTR4/DOB1, which is a known cofactor for an exoribonuclease complex, the exosome. This finding implicated NVL2 in RNA metabolic processes during ribosome biogenesis. In the present study, we found that a series of mutations within the ATPase domain of NVL2 causes a defect in pre-rRNA processing into mature 28S and 5.8S rRNAs. Co-immunoprecipitation analysis showed that NVL2 was associated with the nuclear exosome complex, which includes RRP6 as a nucleus-specific catalytic subunit. This interaction was prevented by depleting either MTR4 or RRP6, indicating their essential role in mediating this interaction with NVL2. Additionally, knockdown of MPP6, another cofactor for the nuclear exosome, also prevented the interaction by causing MTR4 to dissociate from the nuclear exosome. These results suggest that NVL2 is involved in pre-rRNA processing by associating with the nuclear exosome complex and that MPP6 is required for maintaining the integrity of this rRNA processing complex.

1. Introduction

Nuclear VCP-like 2 (NVL2) is a member of the AAA (ATPase associated with diverse cellular activities) protein family that displays a high level of amino acid sequence similarity with VCP/p97 [1]. AAA proteins are chaperone-like ATPases that play important roles in numerous cellular processes such as DNA replication, organelle membrane fusion, and protein unfolding, degradation, and transport [2,3]. AAA-ATPases contain one or two conserved ATPase domains called AAA modules. They consist of canonical Walker A and Walker B motifs, which are critical for nucleotide binding and hydrolysis, respectively. Additionally, AAA-ATPases contain “second region of homology” motifs that distinguish this protein family from other ATPase families [2]. AAA-ATPases usually assemble into ring-shaped homo-hexamers. ATP hydrolysis causes conformational changes within the proteins, which provide a mechanical force to unfold substrate proteins or to disassemble target macromolecular complexes [3]. NVL2, like VCP, consists of an N-terminal regulatory domain followed by two ATPase domains (D1 and D2). Previous studies on VCP have shown that the D2 domain mediates major ATPase activity, which is critical to induce conformational changes in interacting substrate proteins [4]. In contrast, the D1 ATPase activity is very low; however, its ATP-binding is essential for hexamer formation [5]. Since ATP-binding or catalytically dead mutant versions of these ATPases act as dominant-negative inhibitors in cells, they have been extensively used to evaluate cellular functions of these proteins [6–8].

NVL2 mainly resides in the nucleolus and serves in the formation of 60S ribosomes [7,8]. Ribosome biogenesis in eukaryotic cells is a highly coordinated multistep process that occurs primarily in the nucleolus [9]. In mammalian cells, the process is initiated with the synthesis of 47S pre-ribosomal RNA (pre-rRNA) (Fig. 1A). This primary transcript is then processed into smaller intermediates by sequential exo- and endo-nucleolytic cleavages at sites within the 5′- and 3′-external transcribed spacers (5′-ETS and 3′-ETS) and the two internal spacers (ITS1 and ITS2) of the pre-rRNA, resulting in 28S, 5.8S, and 18S rRNAs [10]. At various steps in this processing, the RNAs assemble with ribosomal proteins, thereby resulting in

* Corresponding author. Department of Molecular and Cellular Biochemistry, Meiji Pharmaceutical University, 2-522-1 Noshio, Kiyose, Tokyo 204-8588, Japan.
E-mail address: nagahama@my-pharm.ac.jp (M. Nagahama).

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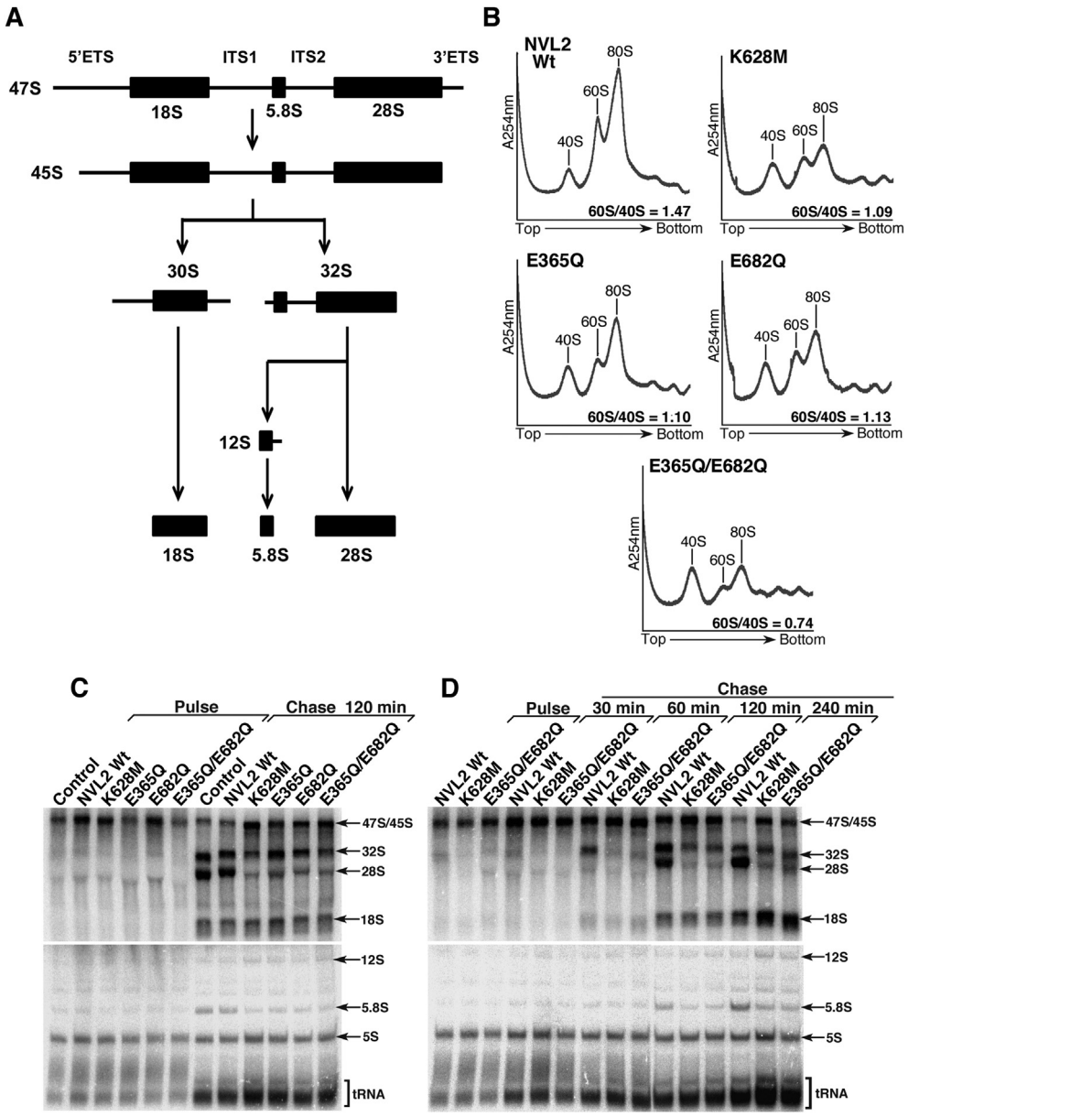

Fig. 1. ATPase domain mutants of NVL2 prevent 60S ribosome synthesis and pre-rRNA processing. (A) Schematic diagram of the major steps in pre-rRNA processing in human cells. The precursor (47S pre-rRNA) contains the sequences of mature 18S, 5.8S, and 28S rRNAs separated by ITS1 and ITS2, and flanked by 5′-ETS and 3′-ETS. After multiple steps of processing, the mature rRNA products (filled bars) are generated. (B) Ribosome profiles ($A_{254\ nm}$) obtained on performing sucrose gradient centrifugation of a cytosolic extract of cells induced with doxycycline to overexpress wild-type NVL2 (Wt) or mutants are shown. The positions of 40S, 60S, and 80S ribosomal subunits are indicated. (C, D) Following induction with doxycycline to express wild-type NVL2 (Wt) or the indicated mutants, the cells were labeled for 30 min with ^{32}Pi (Pulse), and then incubated for indicated periods in nonradioactive medium (Chase). Total RNA was extracted, separated on a denaturing gel made of agarose (upper panel) or polyacrylamide (lower panel), and then subjected to autoradiography.

the formation of mature 40S and 60S ribosomal subunits. Additionally, numerous non-ribosomal *trans*-acting proteins, such as ribonucleases, helicases, chaperones and GTPases, are necessary for ribosome biogenesis but are not incorporated into the mature ribosomes and must be dissociated from precursor particles before maturation [9]. While many of these *trans*-acting factors have been identified in yeast, they are less well characterized in higher eukaryotes despite their high degree of evolutionary conservation. We have previously identified ribosomal protein L5 and RNA helicase MTR4/DOB1 as NVL2-binding factors, which possibly act in ribosome biogenesis under the control of NVL2 activity [7,8].

The RNA exosome, a major 3′-5′ exoribonuclease complex that is present both in the nucleus and cytoplasm, is involved in the metabolism of many RNA species, including pre-rRNAs [11,12]. The eukaryotic exosome consists of nine catalytically inert core components, which form a two-layered barrel-like structure. The core exosome associates with catalytic subunits, which include DIS3 and RRP6 (also called PM/Scl-100). DIS3 contains two distinct exo- and endo-nucleolytic catalytic sites and is found in both the nuclear and cytoplasmic forms of the exosome. In contrast, a second 3′-5′ exonuclease, RRP6, is specifically associated with the nuclear exosome. In addition, a number of exosome-associating cofactors, which participate in the regulation or coordination of its activity, are known to exist. Distinct classes of RNA substrates are assigned to individual catalytic subunits and cofactors; however, the targeting mechanisms in this process are largely unknown [12,13].

In this study, we first investigated whether NVL2 is involved in the biosynthetic processing of the rRNA precursor. We then examined its interaction with the nuclear exosome and its co-factors. Our results suggest a regulatory role for NVL2 in the structural rearrangement of the exosome-containing complexes as an ATP-driven machinery during the biogenesis of 60S ribosomes in mammalian cells.

2. Materials and methods

2.1. Plasmid construction

cDNAs coding for NVL2 mutants (E365Q and E682Q) were generated by polymerase chain reaction (PCR)-based mutagenesis. cDNAs containing each mutation were combined to generate the E365Q/E682Q double point mutant by using unique restriction sites. Generation of the K628M mutant of NVL2 has been described previously [7]. These mutant and wild-type cDNAs were subcloned into pFT103 [14] to stably express C-terminally Strep-tagII-tagged NVL2 constructs in the doxycycline-inducible Flp-In T-REx system (Invitrogen, Carlsbad, CA). The expression plasmid for C-terminally FLAG-tagged NVL2 was described previously [7]. The cDNA for human MTR4 (DOB1) was obtained as previously described [8]. Full-length cDNAs for human RRP6 (PM/Scl-100), RRP4 and MPP6 were amplified from a human kidney cDNA library (Clontech, Palo Alto, CA) by PCR. To express N-terminally FLAG-tagged MTR4 or RRP6 in the Flp-In T-REx system, the cDNAs were inserted into pcDNA5/FRT/TO (Invitrogen) to construct pFT-FLAG-MTR4 or pFT-FLAG-RRP6, respectively. The human RRP4 cDNA was subcloned into pGEX-4T-2 (GE Healthcare, Piscataway, NJ) to express RRP4 as glutathione-*S*-transferase (GST)-fused protein in *Escherichia coli*. The cDNA for MPP6 was subcloned into pEBG [15] to express the protein fused to the N-terminal GST in mammalian cells.

2.2. Antibodies

Generation of rabbit anti-NVL2, anti-L5 and anti-MTR4 antibodies has been described previously [7,8]. The monoclonal anti-FLAG M2 antibody and the polyclonal anti-PM/Scl-100 antibody were purchased from Sigma–Aldrich (St. Louis, MO). To generate the polyclonal anti-RRP4 antibody, rabbits were immunized with GST-fused full-length human RRP4 expressed in *E. coli*.

2.3. Cell culture and transfection

Flp-In T-REx-293 cells (Invitrogen) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 100 μg/ml Zeocin (InvivoGen, San Diego, CA), and 15 μg/ml blasticidin S (Kaken Pharma, Tokyo, Japan). Transfection was performed with Lipofectamine 2000 reagent (Invitrogen) according to manufacturer's instructions. To generate stable cells exhibiting doxycycline-inducible expression of cDNAs, Flp-In T-REx-293 cells were cotransfected with pFT-FLAG-MTR4 or pFT-FLAG-RRP6 and the Flp recombinase plasmid pOG44. Stable integrants were selected by culture in hygromycin B (100 μg/ml) (Wako, Osaka, Japan) and blasticidin S (15 μg/ml) and maintained under the same conditions. cDNA expression was induced with 1 μg/ml of doxycycline (Sigma–Aldrich) before use in the experiments.

2.4. RNA interference

For knockdown of human MPP6, synthetic small interference RNA (siRNA) was purchased from B-Bridge International (Mountain View, CA). The siRNA sequences were as follows: siMPP6-1, 5′-CCAAGAAUCUACUGCGCAU-dTdT-3′; siMPP6-2, 5′-GAGAAAGAGAG UUUCAUAA-dTdT-3′. Stealth RNAi oligonucleotides (Invitrogen) were used for knockdown of human MTR4 and RRP6. The sequences used were as follows: siMTR4-1, 5′-GGGAAUUAACAUGCCA GCUAGAACU-3′; siMTR4-2, 5′-GGAAUGGAUGAUAGAGGAAUU-GUAA-3′; siMTR4-3, 5′-CCCUAUUGAUGAUAUGGGCAUUCAA-3′; siRRP6-1, 5′-GCUGGAACCGUAAGGCAGCAGAAUA-3′; siRRP6-2, 5′-CCCAGUUAUACAGACCUAUAGAAGA-3′; siRRP6-3, 5′-CAACCAGUGG AUCUGUGCCAGUUCA-3′. The siRNA targeting luciferase (GL3) was used as a negative control. The siRNAs were transfected with Lipofectamine 2000 reagent according to the manufacturer's protocols.

2.5. Immunoprecipitation

The interactions between FLAG-tagged bait proteins and cellular proteins were studied by co-immunoprecipitation analysis of extracts prepared from Flp-In T-REx-293 cells, which were doxycycline-induced or transiently transfected before experiments. The cells were lysed at 4 °C for 20 min in lysis buffer (50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 5 mM $MgCl_2$, 2 mM EDTA, 2 mM ATP, 0.5% Nonidet P40, 1 mM dithiothreitol, 10 μg/ml leupeptin, 1 μg/ml pepstatin A, and 1 mM phenylmethylsulfonylfluoride). After brief sonication, the lysate was cleared at 14,000 rpm for 30 min, and then incubated with anti-FLAG M2 agarose beads (Sigma–Aldrich). After incubation for 2 h at 4 °C, the beads were spun down and washed four times with wash buffer (50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 5 mM $MgCl_2$, 2 mM EDTA, 2 mM ATP, 0.05% Nonidet P40, 1 mM dithiothreitol, and 1 mM phenylmethylsulfonylfluoride). The bound proteins were then eluted from the beads with SDS-polyacrylamide gel electrophoresis (PAGE) sample buffer by heating at 95 °C for 3 min and then subjected to SDS-PAGE followed by western blotting. Proteins were detected with an enhanced chemiluminescence reagent (Thermo Fisher, San Jose, CA).

2.6. Ribosome profiling by sucrose gradient velocity centrifugation

Cytoplasmic extracts of cells were analyzed by fractionation on a linear sucrose gradient (10–40%) as described previously [7,8]. After ultracentrifugation, the ribosome profile was determined by UV monitoring.

2.7. Analysis of RNA synthesis and processing

Twenty-four hours after the addition of doxycycline to induce overexpression of wild-type or mutant NVL2, Flp-In T-REx-293-derived cells were metabolically labeled with 20 μCi/ml [^{32}P] orthophosphate (8000–9000 Ci/mmol, PerkinElmer) in complete medium for 30 min, washed twice with phosphate-buffered saline and chased in nonradioactive medium for various times. Total RNA was extracted with Isogen reagent (Nippon Gene, Tokyo, Japan). Aliquots of the extracted RNA (^{32}P labeling: ~10,000 cpm/lane) were resolved on a 1% agarose gel containing formaldehyde and transferred to Hybond-N$^+$ membranes (GE Healthcare): the signal was visualized using a BAS-1500 phosphorimager (Fuji Photo Film, Tokyo, Japan). To separate low molecular weight RNAs, an 8% polyacrylamide-7M urea gel was used.

3. Results

3.1. NVL2 is involved in the pre-rRNA processing for 60S ribosome synthesis

Our previous work suggested that NVL2 is implicated in ribosome biosynthesis. The expression of the ATP-binding

deficient NVL2(K628M) mutant, with an amino-acid substitution in the Walker A motif of the second ATPase domain, was found to lead to reduction in 60S ribosomes in cells [7,8]. To further confirm that NVL2 is active in the ribosome synthesis, we generated additional point mutations in the Walker B motif of either or both of the two ATPase domains (E365Q, E682Q and E365Q/E682Q), which are expected to cause defects in ATP hydrolysis. By using the Flp-In T-REx system, these mutants were expressed in HEK293 cells in a doxycycline-inducible manner. Profiles of cytoplasmic ribosomes were analyzed on sucrose density gradients (Fig. 1B). As previously shown [7,8], in K628M mutant-expressing cells, the levels of 60S and 80S ribosomes decreased while the 40S subunits were not affected. Similarly, both the E365Q and E682Q mutants have reduced 60S ribosomes, with the double point mutant E365Q/E682Q exhibiting the largest decrease. These results suggest that ATP hydrolysis in the two ATPase domains is important and that they work together in 60S subunit synthesis.

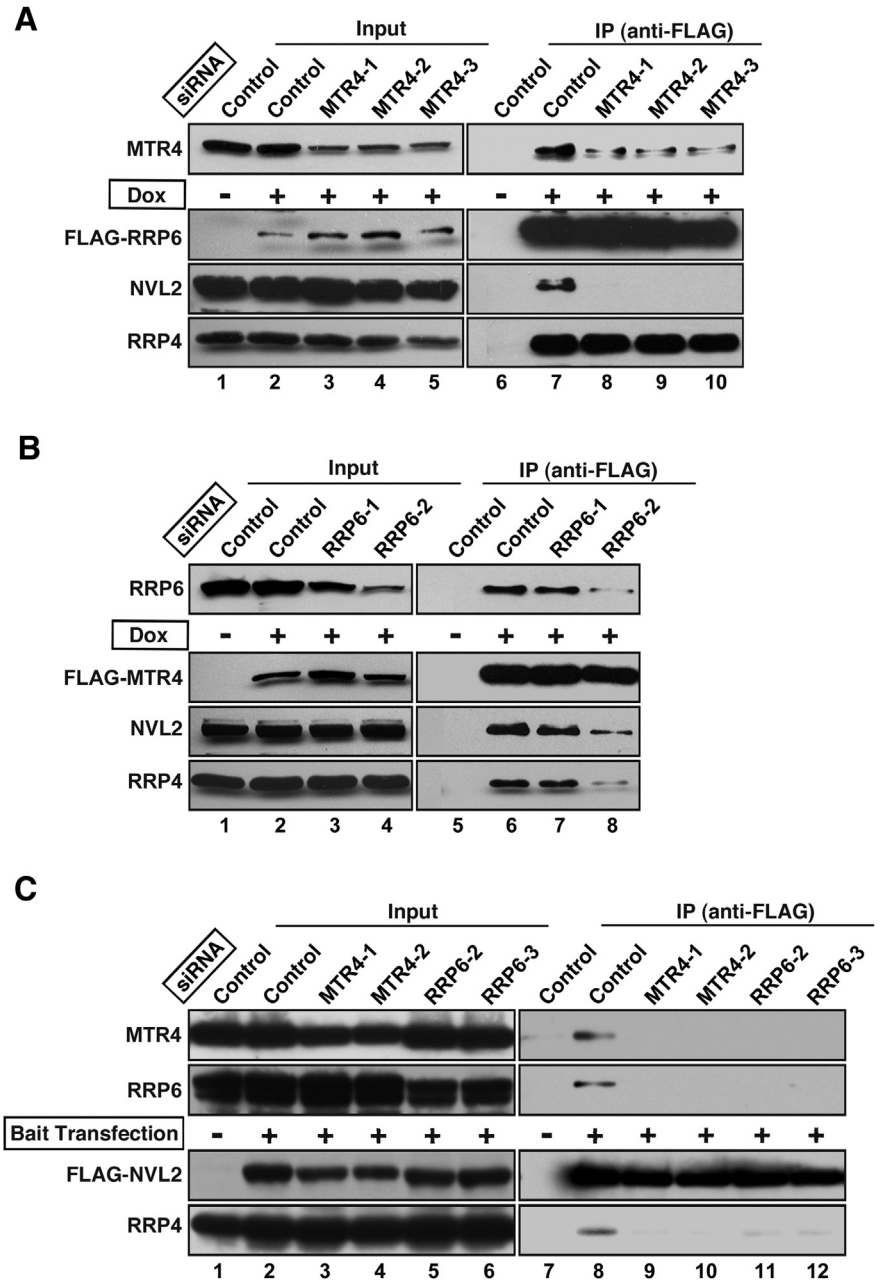

Fig. 2. NVL2 interacts with the nuclear exosome. (A) Effect of MTR4 knockdown on the interaction between NVL2 and the nuclear exosome. HEK293 cells that express FLAG-RRP6 upon induction with doxycycline (Dox) were transfected with control siRNA (Control) or siRNAs against MTR4 (MTR4-1, -2, or -3), followed by treatment with doxycycline. The cell lysates were immunoprecipitated with anti-FLAG agarose beads. The immunoprecipitates (IP) and 2% of the starting material (Input) were resolved by SDS-PAGE and then analyzed by western blotting. (B) Effect of RRP6 knockdown on interaction between MTR4 and NVL2. HEK293 cells that express FLAG-MTR4 upon induction with doxycycline (Dox) were transfected with control siRNA (Control) or siRNAs against RRP6 (RRP6-1 or -2), followed by the treatment with doxycycline. The cell lysates (Input) and immunoprecipitates (IP) were analyzed by western blotting. (C) Effects of knockdown of MTR4 and RRP6 on interaction between FLAG-NVL2 and the nuclear exosome complex. HEK293 cells were transfected with an expression plasmid encoding FLAG-NVL2, followed by transfection with control siRNA (Control), siRNA against MTR4 (MTR4-1 or -2), or siRNA against RRP6 (RRP6-2 or -3). The cell lysates (Input) and immunoprecipitates (IP) were analyzed by western blotting.

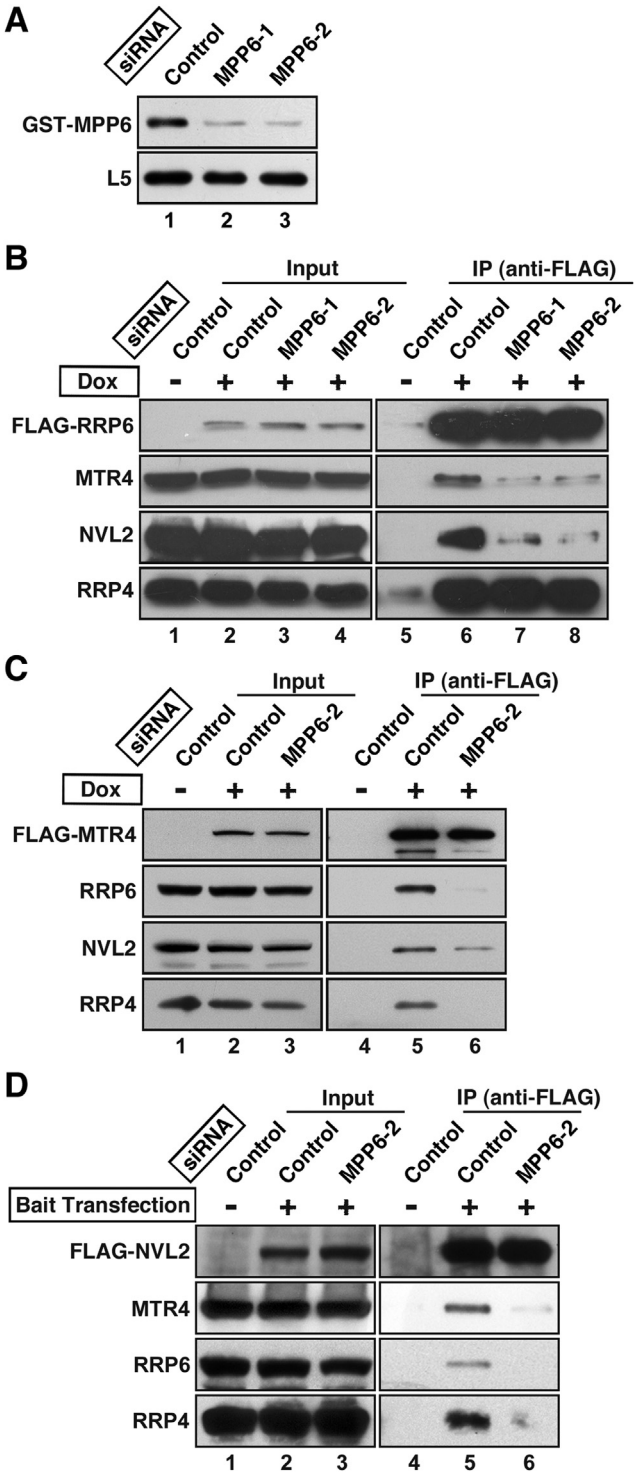

Fig. 3. MPP6 is crucial for the interaction between NVL2, MTR4, and the nuclear exosome. (A) Efficiency of MPP6 knockdown. HEK293 cells were transfected with an expression plasmid encoding GST-MPP6, followed by transfection with control siRNA (Control) or siRNA against MPP6 (MPP6-1 or -2). The cell lysates were resolved by SDS-PAGE and analyzed by western blotting using antibodies against GST or ribosomal protein L5 as a loading control. (B) Effect of MPP6 knockdown on the interaction of FLAG-RRP6 with MTR4 or NVL2. HEK293 cells that express FLAG-RRP6 upon induction with doxycycline (Dox) were transfected with control siRNA (Control) or siRNA against MPP6 (MPP6-1 or -2), followed by doxycycline treatment. The cell lysates (Input) and immunoprecipitates (IP) were analyzed by western blotting. (C) Effect of MPP6 knockdown on the interaction of FLAG-MTR4 with NVL2 and the nuclear exosome. HEK293 cells that express FLAG-MTR4 upon induction with doxycycline (Dox) were transfected with control siRNA (Control) or siRNA against MPP6 (MPP6-2), followed by doxycycline treatment. The cell lysates (Input) and immunoprecipitates (IP) were analyzed by western blotting. (D) Effect of MPP6 knockdown on interaction between FLAG-NVL2 and MTR4 or the nuclear exosome. HEK293 cells were transfected with an expression plasmid encoding FLAG-NVL2, followed by transfection with control siRNA (Control) or siRNA against MPP6 (MPP6-2). The cell lysates (Input) and immunoprecipitates (IP) were analyzed by western blotting.

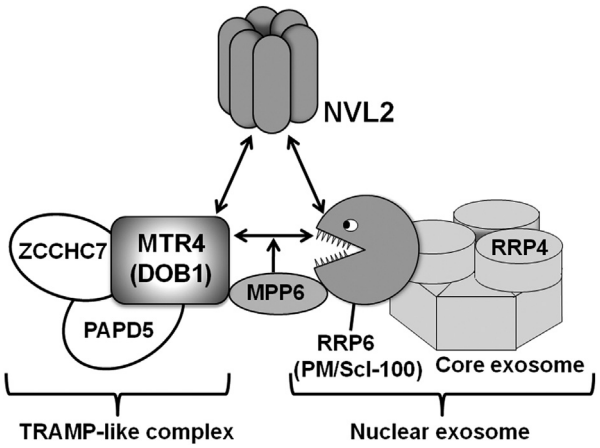

Fig. 4. A model for the interaction between NVL2, MTR4, and the nuclear exosome.

During ribosome biogenesis, the 47S precursor RNA is sequentially processed, resulting in the production of mature 28S, 18S, and 5.8S rRNAs (Fig. 1A). To further investigate the reduction in 60S ribosomes in the NVL2 mutant-expressing cells, we performed pulse-chase experiments to monitor rRNA synthesis and processing in those cells. The mutant expressing-cells were metabolically labeled with [^{32}P]orthophosphate and then chased for 120 min (Fig. 1C). The conversion efficiency from the 47S/45S precursor to mature 28S and 5.8S rRNAs *via* the 32S intermediate was significantly affected in the mutant-expressing cells compared to control (no doxycycline-induced) or wild-type NVL2-expressing cells. In contrast, there was no obvious effect in the formation of 18S rRNA in those cells. Furthermore, a time course experiment revealed that the mutant-expressing cells had slower conversion rates from the 47S/45S precursor to the 32S intermediate and from the 12S intermediates to the mature 5.8S rRNA (Fig. 1D). These results indicate that the dysfunction of NVL2 affects both the early and late stages of the pre-rRNA processing pathway.

3.2. *NVL2 associates with the nuclear exosome through interaction with MTR4 and RRP6*

We previously showed that NVL2 interacts with a DExD/H-box RNA helicase MTR4, which may participate in the mechanism underlying the regulatory role of NVL2 in 60S ribosome biogenesis [8]. MTR4 serves as a cofactor for the nuclear exosome and is known to be involved in the pre-rRNA processing [16–19]. To see if NVL2 might also associate with the nuclear exosome *via* an MTR4-interaction in the pre-rRNA processing pathway, we examined the interactions of these proteins by co-immunoprecipitation analysis. When RRP6, a catalytic subunit of the nuclear exosome, was precipitated as a FLAG-tagged bait protein, both MTR4 and the core exosome subunit RRP4 were coprecipitated as expected (Fig. 2A, lane 7). In the same time, NVL2 was also coprecipitated from the cell extract, indicating that NVL2 interacts with the nuclear exosome. Thus, to test the requirement of MTR4 for this interaction, we performed this same experiment in the presence of a siRNA-mediated knockdown of MTR4. The results showed that MTR4 is

required for the interaction between NVL2 and the nuclear exosome (Fig. 2A, lanes 8–10). Next, we examined the role of RRP6 in the interaction between NVL2 and MTR4 (Fig. 2B). NVL2, RRP6, and RRP4 coprecipitated with FLAG-MTR4, and these interactions were abolished in an RRP6 knockdown. These results suggest that RRP6 mediates the association of MTR4 with the core exosome and that MTR4 and RRP6 are mutually required for the interaction with NVL2. These results were also confirmed by co-immunoprecipitation using FLAG-tagged NVL2 as a bait protein (Fig. 2C).

3.3. MPP6 is crucial for MTR4-RRP6 interaction and required for recruiting NVL2 to the nuclear exosome

Since MTR4 and RRP6 are co-dependent for their interaction with NVL2, a stable association between these two proteins might be important for the functional integrity of the nuclear exosome. Previous work suggests that the exosome cofactor MPP6 binds to the 3′-end of the 12S pre-rRNA and recruits the nuclear exosome and MTR4 to the substrate [20]. In addition, MPP6 has been reported to interact with both the MTR4 and RRP6 in the yeast two-hybrid system [21]. Therefore, MPP6 may stabilize the MTR4-RRP6 interaction on the exosome, contributing to the recruitment of NVL2 to this complex. Thus, we used a co-immunoprecipitation experiment to investigate the effect of a MPP6 knockdown on the interactions between the nuclear exosome, MTR4, and NVL2 (Fig. 3). The efficiency of the MPP6 knockdown was confirmed by western blotting using cells transfected with GST-fused MPP6 (Fig. 3A). In this knockdown condition, the association of FLAG-RRP6 with MTR4 markedly decreased, while the binding to the core exosome subunit RRP4 was not affected (Fig. 3B). The association of NVL2 with FLAG-RRP6 concomitantly decreased. Consistent result was obtained by co-immunoprecipitation experiment using FLAG-MTR4 as a bait protein. The association of RRP6 and NVL2 with FLAG-MTR4 was also diminished by the MPP6 knockdown (Fig. 3C). Additionally, when FLAG-NVL2 was the bait protein in a co-immunoprecipitation experiment, the association of MTR4 and RRP6 with FLAG-NVL2 was diminished by the MPP6 knockdown (Fig. 3D). Therefore, MPP6 plays a crucial role in stabilizing the MTR4-RRP6 binding on the nuclear exosome and is required for the recruitment of NVL2 to the RNA-processing complex.

4. Discussion

Ribosome biogenesis in eukaryotic cells involves the coordinated interaction of more than 200 *trans*-acting factors [9]. Most of these factors have been discovered in the yeast *Saccharomyces cerevisiae* by using genetic and proteomic approaches; however, the factors functioning in metazoan cells have not been fully understood. In this study, we demonstrate the role of human ribosome biogenesis factor NVL2 in the processing of pre-rRNA. We have shown the mechanism underlying the interactions between NVL2, MTR4, and the nuclear exosome that contains RRP6 as a catalytic subunit. These interactions might constitute the molecular basis of the role of NVL2 in pre-rRNA processing. As shown in Fig. 4, NVL2 associates with the nuclear exosome *via* MTR4 and RRP6, both of which are mutually required for this association. Additionally, the exosome cofactor MPP6 is crucial for the interaction between MTR4 and RRP6 and might maintain the integrity of the nuclear exosome and recruit NVL2 to this complex. Recent work has shown that metazoan MTR4 is involved in multiple complexes. In the nucleolus, it forms a TRAMP-like complex with the noncanonical poly(A) polymerase PAPD5 and RNA-binding protein ZCCHC7, and is thought to function in the adenylation-dependent degradation of 3′-end of RNA by the nuclear exosome. In the nucleoplasm, MTR4 forms the NEXT (nuclear exosome targeting) complex with the RNA-binding proteins RBM7 and ZCCHC8 and is thought to function in the degradation of several RNA species [22].

Pulse-chase experiments performed using cells expressing NVL2 dominant-negative mutants showed that generation of mature 28S and 5.8S rRNAs, but not 18S rRNA, was significantly delayed (Fig. 1C, D). Delay was noted in two distinct processing steps: the late processing step to produce mature 5.8S rRNA from the 12S intermediate and the early processing step to produce the 32S intermediate from the 47S/45S precursor. Our finding that NVL2 interacts with the nuclear exosome can explain why the late maturation step of the 12S intermediate is affected by expression of NVL2 mutants, since MTR4 and RRP6 are known to be involved in this processing step [16–20]. The defect in the earlier processing step suggests that MTR4 and the exosome are also involved in this processing step. Alternatively, this could be due to a feedback mechanism from the affected late maturation step and might not be directly due to NVL2 dysfunction. Another possibility is that NVL2 may have multiple targets in the pre-rRNA processing pathway; identification of these unknown targets would aid our understanding of the role of NVL2 in ribosome biogenesis.

The molecular mechanism by which NVL2 modulates the function of the nuclear exosome is unclear. Since AAA-ATPases can act as chaperones that modulate the structures and functions of macromolecular complexes by dissociating their constitutive proteins, NVL2 may regulate the binding state of the exosome subunits or its cofactors, including MTR4 and the TRAMP-like complex. Alternatively, NVL2 may regulate the interaction of the nuclear exosome with other pre-ribosomal proteins to recruit it to an appropriate stage in pre-ribosome maturation. In *S. cerevisiae*, Rix7, a homologue of NVL2, plays an essential role in the biogenesis of 60S ribosomes and functions in releasing another ribosome biogenesis factor, Nsa1, from a late pre-ribosome particle [23,24]. However, the precise mechanism of this interaction and the role of Nsa1 in the ribosome biosynthesis are not understood.

Our study shows a crucial role of MPP6 in the interaction between MTR4 and RRP6 and the recruitment of NVL2 to the nuclear exosome. Previous work has shown an essential role of MPP6 in the 3′-end formation of 5.8S rRNA [17,20,25]. Maintaining proper interactions among NVL2, MTR4, and the nuclear exosome may be the possible role of MPP6 in the pre-rRNA 3′-end processing machinery. Further studies are needed to understand how MPP6 is involved in coordinating this RNA metabolic complex.

Conflicts of interest

The authors have no conflicts of interest to declare.

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Transparency document

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