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Research Article

DEAD-box helicase DDX27 regulates 3′ end formation of ribosomal 47S RNA and stably associates with the PeBoW-complex

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ABSTRACT

PeBoW, a trimeric complex consisting of pescadillo (Pes1), block of proliferation (Bop1), and the WD repeat protein 12 (WDR12), is essential for processing and maturation of mammalian 5.8S and 28S ribosomal RNAs. Applying a mass spectrometric analysis, we identified the DEAD-box helicase DDX27 as stably associated factor of the PeBoW-complex. DDX27 interacts with the PeBoW-complex via an evolutionary conserved F × F motif in the N-terminal domain and is recruited to the nucleolus via its basic C-terminal domain. This recruitment is RNA-dependent and occurs independently of the PeBoW-complex. Interestingly, knockdown of DDX27, but not of Pes1, induces the accumulation of an extended form of the primary 47S rRNA. We conclude that DDX27 can interact specifically with the Pes1 and Bop1 but fulfils critical function(s) for proper 3′ end formation of 47S rRNA independently of the PeBoW-complex.

Introduction

Ribosome biogenesis contributes to 80% of the overall amount of cellular RNA and consumes up to 70% of cellular energy in proliferating cells [1]. In eukaryotes, ribosome biogenesis is restricted mainly to the nucleolus, while few final steps of ribosome maturation occur in the cytoplasm. Hundreds of factors, including ribosomal proteins, ribonucleoprotein particles (snoRNPs) containing small nucleolar RNA (snoRNA), GTPases, ATPases, helicases, kinases, polymerases, as well as many yet uncharacterized factors contribute to the process of ribosome assembly [2–4]. Transcription and processing of ribosomal RNA (rRNA) is the main integral part of ribosome biogenesis. RNA polymerase I (RNAPI) transcribes the primary 47S rRNA comprising the 18S, 5.8S and 28S rRNAs. These are flanked by external 5′ and 3′ transcribed sequences (5′ ETS and 3′ ETS) and interspersed

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by internal transcribed spacers 1 and 2 (ITS1 and ITS2) [5–7]. The 47S rRNA and ribosomal proteins together with rRNA processing factors assemble to the 90S pre-ribosomal particle. After processing of ITS1 sequences, the 90S pre-ribosome splits into pre-40S and pre-60S ribosomal particles, which further mature to 40S particles containing 18S rRNA, and 60S particles containing 5.8S and 28S rRNA [8–11].

We have previously described the trimeric PeBoW-complex, consisting of Pes1 (Pescadillo), Bop1 (block of proliferation), and WDR12 (WD-repeat protein 12), as an essential rRNA processing factor in mammalian cells [12]. Knockdown of PeBoW components by siRNA technology or expression of dominant-negative mutants of Pes1, Bop1, and WDR12 block processing of the 32S pre-rRNA [12–16]. The structure and function of the PeBoW-complex is conserved throughout evolution and a homolog complex consisting of Nop7p (Yph1p), Erb1p, and Ytm1p has been described in yeast. As in mammals, mutation of *nop7* and *ytm1* inhibit rRNA processing and cell cycle progression [17,18].

Even though a function of the PeBoW-complex in processing of the 32S rRNA precursor is well established, the exact composition of the complex and possible functions of Pes1, Bop1, and WDR12 in other cellular processes are largely unknown. Knockout of Pes1 arrests mouse embryos at morula stages of development, accompanied by a strong impairment of nucleoli differentiation and ribosome accumulation, suggesting an essential function of Pes1 for ribosome biogenesis and nucleologenesis [19]. However, Pes1 has also been identified as binding factor and activator of the cadmium response element in the heme oxygenase-1 promoter in renal epithelial cells [20]. Transient depletion of Pes1 results in an increase of abnormal mitoses and aberrant metaphase plates in colorectal adenocarcinoma cells [21]. Bop1 can stimulate actin stress fiber assembly and RhoA activation and drives hepatocellular carcinoma by promoting epithelial-to-mesenchymal transition [22]. Finally, WDR12 binds to the cytoplasmic domain of Notch1, suggesting a function for WDR12 in modulation of Notch signaling activity [23]. Thus, Pes1, Bop1 and WDR12 appear to be involved in multiple cellular processes. It is yet unclear, whether all these processes require the PeBoW-complex.

To get a more precise insight into the composition of the PeBoW-complex and to clarify, if PeBoW constituents do participate in the formation of other cellular complexes, we performed a comprehensive mass spectrometric analysis of the cellular interactomes of Pes1 and Bop1. Using highly specific monoclonal antibodies (mAbs) we strongly enriched Pes1 and Bop1 containing cellular complexes. By this approach, we identified the DEAD-box helicase DDX27 as a new factor stably associated with the PeBoW-complex. Here we report the functional analysis of DDX27 in ribosome biogenesis and its interaction with the PeBoW-complex.

Materials and methods

Cell culture

Raji B-cells and U2OS osteosarcoma cells were cultured in RPMI and DMEM medium supplemented with 10% fetal bovine serum at 5% or 8% CO_2, respectively. Mass spectrometry analysis was performed with extracts of Raji cells expressing a HA-tagged large subunit (Rpb1) of RNA polymerase II [24]. Polyclonal U2OS cell lines were generated by Polyfect (QIAGEN) transfection of 6×10^5 cells with the pRTS-1 plasmid and selection in the presence of 200 μg/ml hygromycin B for 10 to 14 days. Conditional gene expression was induced with 1 μg/ml doxycycline.

Cloning/plasmids

DDX27 cDNA was amplified from human cDNA using primers: DDX27 fwd: 5′-CCACCATGGTACTTGCGCAAAGACG-3′; DDX27 bwd: 5′-CTTCCTCCTCTTGTATCTGG-3′. The open reading frame of DDX27 and a C-terminal tag were cloned in pUC18 vector and thereafter in the doxycycline inducible vector pRTS-1 using the SfiI restriction site [25]. Mutant alleles of DDX27 were created by site directed mutagenesis. All vectors were controlled by sequencing.

siRNA transfection

8×10^4 U2OS cells were seeded in 6-well plates and transfected with 0.9 μl of siRNA/well (100 μM) (Eurofins or Qiagen flexi tube) using Oligofectamine and Opti-MEM (Invitrogen). Cells were incubated for 6 h and transfected on two consecutive days. siRNA sequences (5′ to 3′) were as following: siDDX27: UGAGAAAGUUCGAAAGAAAdTdT; siPes1-UTR: CCAGAGGACCUAAGU GUGAdTdT; siBop1-UTR: UCGUGCUGAAGUCAACAGAdTdT; siWDR12-UTR: CGUACGUUUCCGUGGGCAAdTdT; siLuc (GL2) GUACGCGGAAUACUUCGAdTdT

^{32}P-*ortho*-phosphate metabolic labeling and RNA extraction

2.5×10^5 U2OS cells were incubated in phosphate-free DMEM/10% fetal bovine serum for 1 h and then incubated for 1 h in presence 15 μCi/ml ^{32}P-*ortho*-phosphate (pulse). Labeling medium was removed and cultivation of cells was continued for 3 h in DMEM medium/10% fetal bovine serum (chase). Labeled RNA was extracted using the PeqGOLD total RNA kit (PeqLab). 1 μg total RNA was separated on a 1% agarose-formaldehyde gel. The gel was dried on a Whatman paper using a regular gel drier (Bio-Rad) connected to a vacuum pump for 4 h at 80 °C. Metabolically labeled RNA was visualized by autoradiography and quantified by a PhosphorImager and AIDA software.

Northern blot hybridization

5 μg of U2OS total RNA were separated on a 1% agarose-formaldehyde gel and blotted on Hybond N+ membranes (Amersham Biosciences). 5′ ^{32}P-labeled oligonucleotides served as probes. Probes (5′ to 3′):

1. ITS-2: TACGCGCGGGGAGGGCGAGGAGGACGG CGGGGCCTCG GAGGA
2. 3′ETS: CTCCCAAACCACGCTCCCCGGACCCCGTCCCGGCCCGGAG
3. 3′ ext: GCTAAGTCCGGAGCTCGCGGGCGGCAGCTGGTC
4. 3′ ext: GAGAGGGAGTTCCGCGTGGTCCCAGCTCCACCGCG
5. 3′ ext: CGCGGACGCAAACTCGCGGTGGGGCTGAA

Immunoblotting/antibodies

Cells were lysed with 2× SDS-loading buffer (100 mM Tris–HCl, 200 mM DTT, 4% SDS, 10 mM EDTA, 0.2% bromophenol blue, 20% glycerol) followed by sonification. Cell lysates were separated by SDS–PAGE and blotted on nitrocellulose membranes (GE Healthcare). Immunodetection was performed with anti-HA (3F10; Roche),

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