Supplementary Figures

Supplementary figure 1: Sequence alignments of DEAH/RHA helicases

Sequences were collected on ExPaSy (www.expasy.org), aligned with the clustal software (www.ebi.ac.uk/Tools/msa/clustalw2/), figures were made with the software ESPript (escript.ibcp.fr/ESPript/ESPript)
Supplementary figure 2: Interaction between Prp43 and ssRNA using different nucleotides as substrates

Interaction between a ssRNA (21nt) labeled with a fluorescent dye with Prp43 WT, R159A or F357A mutants. The ssRNA is incubated with Prp43 proteins (0.5 to 200 or 3000 nM) and effect of nucleotide is assessed by addition of 1mM of ATP, CTP, AMPPNP, CDP or ADP in the reaction mix and as a control an experiment with no nucleotide (no NTP) was also performed. For clarity, the signal detected on protein membrane (bound fraction) is only represented. Ratios of bound RNA for each protein concentration were quantified using Odyssey (Li-COR) and plotted: Prp43 WT (purple), Prp43 R159A (blue) and Prp43 F357A (yellow). Theoretical curves obtained for each gnuplot fit are represented.
Supplementary figure 3: Influence of base stacking on Prp43 helicase activity

(A) RNA–DNA hybrid substrate used in the helicase assays as in figure 4. The 3'-5' helicase substrate was incubated at 30°C for the indicated times with Pfa1 (25 nM), the wild-type Prp43 (B) or the R159A (C) or F357A (D) mutant (10 nM) and ATP. The radiolabeled oligonucleotide alone was loaded on the gel to provide the position of the unwound product (star). A sample of the reaction mix before addition of the proteins was loaded on the gel to show the position of the intact substrate (0).
Strain \textit{GAL::PRP43} was transformed with pHA113 plasmids carrying PRP43-WT, PRP43-R159A, PRP43-E216A or PRP43-F357A, or with the empty pHA113 plasmid as a control (see Materials and methods section, Yeast strains and media paragraph for further details). The resulting strains were grown on a galactose-containing medium and shifted to glucose for 12 hours to deplete endogenous Prp43 proteins. Cells were harvested, soluble protein extracts were prepared and analysed by Western blot using either anti-Prp43 antibodies or anti-Nhp2 antibodies (loading control). The absence of Prp43 in the empty vector control attests that the endogenous protein has been fully depleted and that the pre-rRNA processing defects observed in Figure 5 result from the absence of Prp43 (Empty plasmid) or from the expression of the various mutant proteins.
Supplementary figure 5: PRP43 R159A mutation affects growth in yeast

Ten-fold serial dilutions of haploid yeast strains expressing wild-type PRP43 (A) or the R159A (B) or F357A (C) mutants (see Materials and methods section, Yeast strains and media paragraph for further details). Each strain was spotted from left to right on YPD plates and incubated at 30°C. The growth assay has been repeated three times with similar results.
Supplementary figure 6: Stacking of the base with RecA1 domain influences pre-60S particle processing and in vivo helicase activity

Total extracts prepared from strains expressing Prp43 WT (A) or the R159A (B) and F357A (C) mutants were sedimented through 10-50% sucrose gradients. The ribosome profiles corresponding to the absorbance at 254 nm as a function of collection time (fractions) is shown in the upper panel. The 40S, 60S, 80S and polysome peaks are annotated. RNAs extracted from fractions 1 to 20 were analysed by Northern blot and the snoRNAs snR50, snR41, snR39 and U3, as well as the 35S, 33S/32S and 27S pre-rRNAs were detected using specific radiolabelled oligonucleotide probes. Their sedimentation profiles are presented in the lower panel. As a control to normalize exposure times, RNAs extracted from a sample of each total extract loaded on the gradients were analysed in parallel (T). (D) PhosphorImager quantifications of the pool of free snoRNAs (snoRNAs
present in fractions 1-5) relatively to the signal corresponding to the snoRNAs in the input sample (T).
Supplementary materials and methods

RNA binding assays

The fluorescently 5’ IRD800-labeled RNA (5’-IRD800-GACGGGCUGUUGGUGAUGGAC) was ordered to IDT. The ssRNA was diluted to 0.5 nM into binding buffer (PBS, 2 mM MgCl2, 6% glycerol, 0.5 mM DTT, 0.1 mM EDTA, 5 μg/ml of E.coli tRNA, 50 μg of BSA). Binding reactions consisted of 10 μl of ssRNA at 0.5 nM and 5 μl of proteins (final concentration from 0.5 to 3000 nM). Binding reactions were incubated for 15 min at 20°C and then directly applied to filters containing the two membranes under vacuum. Before and after application of the binding reactions, 200 μl of binding buffer was used to equilibrate and rinse the system. Binding was quantified using Odyssey apparatus (Li-COR) and Image Lite program (Li-COR). The intensity was corrected for background and fit for Kd using Gnuplot (http://www.gnuplot.info).

Helicase assays

Wild-type or mutant Prp43 proteins (10 nM) and Pfa1 (25 nM) were pre-incubated on ice with 1 nM of the 3’–5’ helicase substrate in a buffer containing 25 mM HEPES-KOH (pH 8.0), 2.5 mM Mg(CH3COO)2, 100 mM KCl, 0.2 mM DTT, 100 μg/ml BSA (Sigma). ATP was added at a concentration of 1 mM and the reactions were incubated at 30°C for the indicated times. At each time point, reactions were stopped with a solution containing 1 mg/ml proteinase K, 1.25% SDS, 10 mM Tris–HCl, 0.06% bromophenol blue, 0.06% xylene cyanol and 30% glycerol and a 100-fold excess of the trap oligonucleotide. Reaction products were separated on 8% polyacrylamide/1X TBE gels. Gels were dried and quantified using PhosphorImager screens.

Yeast strains

The yeast strains used in Supplementary Figure 4 are derived from the BMA64 background (ura3-1, trp1-Δ2, ade2-1, leu2-3,112, his3-11,15, can1-100). The wild-type or R159A, E216A or F357A mutant PRP43 ORFs were cloned into plasmid pHA113 (centromeric vector with TRP1 auxotrophy marker) under the control of the GAR1 promoter and upstream of a sequence encoding the ZZ tag (ZZ domains from protein A of S. aureus). These plasmids were transformed into a GAL::PRP43 strain, expressing endogenous PRP43 under the control of the conditional galactose promoter. The yeast strains used in Supplementary Figure 5 are haploid BMA64 cells in which full deletion of the chromosomal PRP43 gene is rescued by pHA113 plasmids expressing wild-type PRP43 or the R159A and F357A mutants.

Yeast strains used in Supplementary figures 5 and 6 are derived from the BMA64 background (ura3-1, trp1-Δ2, ade2-1, leu2-3,112, his3-11,15, can1-100). Wild-type or mutant PRP43 ORFs were cloned into plasmid pHA113 (centromeric vector with TRP1 auxotrophy marker) under the control of the GAR1 promoter and upstream of a sequence encoding the ZZ tag (ZZ domains from
protein A of S. aureus). These plasmids were transformed into a diploid strain in which one allele of PRP43 was deleted by replacement by the G418 resistance maker (kan R MX6) according to (Longtine et al., 1998, Yeast). After sporulation and tetrad dissections, haploid clones were selected in which deletion of the chromosomal PRP43 allele is rescued by the plasmid-borne copies of the WT or mutant PRP43 genes. The resulting strains were propagated on a rich medium supplemented with glucose (YPD).

**Soluble protein extracts and Western blot experiments**

Cell pellets corresponding to 50 ml of yeast cultures were resuspended with 600 µl A200 KCl Buffer (20 mM Tris-HCl pH 8.0, 5 mM MgAc, 200 mM KCl, 0.2% Triton X-100, 1 mM DTT) supplemented with 1X complete EDTA-free protease inhibitor cocktail (ROCHE) and 0.1 U/µl RNasin (Promega). Cells were broken by vortexing in the presence of glass beads. Extracts were clarified by two consecutive centrifugations at 13 000 rpm, 5 min, 4°C, the soluble fractions were collected and calibrated using Nanodrop. Equal amounts of total proteins were mixed with SDS loading buffer, separated on SDS-10% polyacrylamide gels and transferred to Amersham Hybond-C Extra membranes (GE Healthcare). Prp43 and Nhp2 were detected using polyclonal anti-Prp43 and anti-Nhp2 antibodies, respectively, both diluted to 1:3000 in a buffer containing [1X PBS, 0.5% TWEEN 20, 5% powder milk] and HRP-conjugated goat anti-Rabbit secondary antibodies (Promega).

**Sedimentations on sucrose gradients**

Yeast cell pellets were broken with glass beads in buffer K (20 mM Tris-Cl pH 7.4, 50 mM KCl, 10 mM MgCl₂, 50 µg/ml cycloheximide, 1 mM dithiothreitol, 1X Roche Complete EDTA-free protease inhibitor cocktail, 0.1 U./µl Promega RNasin). Extracts were clarified by centrifugation, calibrated, loaded on 10% to 50% sucrose gradients and centrifuged at 39 000 rpm for 150 min on a Beckman-Coulter Optima L-100 XP ultracentrifuge using a SW41 rotor. Fractions were collected using a Foxy Jr. gradient collector (Teledyne Isco).