Supplementary Material

The G-patch protein NF-κB-repressing factor mediates the recruitment of the exonuclease XRN2 and activation of the RNA helicase DHX15 in human ribosome biogenesis

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Supplementary Figures

Figure S1. NKRF, DHX15 and XRN2 can interact directly. (A) Recombinant ZZ-tagged DHX15 was incubated with MBP-tagged NKRF and/or XRN2, and complexes were isolated on IgG sepharose. After washing, proteins were eluted and inputs (10%) and eluates were separated by SDS-PAGE and visualised by Coomassie staining. **(B)** Recombinant MBP-tagged XRN2 was incubated with GST-tagged NKRF and/or ZZ-tagged DHX15, and complexes were isolated on amylose resin. After washing, proteins were eluted and inputs (10%) and eluates were separated by SDS-PAGE and visualised by Coomassie staining. The asterisks indicate a contaminant that is present in the MBP-XRN2 protein preparation. The experiments were performed in triplicate and representative data are shown.

Figure S2. RNAi-mediated depletion of NKRF, XRN2 and DHX15. Wild-type HeLa cells (WT) or HeLa cells that had been transfected with non-target siRNAs (siNT) or siRNAs targeting NKRF (siNKRF_1 or siNKRF_2), XRN2 (siXRN2_1 or siXRN2_2) or DHX15 (siDHX15_1 or siDHX15_2) were harvested after 96 h. The levels of the indicated proteins were analysed by western blotting using specific antibodies for detection. Tubilin served as endogenous loading control. A representative image from three independent experiments is shown.

Figure S3. Flag-tagged NKRF localises to the nucleolus. Expression of Flag-tagged NKRF was induced in a HEK293 cell line for 24 h before cells were fixed and immunofluorescence was performed using antibodies against the Flag-tag (green) and UTP14A (red), which served as a nucleolar marker. Nuclei were visualised by DAPI staining (blue) and a selection of representative cells is shown. The scale bar indicates 10 μm.

Figure S4. Pulse chase analysis to determine the levels of newly synthesised precursor or mature rRNAs after depletion of NKRF, DHX15 or XRN2. (A) HeLa cells were transfected with non-target siRNAs (siNT) or siRNAs targeting NKRF (siNKRF), XRN2 (siXRN2) or DHX15 (siDHX15). After 84 h, cells were pulse-labelled with ^{32}P-orthophosphate and then grown in unlabelled media for the indicated times before harvesting. RNA was extracted, separated by agarose-glyoxal gel electrophoresis and transferred to a nylon membrane. Mature 28S and 18S rRNA were detected by methylene blue staining (MB; lower panels) and newly synthesised, labelled precursor and mature rRNAs, and Actin mRNA were visualised using a phosphorimager. **(B)** The levels of newly synthesised 18S and 28S rRNAs at 240 minutes (min) were quantified in three independent experiments (including that shown in A) and the 18S/28S ratio was calculated. Data are presented as mean +/- standard deviation. **(C-D)** The levels of newly synthesised 47/45S pre-rRNAs, normalised to the actin mRNA were determined at 120 **(C)** and 240 **(D)** minutes in three independent pulse-labelling experiments (including the experiment shown in A) and are shown as mean +/- SD.

Figure S5. Gradient profiles of extracts from cells depleted of NKRF, XRN2 or DHX15. (A-D) HeLa cells were transfected with non-target siRNAs (siNT) or siRNAs targeting NKRF (siNKRF), DHX15 (siDHX15) or XRN2 (siXRN2) and harvested after 96 h. Cell extracts were prepared, separated by sucrose density gradient centrifugation and A_{260} measurements of the fractions from each of the gradients were used to generate profiles (siNT – **A**; siNKRF –

B; siDHX15 – **C**; siXRN2 – **D**) on which the positions of the 40S and 60S ribosomal subunits as well as 80S monosomes are indicated.

Figure S6. NKRF is required for localisation of XRN2 to the nucleolus. HEK293 cells containing a tetracycline-inducible expression cassette for GFP-XRN2 were transfected with non-target siRNAs (siNT) or siRNAs targeting NKRF (siNKRF) or DHX15 (siDHX15) and RNAi was performed for 96 h. During the final 24 h, expression of GFP-XRN2 was induced to endogenous levels by addition of tetracycline and then cells were fixed. Immunofluorescence was performed using an antibody against the nucleolar protein UTP14A and the localisation of GFP-XRN2 (green) and UTP14A (red) was determined by fluorescence microscopy. Nuclear material was visualised by DAPI staining (blue). The scale bar indicates 10 μm. Experiments were performed three times and representative cells from one experiment are shown.

Figure S7. Recombinant proteins used in ATPase and unwinding assays. Recombinant MBP-DHX15-His_{14} and MBP-$DHX15_{E261Q}$-His_{14} were expressed in *E.coli* and purified on Ni-NTA. Proteins were separated by SDS-PAGE and visualised with Coomassie staining.

Supplementary methods

Pulse-chase labelling of RNAs

Pulse-chase labelling was performed as previously described (28). HeLa cells were treated with siRNAs for 84 h and were then starved in phosphate-free DMEM for 1 h at 37^{o}C. Metabolic labelling was performed for 1 h at 37^{o}C in phosphate-free DMEM supplemented with 10 μCi/ml ^{32}P orthophosphate. Next, cells were incubated in complete DMEM and at different time points were harvested and total RNA was extracted, separated by agarose-glyoxal gel electrophoresis, transferred to a nylon membrane. The levels of nascent 47/45S

pre-rRNAs, newly synthesised 28S and 18S rRNAs, and actin mRNA were determined by northern blotting of these membranes using specific probes (Supplementary Table S3) and a phosphorimager, and quantified using ImageQuant software (GE Healthcare).

Fluorescence microscopy

Fluorescence microscopy was performed as previously described (47). In brief, HEK293 cells for inducible expression of GFP-XRN2 grown on coverslips were transfected with siRNAs for 96 h and expression of the tagged protein was induced during the last 24 h by addition of tetracycline. Cells were fixed using 4% paraformaldehyde in phosphate buffer saline (PBS) for 20 min at room temperature before permeabilisation using 0.1% Triton-X-100 in PBS for 15 min. Cells were blocked using 10% foetal calf serum (FCS) in PBS with 0.1% Triton-X-100 for 1 h before incubation with an antibody against UTP14A (Supplementary Table S2) for 2 h at room temperature. Cells were washed and incubated with an Alexa Fluor 594-conjugated secondary antibody for 2 h at room temperature. Coverslips were mounted onto slides using mounting medium containing DAPI and fluorescence was detected by confocal microscopy.

Supplementary references

28. Sloan,K.E., Mattijssen,S., Lebaron,S., Tollervey,D., Pruijn,G.J.M. and Watkins,N.J. (2013) Both endonucleolytic and exonucleolytic cleavage mediate ITS1 removal during human ribosomal RNA processing. *J.Cell Biol.*, **200**, 577-588.

47. Warda,A.S., Freytag,B., Haag,S., Sloan,K.E., Görlich,D. and Bohnsack,M.T. (2017) Effects of the Bowen-Conradi syndrome mutation in EMG1 on its nuclear import, stability and nucleolar recruitment. *Hum. Mol. Genet.*, doi: 10.1093/hmg/ddw351

Supplementary Tables

Supplementary Table S1. Small interfering (si)RNAs used in this study

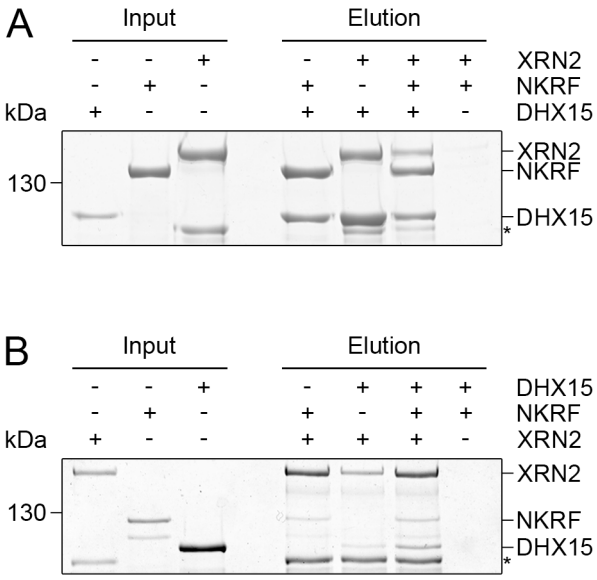
Name	Sequence (5'-3')
siNT	UCGUAAGUAAGCGCAACCC
siNKRF_1	GGCUAUGCUUGUGAAGUUA
siNKRF_2 or siNKRF	GUAUUGAAGUUAGAGUUGU
siXRN2_1 or siXRN2	GGGAAGAAAUAUUGGCAAA
siXRN2_2	AAGAGUACAGAUGAUCAUG
siDHX15_1 or siDHX15	GGUUAUAGUUAUGAGCGCUACUCUA
siDHX15_2	GAGAAGGAGUUGCGAGCUU

Supplementary Table S2. Antibodies used in this study

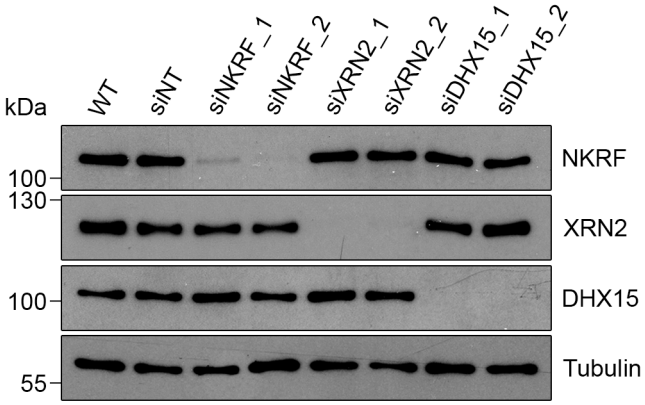
Antibody	Source
NKRF	Bethyl (A304-016A)
DHX15	Bethyl (A300-390A)
XRN2	Bethyl (A301-103A)
Tubulin	Sigma-Aldrich (T6199)
PCNA	Santa Cruz Biotechnology (sc-56)
Fibrillarin	Santa Cruz Biotechnology (sc-25397)
Nucleophosmin	Sigma-Aldrich (B0556)
UTP14A	ProteinTech (11474-1-AP)
NSUN5	Santa Cruz (Sc-376147)
PWP2	GeneTex (GTX105344)
RPL15	Aviva Systems Bio (ARP65141)
RPS3A	ProteinTech (14123-1-AP)
PES1	Bethyl (A300-903A)
WBSCR22	Abgent (AP20254b)
Flag	Sigma-Aldrich (F3165)

Supplementary Table S3. Northern blotting probe sequences

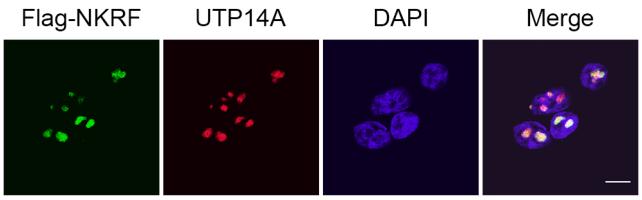
Probe	Sequence (5'-3')
ETS1	CGGAGGCCCAACCTCTCCGACGACAGGTCGCCAGAGGA CAGCGTGTCAGC
ETS3	ACCGGTCACGACTCGGCA
5'ITS1	CCTCGCCCTCCGGGCTCCGTTAATGATC
ITS1	AGGGGTCTTTAAACCTCCGCGCCGGAACGCGCTAGGTAC
ITS2	GCTCTCTCTTTCCCTCTCCGTCTTCC
Actin	AGGGATAGCACAGCCTGGATAGCAAC



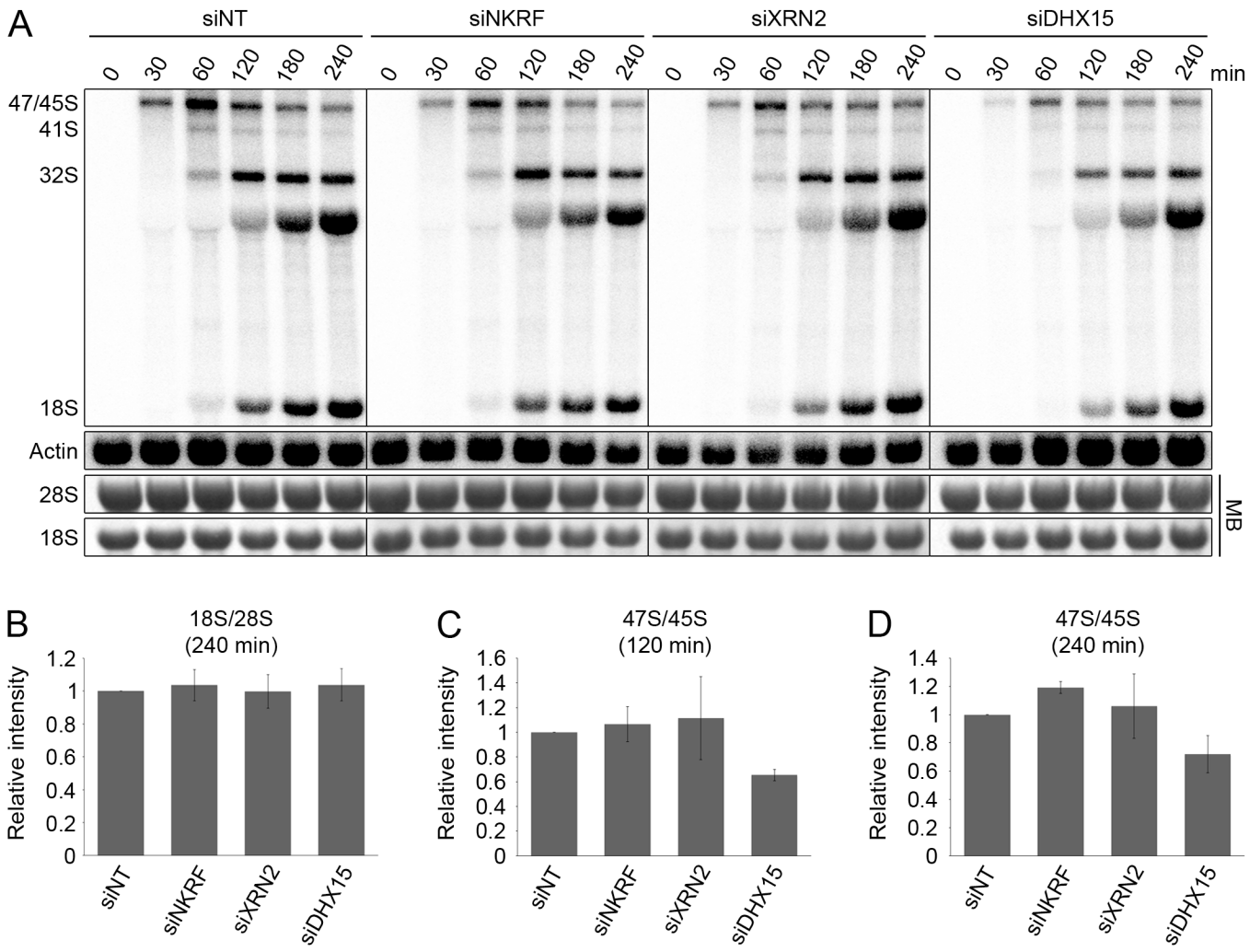
Memet *et al.*, Figure S1



Memet *et al*., Figure S2



Memet *et al*., Figure S3



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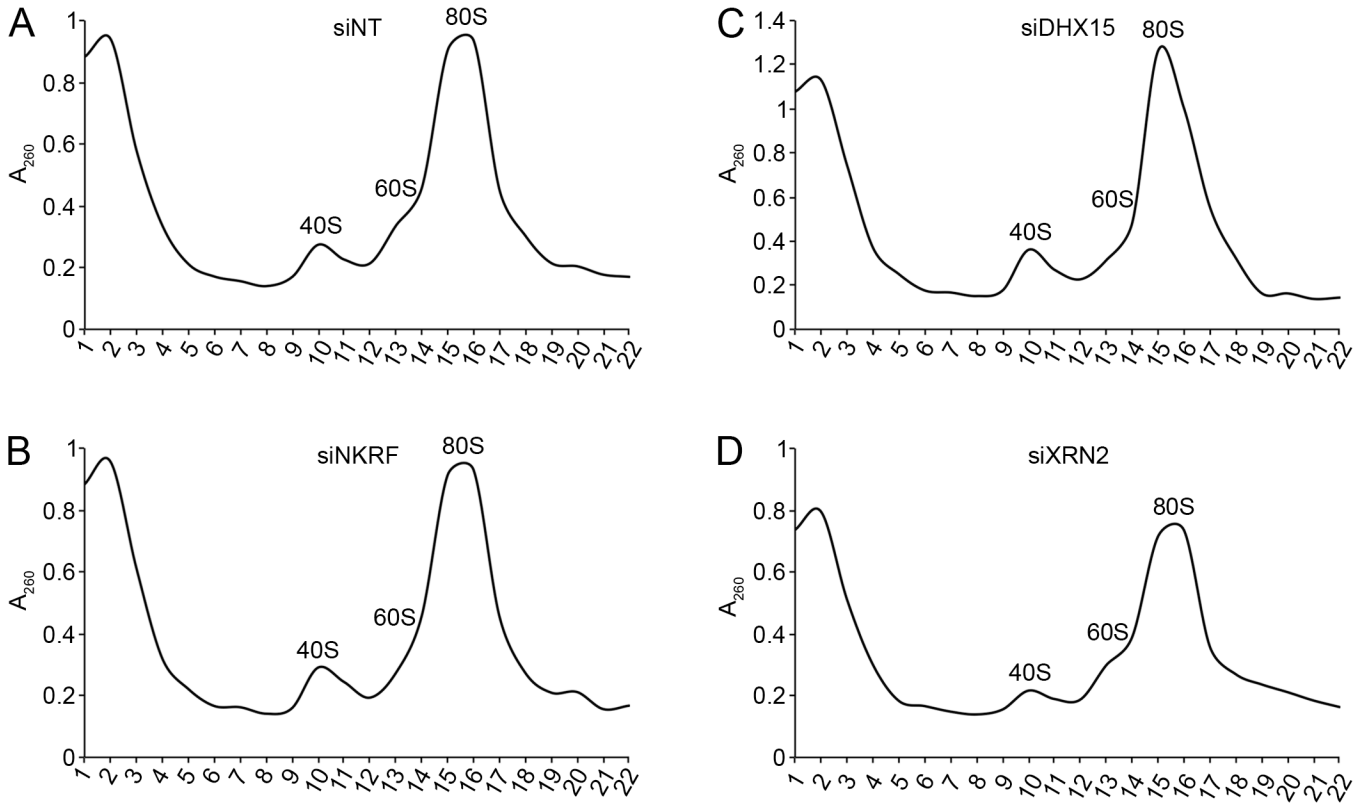
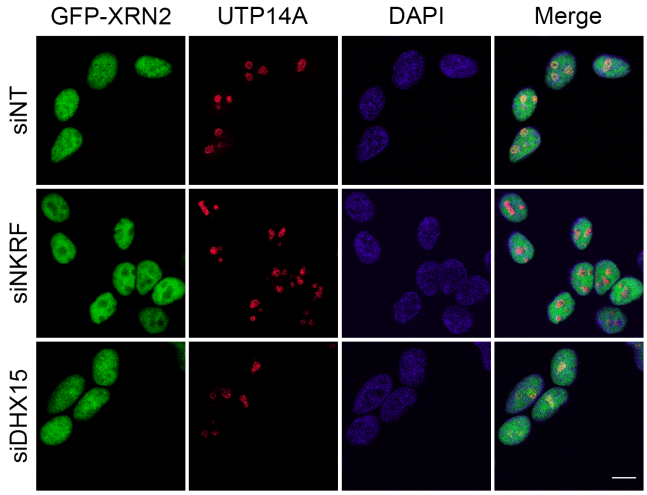


Memet *et al*., Figure S5



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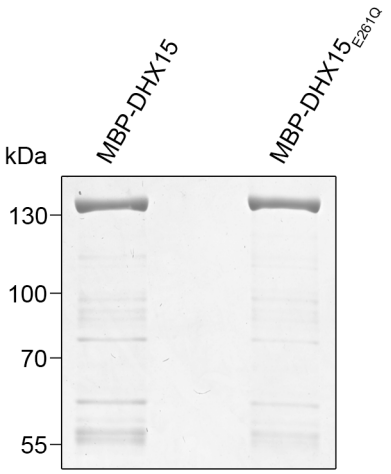

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