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WDR74 participates in an early cleavage of the pre-rRNA processing pathway in cooperation with the nucleolar AAA-ATPase NVL2

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ABSTRACT

WD repeat-containing protein 74 (WDR74), a nucleolar-localized protein, is the mammalian ortholog of Nsa1, a 60S ribosome assembly factor in yeast. We previously showed that WDR74 associates with MTR4, the nuclear exosome-assisting RNA helicase, whose dissociation is prohibited by an ATPase-deficient mutant of the AAA-type chaperone NVL2. However, the functions and regulation of WDR74 during ribosome biogenesis in cooperation with NVL2 remains unknown. Here, we demonstrated that knockdown of WDR74 leads to significant defects in the pre-rRNA cleavage within the internal transcribed spacer 1 (ITS1), occurring in an early stage of the processing pathway. Interestingly, when the dissociation of WDR74 from the MTR4-containing exonuclease complex was impaired upon expression of the mutant NVL2, the same processing defect, with partial migration of WDR74 from the nucleolus towards the nucleoplasm, was observed. In the nucleoplasm, an increased interaction between WDR74 and MTR4 was detected by *in situ* proximity ligation assay. Therefore, the dissociation of WDR74 from MTR4 in a late stage of rRNA synthesis is thought to be required for appropriate maturation of the pre-60S particles. These results suggest that the spatiotemporal regulation of ribosome biogenesis in the nucleolus is mediated by the ATPase activity of NVL2.

1. Introduction

Eukaryotic ribosomes, composed of small 40S and large 60S subunits, are assembled through a dynamic multi-step process that is fundamental to various cellular processes such as cell growth and proliferation [1,2]. Ribosome biogenesis is initiated in the nucleolus where a single precursor rRNA (47S pre-rRNA in humans) is transcribed by RNA polymerase I. After transcription, the pre-rRNA undergoes multiple modifications, leading to the generation of 18S, 5.8S, and 28S mature rRNAs [3,4]. In the pre-rRNA processing pathway, external and internal transcribed spacer sequences are removed from the 47S pre-rRNA by an ordered series of endo- and exonucleolytic cleavages (Fig. 1A). In the nucleolus, the primary pre-rRNA transcript forms a 90S pre-ribosomal particle by associating with many ribosomal and nonribosomal proteins, which is then converted to a series of pre-40S and pre-60S particles. Maturation of pre-ribosomal particles proceeds with their migration through the nucleolus and nucleoplasm, and into the cytoplasm. This process involves more than 200 nonribosomal factors, including different families of energy-consuming enzymes, such as RNA helicases, AAA-ATPases, and GTPases. These proteins confer directionality and accuracy to this process [1,2].

The AAA (ATPases associated with diverse cellular activities) family of chaperone-like proteins is characterized by a structurally conserved ATPase domain (AAA domain) [5,6]. It contains Walker A and Walker B motifs responsible for ATP binding and hydrolysis, respectively. During ribosome biogenesis in yeast, three AAA-ATPases—Rix7, Drg1, and Rea1—are known to be involved at different stages of pre-ribosome maturation to strip specific factors from the evolving intermediate particles [1,7]. Rix7 and Drg1 function in the nucleolus and cytoplasm, respectively, and Rea1 plays dual roles in the nucleoplasm.

Nuclear VCP-like protein 2 (NVL2), a member of the AAA-ATPase family, shows specific localization to the nucleolus and is involved in 60S ribosome biogenesis [8]. NVL2 belongs to the type II AAA-ATPase and exhibits a high degree of amino acid sequence similarity to the well-characterized ubiquitin-selective chaperone VCP/p97 [9]. Our previous work showed an ATP-dependent interaction of NVL2 with MTR4 (DOB1), a DExD/H-box RNA helicase assisting

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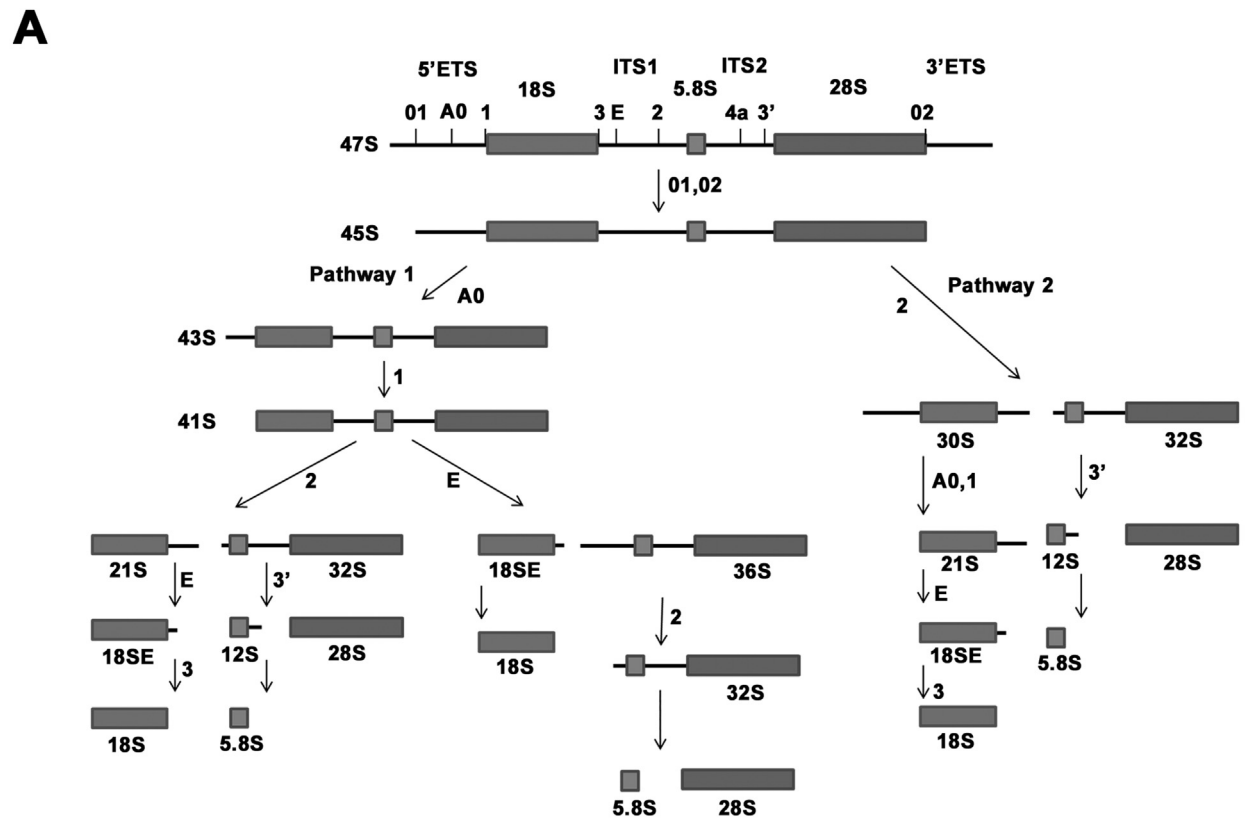

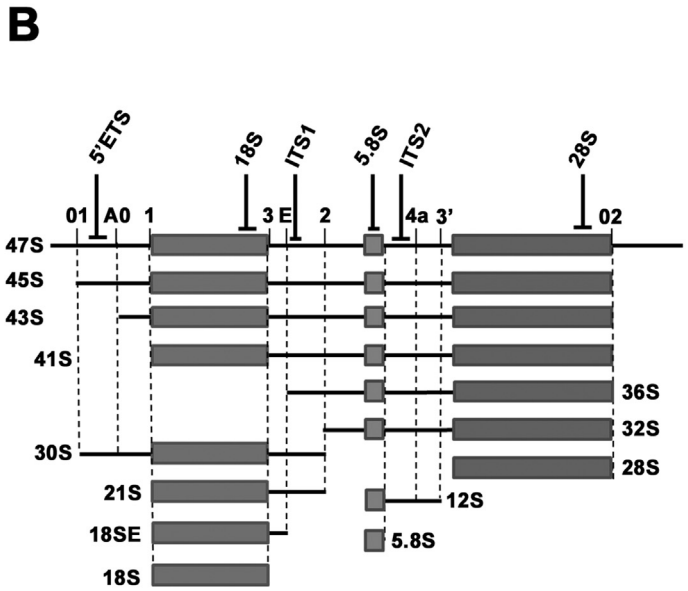

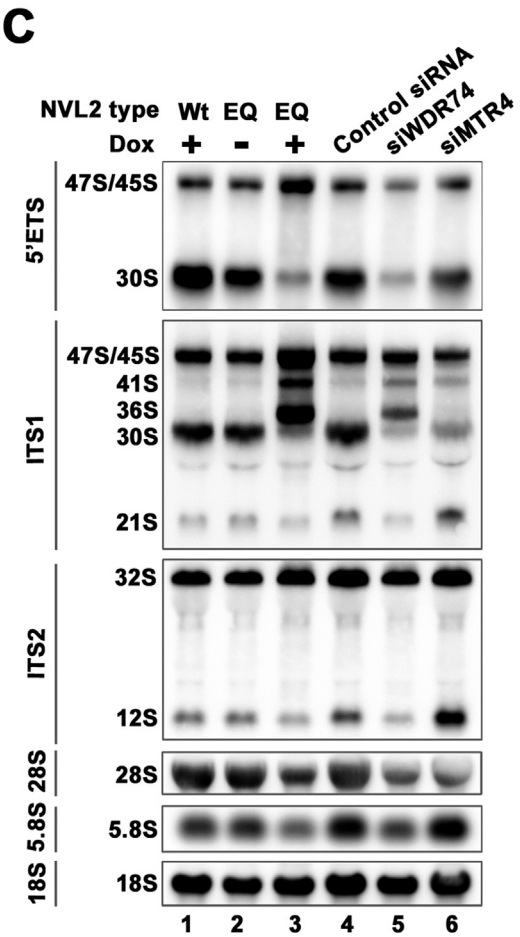

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