A guard protein mediated quality control mechanism monitors 5'-capping of pre-mRNAs

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ABSTRACT

Efficient gene expression requires properly matured mRNAs for functional transcript translation. Several factors including the guard proteins monitor maturation and act as nuclear retention factors for unprocessed pre-mRNAs. Here we show that the guard protein Npl3 monitors 5'-capping. In its absence, uncapped transcripts resist degradation, because the Rat1–Rai1 5'-end degradation factors are not efficiently recruited to these faulty transcripts. Importantly, in *npl3Δ*, these improperly capped transcripts escape this quality control checkpoint and leak into the cytoplasm. Our data suggest a model in which Npl3 associates with the Rai1 bound pre-mRNAs. In case the transcript was properly capped and is thus CBC (cap binding complex) bound, Rai1 dissociates from Npl3 allowing the export factor Mex67 to interact with this guard protein and support nuclear export. In case Npl3 does not detect proper capping through CBC attachment, Rai1 binding persists and Rat1 can join this 5'-complex to degrade the faulty transcript.

INTRODUCTION

Eukaryotic transcripts are synthesized as precursor (pre-)mRNAs by RNA-polymerase II (RNAP II) and undergo different processing events, such as 5'-capping, splicing and 3'-polyadenylation before they are exported to the cytoplasm as mature mRNAs where they are translated [1–3]. These processes are monitored and defective RNAs are eliminated from cells to ensure highly efficient and correct gene expression. In *Saccharomyces cerevisiae* mRNA surveillance includes the four guard proteins Npl3, Gbp2, Hrb1 and Nab2, which retain transcripts in the nucleus until maturation is completed [4,5]. Consequently, the lack of any guard protein results in the leakage of faulty transcripts to the cytoplasm [4]. On correctly processed mRNAs the guard proteins recruit the export receptor heterodimer Mex67-Mtr2 (TAP-p15 in human) [6] to enable the transit through the nuclear pore complex (NPC). Whether an mRNA can indeed pass through the NPC is finally controlled by the nuclear basket protein Mlp1 (human TPR). It supports a fast passage of transcripts on which all guard proteins are properly covered with Mex67 and delays passage if this is not the case and the ribonucleoparticle (RNP) differs from the norm [4,5,7–9]. This nuclear quality control system ensures an efficient elimination of faulty transcripts from the cell by the nuclear, Rrp6 containing exosome [10,11], which is guided to faulty RNAs by assisting complexes, such as the TRAMP (Trf4/5, Air1/2, Mtr4 polyadenylation) complex that marks faulty RNAs by adding a 3–5 nucleotide long oligo(A) tail [3,12–15].

The different guard proteins act at different mRNA maturation stages. Gbp2 and Hrb1 are preferentially loaded onto transcripts that will be spliced. They interact with the late splicing machinery and their deletion leads to the leakage of intron-containing pre-mRNAs to the cytoplasm. Therefore, a quality control function for Gbp2 and Hrb1 was suggested for proper intron excision [8]. In fact, subsequent studies have shown that they continue their quality control function in the cytoplasm and participate in the nonsense mediated decay (NMD) of premature termination codon (PTC) containing mRNAs that often arise from defects in intron removal [16]. Nab2 binds to transcripts close to the polyadenylation site [17] and it was shown to control the length and quality of 3'-tails [18]. However, for Npl3 a particular guard function has not been identified. It has been shown that Npl3 is recruited early during transcription via the C-terminal domain (CTD) of RNAP II [19]. Transcriptome wide studies indicate that Npl3 binding is mostly detected at the transcription start side of emerging transcripts, but that it also peaks at 3'-ends close to polyadenylation sites [17,20]. Additionally, it shows genetic and physical interactions with the cap-binding complex (CBC) [21]. Therefore, it seems possible that Npl3 could be a quality control factor for proper 3'-end maturation and 5'-capping. Correct transcript capping is important for mRNA stabil-

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ity, it promotes transcript maturation, such as pre-mRNA splicing and 3'-end processing, and it supports nuclear export and translation (22,23).

5'-caps are typically established on emerging pre-mRNAs, when ∼20 nucleotides have been synthesized (24). Then, a 7-methylguanosine is added to the first transcribed nucleotide via the 5' hydroxyl group, through a triphosphate linkage (5'–5') by the essential capping enzymes Cet1, an RNA 5'-triphosphatase and Ceg1, a guanylyltransferase (25). Methylation of the cap is finally mediated in yeast by the methyltransferase Abd1 and these mature, methylated caps are the prerequisite for CBC binding in the nucleus (26). CBC is composed of Cbp20 and Cbp80 and supports mRNA, snRNA and telomerase RNA *TLC1* export by the recruitment of the export factor Xpo1 (27) (28). On mR-NAs, CBC is exchanged in the cytoplasm by eIF4E, which is important for translation initiation and circularization of mRNAs supporting continuous translation of an mRNA through a repeated cycling of the ribosomes (24).

Importantly, improperly capped transcripts are recognized by the Rat1–Rai1 complex that initiates de-capping and subsequent 5'-3' degradation of the mRNA (29). Additionally, a Rai1 homologue, Dxo1, was shown to possess both decapping and exonuclease activities so that it can act independently of Rat1 (30). Although all three enzymes can eliminate mRNAs with defective 5'-caps, it is currently unclear how improper capping is monitored and how this degradation machinery is recruited to these transcripts. Here we show that the guard protein Npl3 monitors 5'-capping through interaction with the CBC and retains improperly capped transcripts in the nucleus. Uncapped transcripts or pre-mRNAs with faulty caps do not bind to Cbp80, allowing Npl3 and Rai1 to recruit Rat1 onto faulty transcripts, leading to the elimination of these RNAs. In contrast, on correctly capped mRNAs Npl3 contacts Cbp80, which leads to the dissociation of Rai1 and allows the recruitment of the export factor Mex67. The export receptor binding marks these transcripts as correctly capped and allows their nuclear export.

MATERIALS AND METHODS

Yeast strains, Plasmids and Oligonucleotides

All yeast strains used in this study are listed in Supplementary Table S1, plasmids in Supplementary Table S2 and oligonucleotides in Supplementary Table S3. Strains were cultivated in standard media at 25°C. All newly created double-mutant strains were generated by crossing of the respective parental strains of the opposite mating type in the presence of a rescue plasmid, encoding for one of the deleted or mutated genes bearing an *URA3* marker. Diploid strains were then sporulated and subjected to tetrad dissection. All haploid spores were analyzed according to their genetic markers. For the generation of plasmid pHK1574, the *CBP80* ORF was amplified by PCR from gDNA using the primers HK2507 and HK2508 and inserted via Gibson assembly reaction into pHK750 linearized via EcoRI and SacI digestion, replacing the NLS, NES, and ADH1 promotor (P_{ADH}:*NLS-NES-MYC-MYC-MYC CEN, URA3*).

Drop dilution analysis

Cells were grown to log phase (2×10^7 cells/ml) and diluted to 1×10^7 cells/ml. Ten-fold serial dilutions to 1×10^3 cells/ml were prepared and 10μl of each dilution was spotted onto either full medium (YPD) agar plates, selective plates, or FOA plates. The plates were subsequently incubated for 3 days at the indicated temperatures. Pictures were taken after 2 and 3 days with the Intelli Scan 1600 (Quanto technology) and the SilverFast Ai program.

Co-immunoprecipitation (IP)

The experiments were essentially performed as published earlier (8). All yeast strains were grown to mid log phase (2×10^7 cells/ml) before usage. The cells were harvested and lysed in 1 pellet volume of PBSKMT buffer (13.7 mM NaCl, 3.27 mM KCl, 2.5 mM MgCl$_2$, 0.18 mM KH$_2$PO$_4$, 1 mM Na$_2$HPO$_4$, 0,5% (v/v) Triton X-100, pH 7.4) and one pellet volume of glass beads. Cells were lysed by vigorous vortexing three times for 30 sec at 6 m/s using the FastPrep®-24 instrument (MP Biomedicals). For immunoprecipitation of GFP-tagged proteins, 10 μl GFP-selector beads slurry (NanoTag) were used and incubated with 1 ml lysate rotating at 4°C for 1.5 h. For myc-tagged proteins and Mex67 immunoprecipitation, 20 μl G-Sepharose beads slurry (GE Healthcare) was used per reaction and incubated with the respective antibody and 1 ml lysate rotating for 3 h. Where indicated, 0.2 mg/ml RNase A (AppliChem) were added for 30 min at 25°C and then 1.5 h at 4°C. The proteins were separated on 10% SDS-polyacrylamide and analysed via western Blot.

Western blot analyses and detection

All antibodies used in this study are listed in Supplementary Table S4. Antibodies against GFP (GF28R, mouse) were used in a dilution of 1:5,000 (Thermo Fisher Scientific), anti-GFP PABG1 (rabbit) 1:4,000 (Chromotek). Anti-myc 9E10 (mouse) or anti-myc A-14 (rabbit) antibodies were used in a dilution of 1:1,000 (Santa Cruz). Antibodies against Hem15 (rabbit) and Dre2 (rabbit) (courtesy of U. Mühlenhoff, Marburg, Germany), Mex67 (rabbit) (courtesy of C. Dargemont, Paris, France), Nop1 28F2 (mouse) (Santa Cruz), Npl3 (rabbit) (custom-made, H. Krebber), Zwf1 (rabbit) (Santa Cruz), Gbp2, Hrb1 (custom-made, H. Krebber) were used in dilutions of 1:10,000, 1:20,000, 1:20,000, 1:4,000, 1:5,000, 1:4,000, 1:50,000, 1:20,000, respectively. Secondary anti-mouse IgG-HRP and anti-rabbit IgG-HRP were diluted 1:20,000 (Dianova) and detected with WesternBright Chemilumineszenz Substrat Quantum (Biozym) and detected with a FUSION-SL or FUSION FX chemiluminescence detection system (Peqlab). Western Blot signals were quantified with the Bio1D software (Peqlab) or the Fiji-software. The signal intensity of the co-precipitated protein bands was related to the intensity of the pulled-down protein bands. The ratio between mutant strains was compared to wild type.

Xrn1 digestion

Total RNA was isolated from wild type, *cet1-2,* and *cet1-2 npl3Δ* mutant cells using the Macherey Nagel NucleoSpin

RNA Kit that were shifted for 2 h to 37°C. The isolated RNA was incubated with the 5′-3′ exonuclease Xrn1 (New England Biolabs) for 2 h at 37°C. After Xrn1 digestion, the amount of specific mRNAs was compared between wild type and *cet1-2* using qRT-PCR.

GFP microscopy

Cells were grown to mid log phase (2×10^7 cells/ml) prior to a temperature shift to 37°C for 2 h. Green fluorescent protein (GFP)-tagged proteins were analyzed under a fluorescence microscope upon a 1 min fixation in 2.5% formaldehyde, and subsequent washing once with 0.1 M phosphate buffer pH 6.5 and once with P-solution (0.1 M phosphate buffer pH 6.5 and 1.2 M sorbitol). Cells were permeabilized with 0.5% Triton X-100 in P-solution, washed once with P-solution and once with Aby wash 2 (0.1 M Tris pH 9.5, 0.1 M NaCl). Afterwards the nucleus was stained with Hoechst 33342 (Sigma), followed by three washing steps with Aby wash 2. Microscopy studies were performed with a Leica AF6000 microscope and pictures were obtained by using the LEICA DFC360FX camera and the LAS AF 2.7.3.9 software (Leica).

In vivo RNA co-immunoprecipitation experiments (RIP)

All yeast strains were grown to mid log phase (2×10^7 cells/ml). For RIP experiments with temperature-sensitive mutants, cells were shifted to non-permissive temperature for 1 h 37°C or 16°C, respectively. For all other RIP experiments, cells were cultured at 25°C. The cells were harvested and lysed in 1 pellet volume RIP buffer (25 mM Tris–HCl, pH 7.5, 150 mM NaCl, 2 mM MgCl₂, 0.2% (v/v) Triton X-100, 0.2 mM PMSF, 0.5 mM DTT, 10U RiboLock™ RNase Inhibitor (Thermo Scientific)) and 1 volume of glass beads. Cells were lysed by vigorous vortexing three times for 30 sec at 6 m/s using the FastPrep®-24 instrument (MP Biomedicals). Input control samples were taken for Protein detection via western blot and RNA-Isolation via Trizol-chloroform (Ambion® RNA by Life technologies ™) extraction. Co-immunoprecipitation experiments were performed at 4°C by incubating the lysates with Protein G sepharose beads (Amersham Biosciences) conjugated to monoclonal c-myc (9E10)-antibodies (Santa Cruz) for 2 h, or GFP-selector beads (NanoTag) for 1 h. For DNaseI digestion the lysates were incubated with 15 μl (40 Kunitz units) DNaseI (Qiagen) at 25°C for 30 min. Afterwards the beads were washed five times with RIP buffer and split into two portions after the last washing step. Proteins were detected by western blot. Eluates were purified via Trizol–chloroform extraction similar to lysates. Identical volumes of purified lysate or eluate RNA were finally used as template for reverse transcription by FastGene Scriptase II (Nippon Genetics) for subsequent qPCR analyses. Eluted RNA levels were measured by qPCR and normalized to *21S* rRNA, except for Cbp80-*myc* in *cet1-2.* All samples were related to the no tag control.

Fluorescent *in situ* hybridization experiments (FISH)

The experiments were essentially performed as described earlier ([8]). To detect the poly(A)⁺RNA a Cy3-labelled oligo d(T)₅₀ probe (Sigma) was used. To detect single GFP mRNA, specific Cy3-labelled oligos were used. Cells were grown to log phase and then shifted to 37°C for either 30 min, 2 or 3 h. Samples were fixed by adding formaldehyde to a final concentration of 4%. Cells were spheroplasted by adding zymolase, subsequently permeabilized in 0.1 M potassium phosphate buffer pH 6.5, 1.2 M sorbitol, 0.5% Triton® X-100 and then prehybridized with Hybmix (50% deionized formamide, 5× SSC, 1× Denhardts, 500 μg/ml tRNA, 500 μg/ml salmon sperm DNA, 50 μg/ml heparin, 2.5 mM EDTA pH 8.0, 0,1% Tween® 20, 10% dextran sulfate) on a polylysine coated slide. Hybridization was performed over night at 37°C. After hybridization cells were washed with 2× SSC and 1× SSC at room temperature, each for 1 h and 0.5× SSC at 37°C and room temperature, each for 30 min, respectively. DNA was stained with Hoechst 33342 (Sigma). Microscopy studies were performed with a Leica AF6000 microscope and pictures were obtained by using the LEICA DFC360FX camera and the LAS AF 2.7.3.9 software (Leica) and quantified by using the Fiji-software. The % nuclear accumulation was calculated by dividing the nuclear fluorescent signal by the fluorescent signal of the whole cell and relating the amounts of the mutant to those of the wild type.

The quantification of the signal intensities were measured in 10 cells per strain and experiment. Thus, each bar represents at least 30 measured cells.

Nucleo-cytoplasmic fractionation experiments

The experiments were essentially performed as described earlier ([28]). Cells were grown to mid log-phase (2×10^7 cells/ml), harvested by centrifugation for 5 min at 4000 rpm, were washed once with 1 ml YPD/1 M Sorbitol/2 mM DTT and resuspended in YPD/1 M Sorbitol/1 mM DTT. Cells were spheroblasted using 1 mg zymolyase (100 mg/ml) and after that diluted in 50 ml YPD/1 M Sorbitol for 30 min at 25°C for recovery. Subsequently, the cells were shifted to 37°C for 1 h. Cells were placed on ice, centrifuged at 2000 rpm for 10min and the pelleted cells were resuspended in 500 μl Ficoll buffer (18% Ficoll 400, 10 mM HEPES pH 6.0) and 1 μl Ribolock. Cells were lysed by addition of 1ml buffer A (50 mM NaCl, 1 mM MgCl₂, 10 mM HEPES pH 6.0). The suspension was mixed and centrifuged at 4000 rpm for 15 min. The supernatant was used for cytoplasmic analyses. RNA was isolated using the Nucleo-Spin RNA Kit (Macherey and Nagel) and reverse transcribed with Fast-Gene Scriptase II (Nippon Genetics) for subsequent qPCR analyses. All values were normalized to the amount of the *21S rRNA*. To verify no nuclear contamination in the cytoplasmic fraction, aliquots of the samples were analyzed in western blots for the presence of the cytoplasmic Zwf1 protein and the absence of the nuclear Nop1 protein.

Total RNA isolation

Total RNA isolation was carried out with the NucleoSpin RNA Kit from Macherey-Nagel. All steps were conducted according to the manufacturer's description. An additional DNA digestion step was carried out after elution of the RNA. For this, the eluted RNA was mixed with a 10th vol-

ume of the reaction buffer and 1 μl DNaseI (Qiagen), according to the manufacturer's description. The digest was incubated for 10 min at 37°C. Afterwards, the RNA was precipitated through sodium acetate ethanol precipitation. For this 0.1 volume 3 M sodium acetate, pH 5.2, 2.5 volumes of 99% pure ethanol and 1 μl Glycoblue were added and incubated over night at –20°C. The collected RNA pellets after centrifugation were resuspended in RNase-free H_2O. The purified RNA was measured via Nanodrop. For cytoplasmic fractionation and Xrn1 digestion experiments, a defined amount of RNA was reverse transcribed with Fast-Gene Scriptase II (Nippon Genetics) for subsequent qPCR analyses.

mRNA isolation

mRNA isolation from purified total RNA was carried out with the Dynabeads mRNA DIRECT Purification Kit (Ambion® by Life technologies™). mRNA was isolated from total RNA according to the manufacturer's instructions. The total RNA was incubated for 10 minutes with binding buffer to allow the binding of the poly(A) tails. The mRNA concentration was measured via NanoDrop.

Statistical analyses

All experiments shown in this work were repeated at least three times independently. Quantifications of immunoprecipitation, Xrn1 digest, and fluorescence in situ hybridization experiments were analysed for significance by Student's two-tailed, two-sample, unequal variance *t*-test. Error bars represent the standard deviation. P values are indicated as follows: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

RESULTS

Npl3 binds to the mRNA independent of the 5'-capping

Attachment of the 7-methylguanosine to the 5'-end of an emerging transcript is mediated by the triphosphatase Cet1 and the guanylyltransferase Ceg1 (25). To investigate a potential function of Npl3 in monitoring 5'-capping, we analyzed whether this guard protein shows genetic and physical interactions with the capping machinery. Combination of the temperature sensitive *cet1-2, ceg1-3* and *ceg1-34* mutants with the deletion of *NPL3* leads to a slower growth phenotype, with *cet1-2* and *ceg1-3* showing a more severe synthetic growth defect with *npl3Δ* than *ceg1-34* at 25°C, pointing to a possible functional connection of Npl3 with 5'-capping. While the *ceg1-3* mutant is known to display a severe temperature sensitive phenotype resulting from three point mutations in its catalytic domain (F51Y, N190Y, Y226F) (31), the milder *ceg1-34* allele contains a single point mutation in a region that mediates Cet1 interaction (P346L) (32). These differences might explain the diverse genetic interactions with *NPL3*. The mild phenotype of *ceg1-34,* which is impaired in its interaction with Cet1 but retained its enzymatic function at the restrictive temperature might be tolerable when Npl3 is missing, while in the combination of *ceg1-3* with *npl3Δ* in which *ceg1-3* has defects

in its enzymatic function growth is more severely inhibited. This is specific for *npl3Δ*, as the triple mutant strain *cet1-2 gbp2Δ hrb1Δ* did not show a synthetic growth defect. Combination of a deletion of *NPL3* and deletions of the CBC are synthetically lethal (Figure 1A). To analyze whether Npl3 also physically interacts with Cet1 and/or Ceg1, co-immunoprecipitation (IP) analyses were carried out. Although Cet1-GFP and Ceg1-GFP were clearly present in the eluates, Npl3 was not co-precipitated, indicating that no physical contact of Npl3 with the capping machinery occurs (Figure 1B, Supplementary Figure S1A). However, as an interaction of Npl3 with the CBC was shown earlier (21), it is possible that Npl3 might be recruited to pre-mRNAs after the capping machinery has left the pre-mRNA. In fact, a quality control factor that monitors proper capping should be loaded independently of the capping event to emerging transcripts. Thus, one would expect to find Npl3 also bound to transcripts generated in a *cet1* mutant. Upon shifting *cet1-2* to its non-permissive temperature newly synthesized transcripts should be uncapped. To determine the amount of the pre-mRNAs that are uncapped, after a 1 h incubation at 37°C, we analyzed the mRNAs produced in *cet1-2* in an *in vitro* experiment. We isolated the RNA from wild type and *cet1-2* cells and incubated it with recombinant Xrn1. This exonuclease degrades RNAs from their 5'-ends, if not protected by a 5'-cap (33,34). Thus, mutations in *CET1* should increase the amount of the degraded mRNAs. The experiment revealed that approximately 50% of the analyzed transcripts were uncapped in comparison to RNA that was isolated from wild type cells treated equally (Figure 1C). To investigate whether Npl3 associates with these faulty transcripts and might accumulate on these retained transcripts in the nuclei of *cet1-2* mutants, we made use of an import defective mutant of Npl3, Npl3c that is localized to the cytoplasm at steady state (35,36). Npl3c is still capable of shuttling between both compartments, but its import rate is slower than its export rate. In contrast, the wild type protein is imported faster and thus nuclear at steady state. Mutations that trap bulk mRNAs in the nucleus, such as in *mex67-5*, lead to a nuclear accumulation of Npl3c (Figure 1D) and (36). Clearly, the same phenotype is also visible in *ceg1-3* and *cet1-2* mutants (Figure 1D, Supplementary Figure S1B). This result could either mean that Npl3 is not recruited to transcripts that lack 5'-caps and is accumulating in the nucleus because not enough mRNAs are generated that are exported, or it could suggest that it accumulates on uncapped pre-mRNAs in the nucleus that await degradation. To distinguish between these two possibilities, we analyzed the binding of Npl3 to transcripts in *cet1-2* mutants shifted to 37°C for 1h by RNA-co-IP experiments (RIPs). This two-step procedure first pulls down Npl3 from cells and in a second step isolates the Npl3-bound mRNA. The experiment revealed that the binding of Npl3 to randomly chosen transcripts increased by ~3–9-fold in the *CET1* mutant (Figure 1E, Supplementary Figure S1C). Together, these results suggest that Npl3 binds to emerging transcripts independent of the 5'-cap formation and it remains associated with uncapped transcripts in the nucleus until they are either correctly processed and exported or eliminated from the cells in case capping fails.
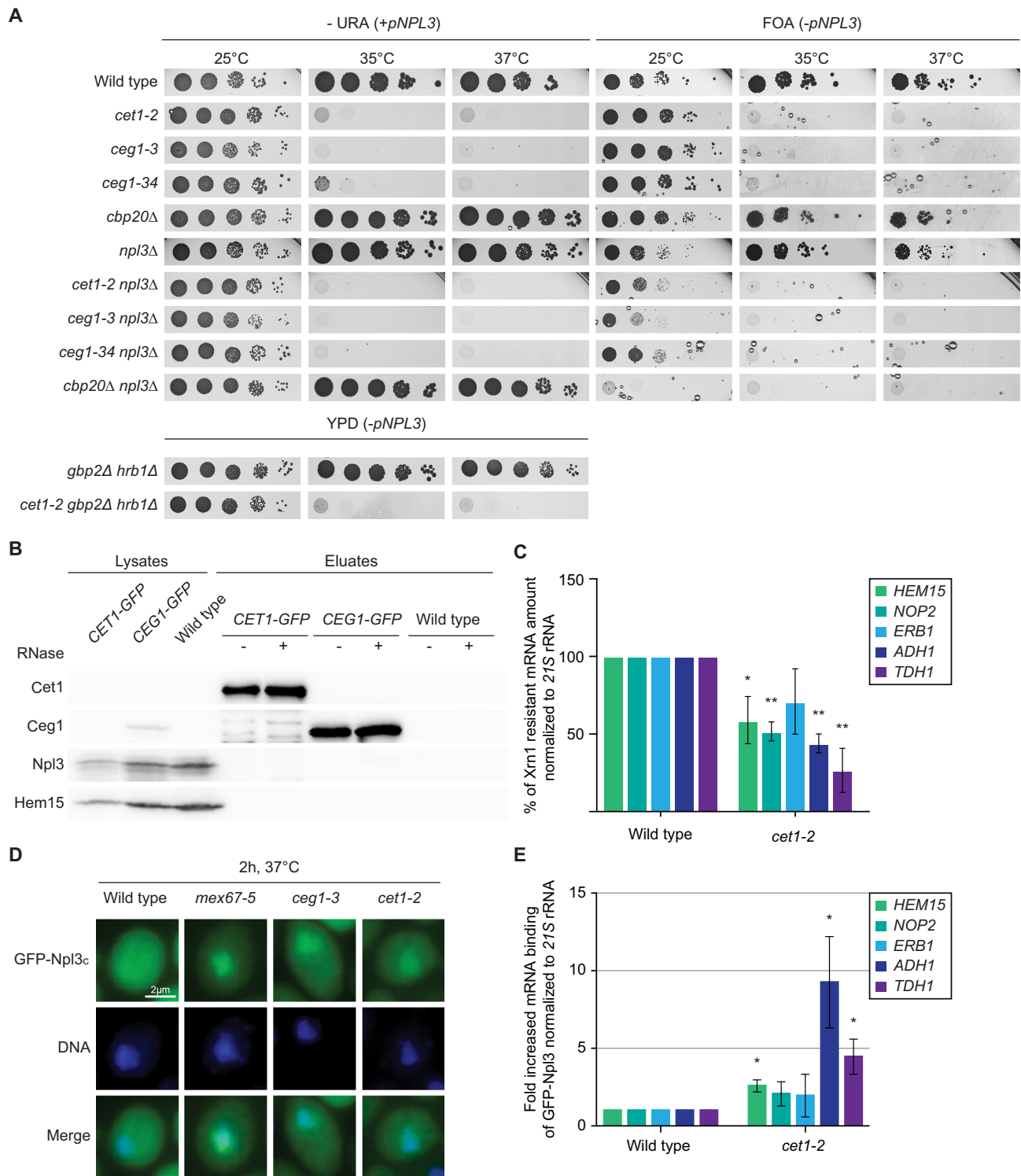
Figure 1. Npl3 binds pre-mRNAs with defective 5'-caps. (**A**) Deletion of *NPL3,* but not of *GBP2* and *HRB1,* is synthetically lethal with mutations in 5'-capping factors. Ten-fold serial dilutions of the indicated strains were spotted onto –URA plates to select for the covering plasmid and onto FOA plates to assay the growth upon the loss of the covering plasmid. The plates were incubated for three days. *n = 3.* (**B**) Npl3 does not interact with the capping enzymes Cet1 and Ceg1. Western blot analysis of co-immunoprecipitation (co-IP) experiments with GFP-tagged Cet1 or Ceg1 and Npl3 are shown. Hem15 served as a negative control. *n = 3.* (**C**) The *cet1-2* mutation produces uncapped mRNAs. Log phase cells were shifted for 1 h to 37°C. The RNA was isolated and incubated *in vitro* with the recombinant 5'-3' degrading enzyme Xrn1. The remaining RNA was subsequently analyzed in qPCRs. *n = 3.* (**D**) Npl3 nuclear export is inhibited in mutants of the 5'-capping machinery. An at steady state cytoplasmic version of Npl3 (GFP-Npl3c) that is slower in nuclear re-import, was localized in the indicated strains after a 2 h temperature shift to 37°C. (**E**) The binding of Npl3 to mRNA is increased in mutants of the capping enzyme. RNA co-immunoprecipitation (RIP) experiments with Npl3 were carried out with wild type and *cet1-2* mutants after a 1 h incubation at 37°C. Subsequent qPCRs analyzed the indicated transcripts. *n = 3*; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Npl3 retains uncapped pre-mRNAs in the nucleus

Mutations in the nuclear exosome result in degradation defects of faulty mRNAs, which visibly accumulate in the cell nuclei as shown for *rrp6Δ* earlier (4,8). Interestingly, the simultaneous deletion of any of the guard proteins leads to the leakage of these faulty mRNAs to the cytoplasm (4). However, while mutations in the exosome lead to the accumulation of all kinds of defective mRNAs, mutations of the capping machinery specifically increase the number of cap-defective transcripts, even though defects in *CET1* and *NPL3* lead to an overall decreased RNA level (Supplementary Figure S2A, C). However, in relation to the *cet1-2* mutant, the mRNA levels of the double mutants *cet1-2 rat1-1* and *cet1-2 rai1Δ* are significantly elevated, suggesting that the decrease in mRNA levels in capping mutants is most likely due to the degradation activity of Rat1–Rai1 (Supplementary Figure S2B). To investigate the potential leakage of these uncapped RNAs, we cultivated the *cet1-2 npl3Δ* double mutant at 25°C, at which it grows slowly (Figure 1A, Supplementary Figure S2D) and then shifted it to 37°C, where capping is defective and determined the mRNA localization. Strikingly, while defective mRNAs accumulated in the nuclei of *cet1-2* cells, we found approximately 25% of the mRNA to leak into the cytoplasm when *NPL3* was additionally deleted (Figure 2A-B and Supplementary Figure S2E-F). This cannot be observed when *GBP2* and *HRB1*, the two splicing guards, are deleted, suggesting that cap defective transcripts might only be retained through the action of Npl3.

The method chosen for the detection of the cap-defective mRNAs leakage is based on the detection of polyadenylated mRNAs with a labeled oligo d(T) probe (Supplementary Figure S2E). However, cap-defective RNAs might have a reduced chance of getting further processed and obtain a poly(A) tail. Therefore, we repeated the experiment with a highly expressed single mRNA encoding two GFP sequences in a row. Although the overall signal was fainter due to the selected single template, the nuclear signal in *cet1-2* was clearly detectable and also reduced to ∼70% in *cet1-2 npl3Δ* (Figure 2C, D and Supplementary Figure S2G).

To confirm the mRNA leakage from the nucleus with a different method, we fractionated cells of wild type, *cet1-2* and *cet1-2 npl3Δ* strains, prepared the mRNA from the cytoplasmic fractions and compared the amount to the wild typical RNA-content in the cytoplasm. As expected, due to the functional nuclear quality control in the presence of Npl3, the nuclear export of the cap-defective mRNAs is inhibited and after a 1h incubation of *cet1-2* at the non-permissive temperature, only ∼50% of the mRNAs are detectable in the cytoplasm as compared to wild type (Figure 2E and Supplementary Figure S2H). Strikingly, mRNA export is increased to up to ∼150% in the *cet1-2 npl3Δ* double mutant, which manifests the leakage of cap-defective transcripts to the cytoplasm and identifies Npl3 as a nuclear retention factor for uncapped transcripts. Once in the cytoplasm, other quality control mechanisms seem to exist that eliminate these uncapped RNAs, because in the *cet1-2 npl3Δ* double mutant where leakage occurs, fewer of the overall transcripts are Xrn1 sensitive (Figure 2F).

To investigate whether the CBC might also have a guarding function which is similar to Npl3 and is capable of retaining faulty mRNAs in the nucleus, we repeated the leakage assay with *cbp80Δ*. We found that Cbp80 did not act as a retention factor. Neither in combination with *rrp6Δ*, nor with *cet1-2* did *cbp80Δ* show leakage of mRNAs into the cytoplasm (Figure 2G-H and Supplementary Figure S2I). This highlights the unique nuclear mRNA retention function of Npl3 for uncapped transcripts.
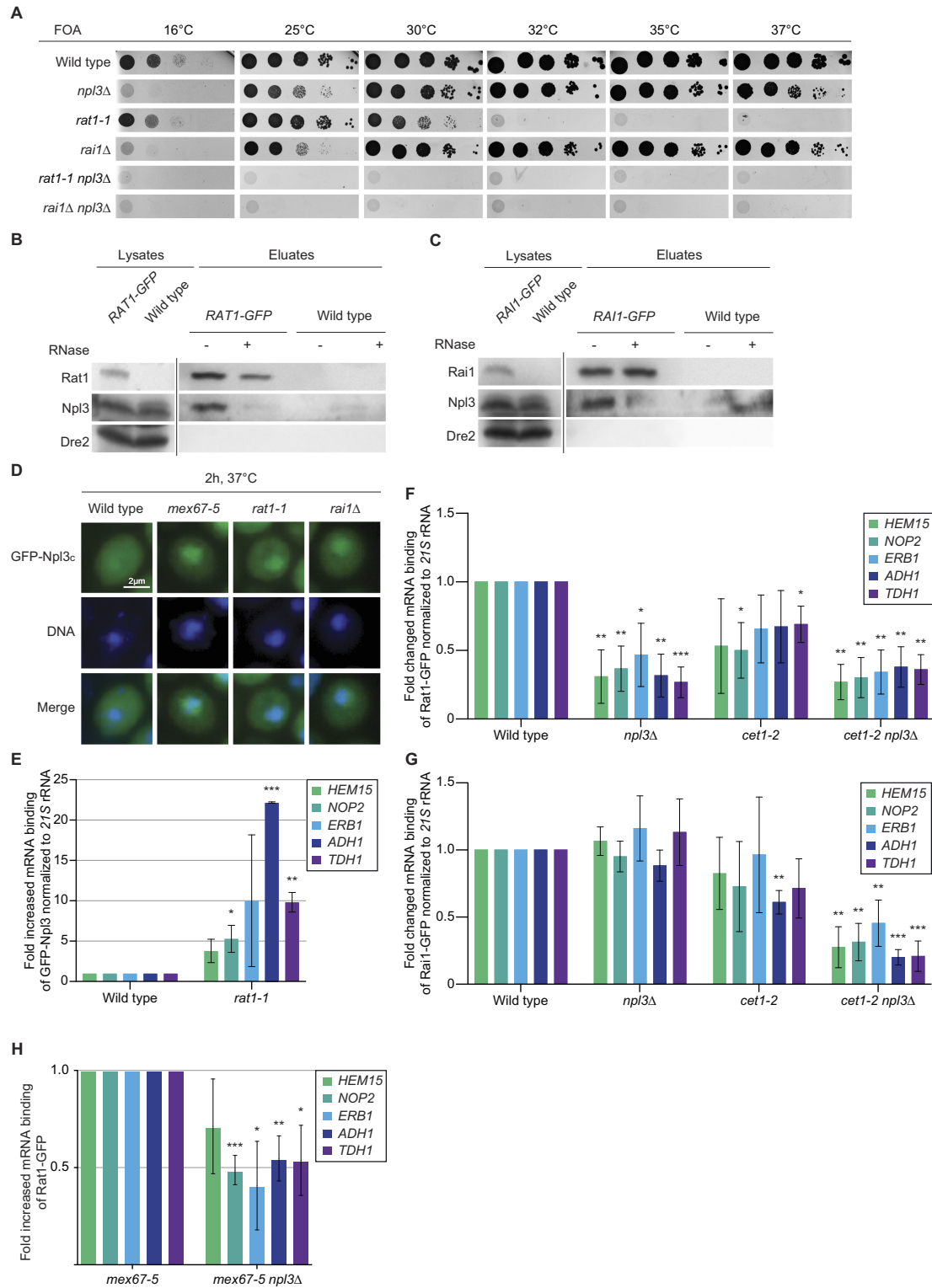
Npl3 recruits the 5'-degadation machinery to uncapped transcripts

Npl3 retains cap-defective transcripts for their subsequent degradation in the nucleus. Dxo1 on its own and Rat1–Rai1 as a complex have been shown to degrade faulty mRNAs from their 5'-ends in the nucleus (29,30). However, it is currently unclear how these enzymes identify faulty transcripts and how they are recruited to these pre-mRNAs. As a guard protein, Npl3 might be involved in the guidance of Dxo1 and/or the Rai1-Rat1 complex to cap-defective mRNAs. To analyze a potential connection between the guard Npl3 and the 5'-degradation factors, we investigated first whether Dxo1 would genetically and physically interact with Npl3, but we found neither a physical interaction between these proteins, nor a nuclear export defect of Npl3c in the *DXO1* deletion mutant (Supplementary Figure S3A–C), suggesting that Npl3 does not act in the Dxo1-mediated degradation of faulty transcripts.

Secondly, we investigated whether *RAT1–RAI1* mutants genetically interact with the deletion of *NPL3*. Indeed, while both single mutants grew well at 25°C, the *rat1-1 npl3Δ* and *rai1Δ npl3Δ* double mutants were synthetic lethal (Figure 3A). Similarly, a negative interaction was shown for the combination of *xrn1Δ npl3Δ* in high throughput analyses, suggesting that RNA degradation in general is critical in the absence of Npl3 (37). Additionally, Rat1 and Npl3 as well as Rai1 and Npl3 physically interact in an RNA-dependent manner as shown by co-IPs (Figure 3B, C and Supplementary Figure S3D). We find a similar binding also for Gbp2 but not Hrb1 (Supplementary Figure S3E, F), however, this could be in regard to their guard protein function for correct splicing. Moreover, we show that Npl3 accumulates in the nucleus of cells mutated in *RAT1* and to a lower extend also in *RAI1* mutants, possibly on retained transcripts that are waiting to be degraded (Figure 3D and Supplementary Figure S3G). To investigate that directly, we carried out RNA-co-IPs of Npl3 in *rat1-1* cells shifted to 37°C for 1h and found that the transcript binding of Npl3 is significantly increased (Figure 3E and Supplementary Figure S3H). These data support a model in which Npl3 accumulates on faulty transcripts that cannot be degraded by the Rat1–Rai1 complex. To investigate whether Npl3 is involved in the recruitment of Rat1 or Rai1 to faulty transcripts, we carried out RIP-experiments that showed that Rat1 but interestingly not Rai1 contacts ∼50% less mRNAs when Npl3 is absent (Figure 3F, G and Supplementary Figure S3I, J). In a *cet1-2* mutant, where newly synthesized RNAs are not capped after a temperature shift, we see slightly less interaction of Rat1 or Rai1 with the transcripts, possibly because an overwhelming number of un-
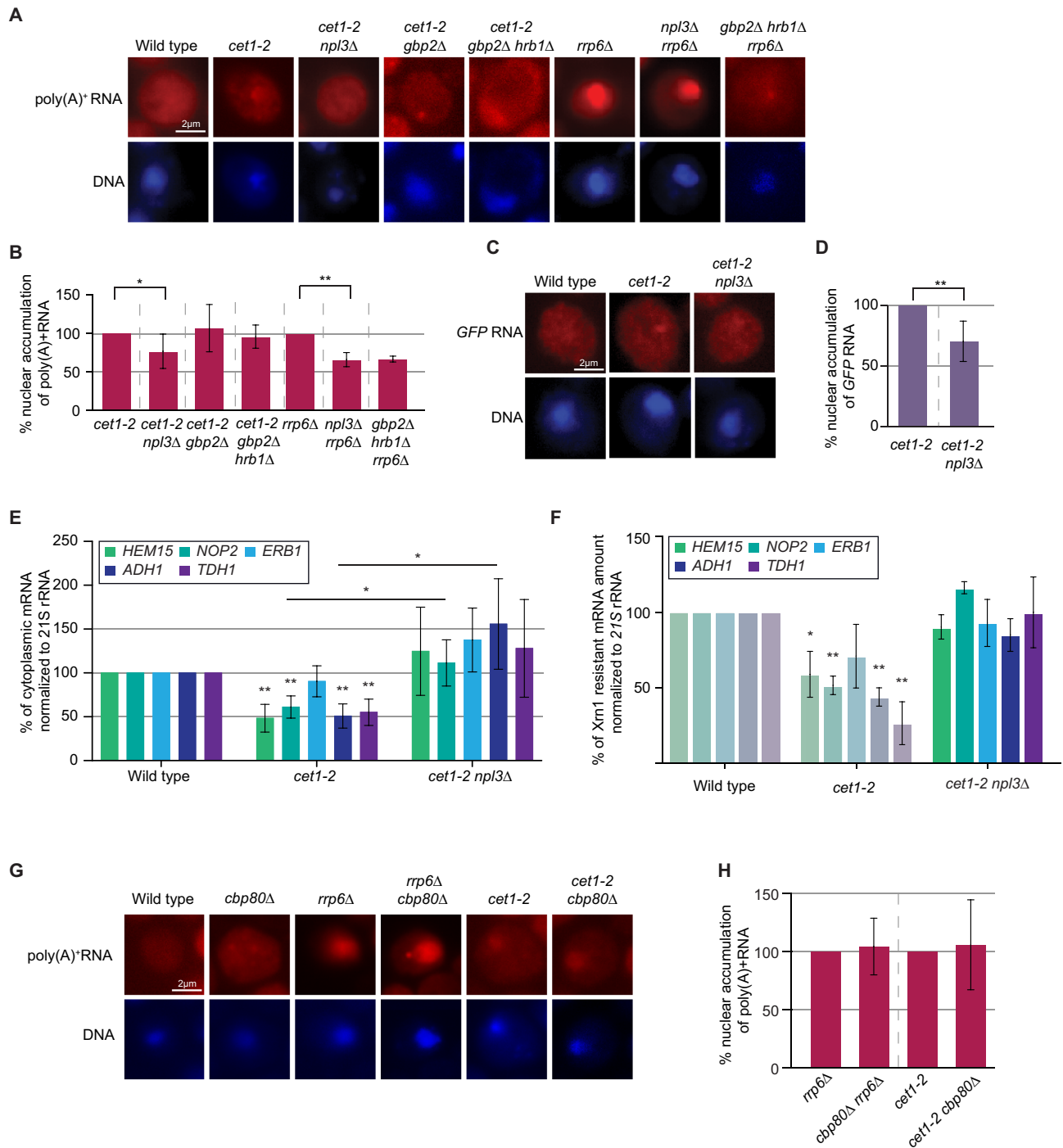
Figure 2. Npl3 mediates the nuclear retention of pre-mRNAs with defective 5'-caps. (**A**) A leakage assay reveals mRNA leakage of usually retained false 5'-capped pre-mRNAs into the cytoplasm in cells deleted for *NPL3,* but not for *GBP2* and *HRB1*. *In situ* hybridization (FISH) experiments of bulk mRNAs are shown in the indicated strains that were shifted for 3 h to 37°C. The poly(A)$^+$RNA was visualized with a Cy3 labeled oligo d(T)$_{50}$ probe. The DNA was stained with Hoechst (blue). (**B**) Quantification of the measured nuclear and cytoplasmic signals shown in (A). $n = 5$. (**C**) FISH experiment with a single RNA species, the *GFP* RNA, expressed from the strong *ADH1* promoter is shown in the indicated strains, shifted for 2 h to 37°C. The GFP transcript was visualized with a gene specific Cy3 labeled probe (red). (**D**) Quantification of the experiment shown in (C). $n = 3$. (**E**) Nucleo-cytoplasmic fractionation experiments reveal the leakage of transcripts to the cytoplasm in cells deleted for *NPL3*. The indicated strains were grown to log phase before they were shifted to 37°C for 1h. The cytoplasm was isolated and the containing mRNAs were analyzed in qPCRs. $n = 4$. (**F**) A deletion of *NPL3* in *cet1-2* shows RNA levels close to wild type. Cells were shifted for 1 h to 37°C. The RNA was isolated and incubated *in vitro* with the recombinant 5'-3' degrading enzyme Xrn1. The remaining RNA was subsequently analyzed in qPCRs. G, The CBC is not a retention factor for mRNAs. A leakage assay as carried out in (Figure 2A) with *cbp80Δ* is shown. H, Quantification of the measured nuclear and cytoplasmic signals shown in (F). $n = 3$, *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Figure 3. Npl3 recruits the 5'-degradation machinery to uncapped transcripts. (**A**) *NPL3* genetically interacts with mutants of *RAT1* and *RAI1*. Serial dilutions of the indicated strain were spotted onto FOA plates and incubated at the indicated temperatures. *n = 5*. (B and C) Physical interaction of Npl3 with the Rat1–Rai1 complex. Western blot analysis of co-IPs with GFP-tagged Rat1 (**B**) or Rai1 (**C**) and Npl3 are shown. *n = 3*. (**D**) Npl3 is retained in the nuclei of mutants defective in the 5' directed RNA-degradation. GFP-Npl3c was localized in the indicated strains after a 2 h temperature shift to 37°C. *n = 5*. (**E**) Increased mRNA binding of Npl3 is detected in *rat1-1*. Cells were shifted for 1 h to 37°C before the Npl3-bound RNAs were isolated. The indicated mRNAs were analyzed in qPCRs. *n = 3*. (F and G) Efficient interaction of Rat1 but not Rai1 with the mRNAs requires Npl3. qPCRs are shown from RIP experiments of Rat1 (**F**) or Rai1 (**G**) for the indicated transcripts in wild type and *npl3Δ*. RIP experiments were performed with Rat1-GFP and Rai1-GFP, respectively. *n = 4*. (F) *n = 4* (G). (**H**) A nuclear mRNA export block does not change the reduced binding of Rat1 to faulty transcripts in *npl3Δ*. qPCRs are shown from RIP experiments of Rat1 for the indicated transcripts in *mex67-5* and *mex67-5 npl3Δ*. *n = 4*; *$*P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

capped transcripts are suddenly produced. However, in support of our model these transcript associations further decrease to ∼30% when *NPL3* is in addition missing (Figure 3F, G). These data can either be interpreted in a way that Npl3 is responsible for the Rat1–Rai1 coordinated elimination of the transcripts or it is possible that through the leakage of the transcripts into the cytoplasm in the absence of Npl3 Rat1–Rai1 cannot contact them for degradation. To distinguish between these two possibilities, we repeated the RIP experiment in *mex67-5 npl3Δ*. In this strain all mRNAs are retained in the nucleus at the non-permissive temperature through the defect of the export receptor. Thus, leakage caused by the missing Npl3 is prevented in this strain. Interestingly, these nuclear transcripts show a reduced binding of Rat1 to these transcripts as well. These data clearly show that not the nuclear escape but the absence of Npl3 is responsible for the Rat1 recruitment (Figure 3H and Supplementary Figure S3K).

Taken together, our data suggest that Rai1 and Npl3 interact on transcripts for surveillance of 5'-capping and they subsequently recruit Rat1 to cap-defective mRNAs for their elimination.

### The interaction of Npl3 with the CBC is disturbed when capping is defective

We have shown that Npl3 retains uncapped transcripts in the cell's nucleus and we provided evidence that Npl3 recruits the 5'-degrading exonuclease Rat1 to cap-defective mRNAs. However, it remains unclear how pre-mRNAs without a correct 5'-cap are identified. It seems likely that CBC might not associate with a defective cap and that its absence alerts the degradation pathway. In wild type cells, Npl3 physically interacts with the CBC (21). We show that this interaction is partially RNA dependent, which could either suggest that Npl3 needs RNA binding to efficiently interact with Cbp80 or that only those Npl3 molecules that bind to the 5'-end of the transcript directly interact with the CBC (Figure 4A and Supplementary Figure S4A). Therefore, it is possible that Npl3 and the CBC associate with the emerging mRNA as a preformed complex. Alternatively, CBC could bind independently of Npl3. To distinguish between these possibilities, we used the *cet1-2* mutant, in which the 5'-cap is defective (Figure 1C). First, we investigated whether the association of CBC with the RNA is disturbed in *cet1-2* cells. Indeed, RIP experiments revealed an approximately 80% reduced transcript binding to Cbp80 in this mutant as compared to wild type cells after a 1h incubation at the non-permissive temperature (Figure 4B, Supplementary Figure S4B). This indicates that CBC binding requires an intact 5'-cap and confirms that the accumulating mRNAs in *cet1-2* mutants are not CBC bound. In contrast, we have shown that the binding of Npl3 to uncapped transcripts is not disturbed but rather increased in *cet1-2* cells (Figure 1E). Therefore, we assume that the interaction between Npl3 and Cbp80 is reduced in *cet1-2* cells, as CBC requires an intact cap to bind. This was verified by co-IP experiments, in which we show that the interaction of Npl3 and Cbp80 in *cet1-2* upon a 1h temperature shift to 37°C was reduced to ∼60% as compared to wild type (Figure 4C-D). Consequently, the absence of the indicator for a cor-

rect 5'-cap as in *cbp80Δ* already leads to a reduced growth rate of cells, while the additional loss of the 5'-degradation machinery is synthetically lethal (Figure 4E). These experiments suggest that the loading of CBC and Npl3 to emerging transcripts is independent of each other. Furthermore, they show that Npl3 and CBC physically interact and that they interact only on mRNAs with correct 5'-cap structures.

Npl3 represents a switch between degradation and export

From these data it seems possible that a potential mechanism of how Npl3 would detect defects in 5'-capping could be that it recognizes the absence of the CBC while it remains bound to Rai1 of the 5'-degradation machinery. It was published earlier, that Npl3 interacts with Mex67 to promote nuclear export (35). Therefore, Npl3 might function as a switch that might only interact with Mex67 when Rai1 dissociates from Npl3 when present in a complex with CBC. To strengthen such a model, we carried out co-IP studies in mutants of *CET1* or *RAT1*, because in these strains less Mex67 should interact with Npl3. While the interaction of Mex67 and Npl3 was clearly visible in wild type cells, it dropped approximately by 20% in cells that have an increased presence of uncapped pre-mRNAs (Figure 5A, B). Although this value does not seem to be high on first sight, one has to keep in mind that Npl3 might have additional functions as guard on the mRNA and bind multiple times, for instance at its 3'-end. Moreover, when we precipitated export competent mRNAs via Mex67 IP, we detected Npl3 but not the Rat1–Rai1 complex (Figure 5C). Vice versa, Npl3 complexes that were co-precipitated with Rat1 or Rai1 contained no CBC and no Mex67 (Figure 5D, E). These findings indicate that Npl3 might either interact with CBC and Mex67, or with Rai1 that supports Rat1 recruitment and support a model in which Npl3 influences the fate of a newly synthesized mRNA in one or the other direction. This decision is most likely made through the contact of Npl3 with CBC. If this model would be correct, one would expect that the correctness of the 5'-cap structure is not directly monitored, but rather through detection of the binding of CBC. To test that, we used the *CBP80* deletion strain, in which no CBC is formed, although these mRNAs have intact 5'-cap structures, and monitored the behavior of Rat1 and Rai1. We detected a decreased binding of Rat1 to a variety of transcripts, while the binding of Rai1 was increased (Figure 5F, G and Supplementary Figure S5A, B). The missing CBC allowed Rai1 to persist on all mRNAs, which is an overwhelming number. On all these RNAs it waits for Rat1 to join and degrade the transcripts as CBC is absent. However, since these are so many transcripts that signal Rat1 to join, this exonuclease might not be able to complete degradation of all transcripts immediately.

In summary, our data reveal a novel function for Npl3 in the nuclear quality control of 5'-capping. We suggest a model in which Npl3 is loaded to the emerging pre-mRNA via RNAP II, possibly in association with Rai1 (Figure 6). This interaction is destabilized upon CBC contact. Rai1 dissociates and the recruitment of Rat1 is prevented. Instead, Npl3 binds to Mex67 allowing the nuclear export of the transcript. In case the 5'-cap was not properly at-
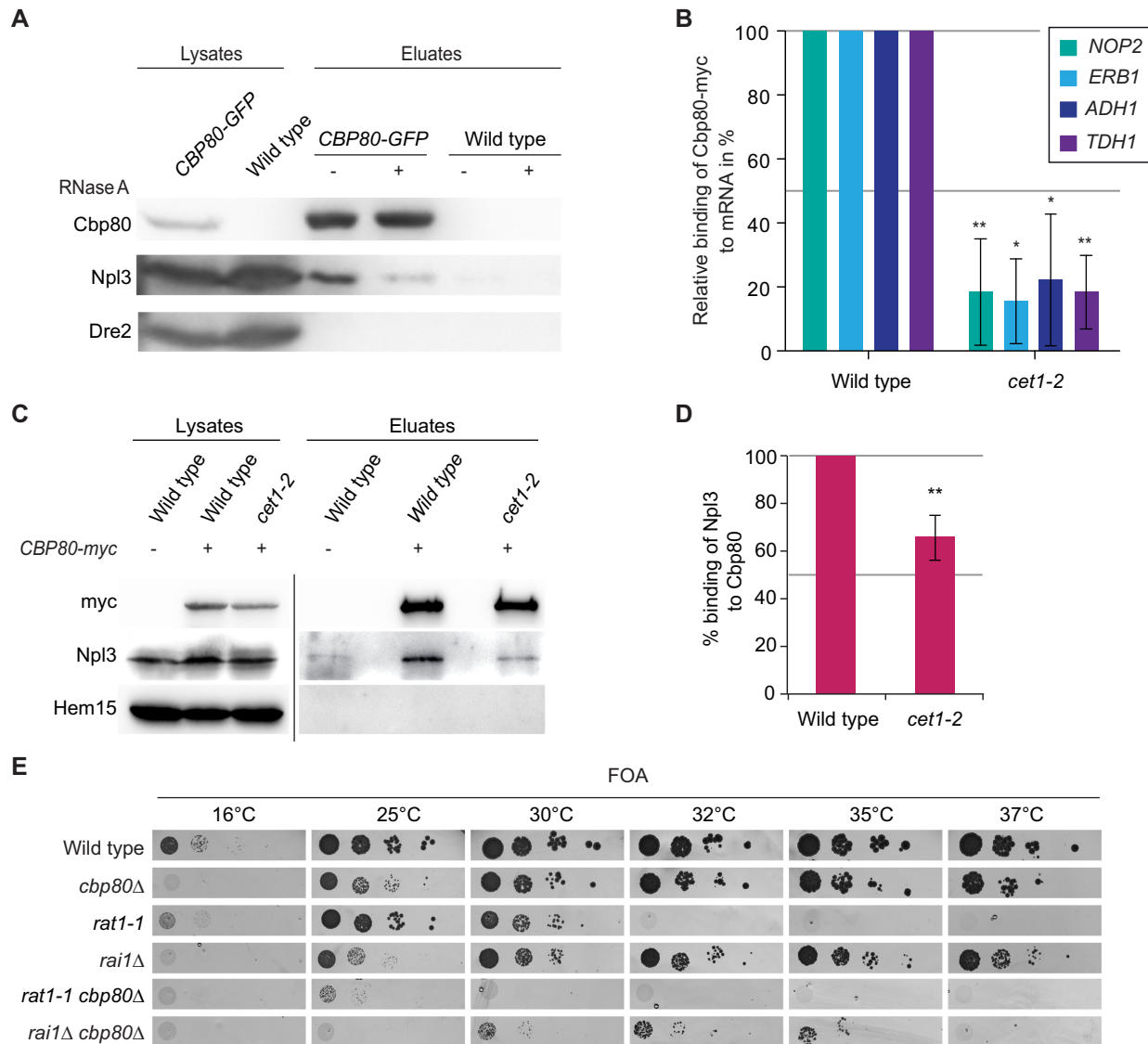
Figure 4. Proper 5'-capping is prerequisite for the interaction of CBC and Npl3. (**A**) Npl3 and Cbp80 physically interact. Co-IPs of Npl3 with GFP-tagged Cbp80 are shown with and without the addition of RNase Dre2 served as a negative control. $n = 4$. (**B**) The transcript binding of Cbp80 is reduced in *cet1-2* mutants shifted to 37°C for 1 h. RIP-experiments with Cbp80 and subsequent qPCRs were carried out. $n = 3$. (**C**) The interaction of Npl3 and Cbp80 is disturbed in the presence of uncapped transcripts. Western blot analysis of co-IPs with Cbp80 and Npl3 was compared in the indicated strains. Hem15 served as a negative control. (**D**) Quantification of three different experiments one of which is shown in (C). $n = 5$. (**E**) Genetic interactions of *cbp80Δ* with the indicated strains are shown. Serial dilutions of the indicated strains are shown on FOA plates. $n = 3$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

tached, Npl3 does not detect and interact with CBC so that Rai1 can persist on the mRNA. Rat 1 is recruited to degrade the faulty transcript and Npl3 dissociates. In the absence of Npl3, uncapped pre-mRNAs are not recognized and can leave the nucleus via other guard proteins that have recruited Mex67.

DISCUSSION

The nuclear mRNA quality control has to monitor all different processing steps on the way of a primary transcript to its mature form that is optimized for efficient translation. This requires not only the elimination of introns from a pre-mRNA, but also the proper formation of the 5'-cap and the 3'-poly(A) tail, as both ends participate in circu-

larization of the mRNA to optimize translation (38). Several mechanisms have been reported to take part in the nuclear quality control (3,39,40). One important group of nuclear RNA surveillance factors is that of the guard proteins (5). These factors are loaded onto the pre-mRNA in the nucleus, directly contact the transcript via their RNA-binding domains, interact on correctly processed mRNAs with the export receptor heterodimer Mex67-Mtr2 and shuttle with the mRNA into the cytoplasm (7,8,36,41,42). Notably, their artificial overproduction in the nucleus actively retains also intact mRNAs (4,36,42). Retention of quality proved transcripts is released through Mex67 binding. Proper Mex67-coverage is detected by Mlp1 at the NPC and those RNAs that have not received the appropriate
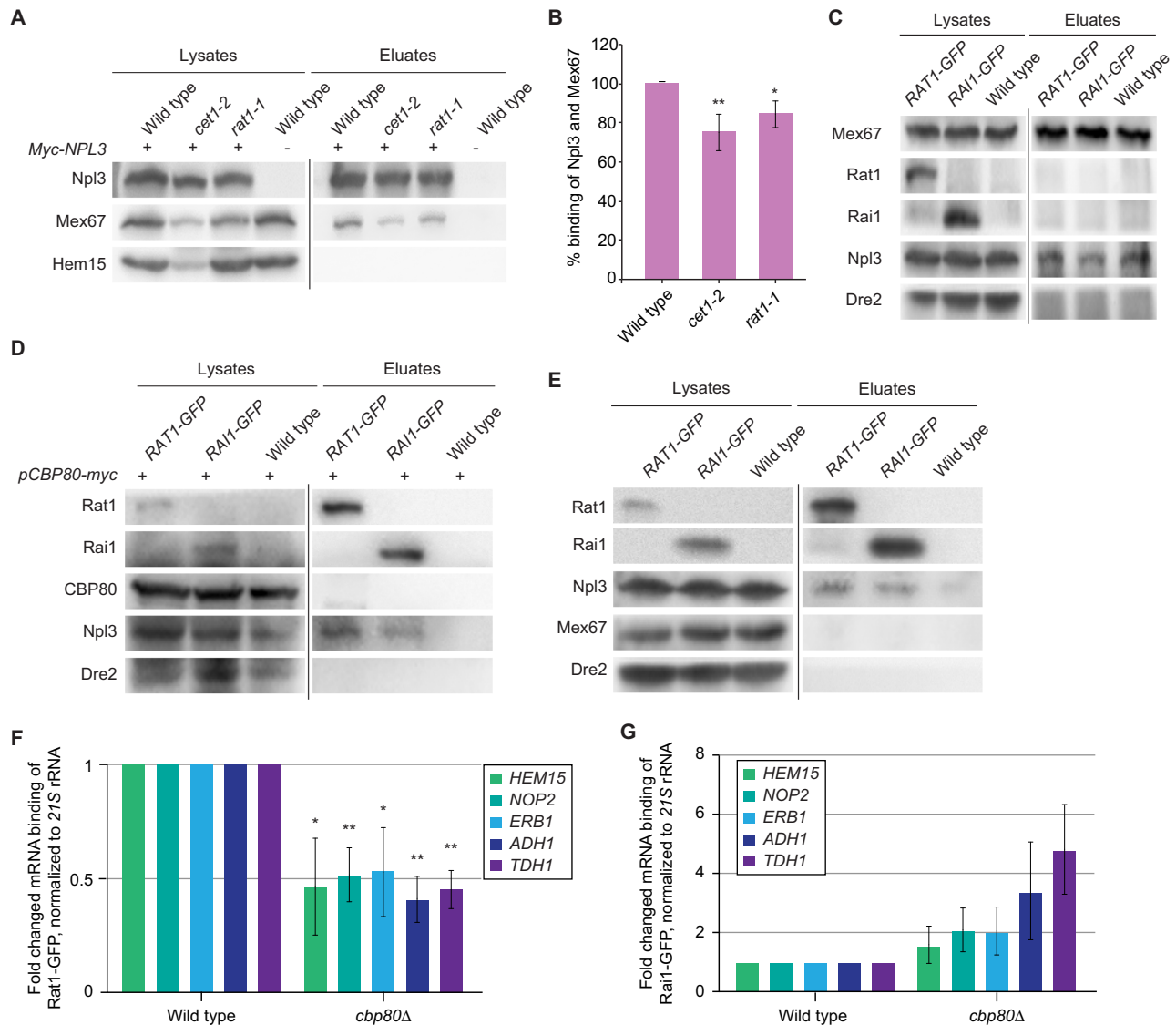
Figure 5. Npl3 binding to Mex67 or Rat1–Rai1 is mutually exclusive. (**A**) Mex67 binding to Npl3 is reduced in 5'-capping and 5'-degradation mutants. Western blot analysis of Mex67 co-IPs with Npl3 is shown. The indicated strains were shifted to 37°C for 2 h before lysis. (**B**) Quantification of experiments, one of which is shown in (A). $n = 8$ for wild type, $n = 8$ *for cet1-2*, $n = 6$ for *rat1-1*. (**C**) Mex67-bound Npl3 does not interact with Rat1 and Rai1. A western blot of Npl3, Rat1 and Rai1 co-IPs with Mex67 is shown. (**D**) Rat1–Rai1-bound Npl3 shows no interaction with Cbp80. A western blot of Npl3 and Rat1 or Rai1 co-IPs with Npl3 and Cbp80 is shown. (**E**) Rat1- and Rai1-bound Npl3 is not Mex67 bound. A western blot of a Mex67 and Npl3 co-IPs with Rat1 and Rai1 is shown. (F and G) In the absence of *CBP80*, the Rat binding to transcripts is decreased while Rai1 binding is increased. RIP experiments with Rai1-GFP and subsequent qPCRs are shown for the indicated transcripts. $n = 3$ for (**F**), $n = 4$ for (**G**); *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Mex67-export 'tickets' have difficulties passing through the NPC. On faulty mRNAs, the guard proteins do not allow Mex67 to bind, but instead recruit the nuclear degradation machinery. Gbp2 and Hrb1 for instance recruit the TRAMP-complex, which initiates degradation through the nuclear exosome of intron-containing transcripts that were not properly spliced (8).

For Npl3 no particular function in the nuclear mRNA quality control had so far been identified. Here we present evidence that it controls proper 5'-capping. It accumulates on uncapped transcripts (Figure 1D, E) and shows several genetic and physical interactions with 5'-cap associated proteins, including the 5'-degradation machinery (Figure 1A, 3A–C). Most importantly, its deletion leads to the leakage

of uncapped mRNAs into the cytoplasm (Figure 2). Interestingly, CBC itself is not a retention factor, as its absence does not result in mRNA leakage into the cytoplasm (Figure 2G, H). CBC rather seems to function as a marking signal for proper capping, because in its absence more RNAs stay associated with Rai1 even though not more RNA is defective (Figure 5G). Reassuringly, we could show that the leakage of uncapped transcripts to the cytoplasm is not the cause for the reduced Rat1 association as seen in the absence of NPL3 (Figure 3F, G), as in *mex67-5 npl3Δ* cells the same phenotype is observed (Figure 3H). Therefore, we suggest a model in which Npl3 interacts with Rai1 on mRNAs and either supports Mex67 recruitment when CBC is bound, or Rat1 binding to Rai1 and thus subsequent degra-
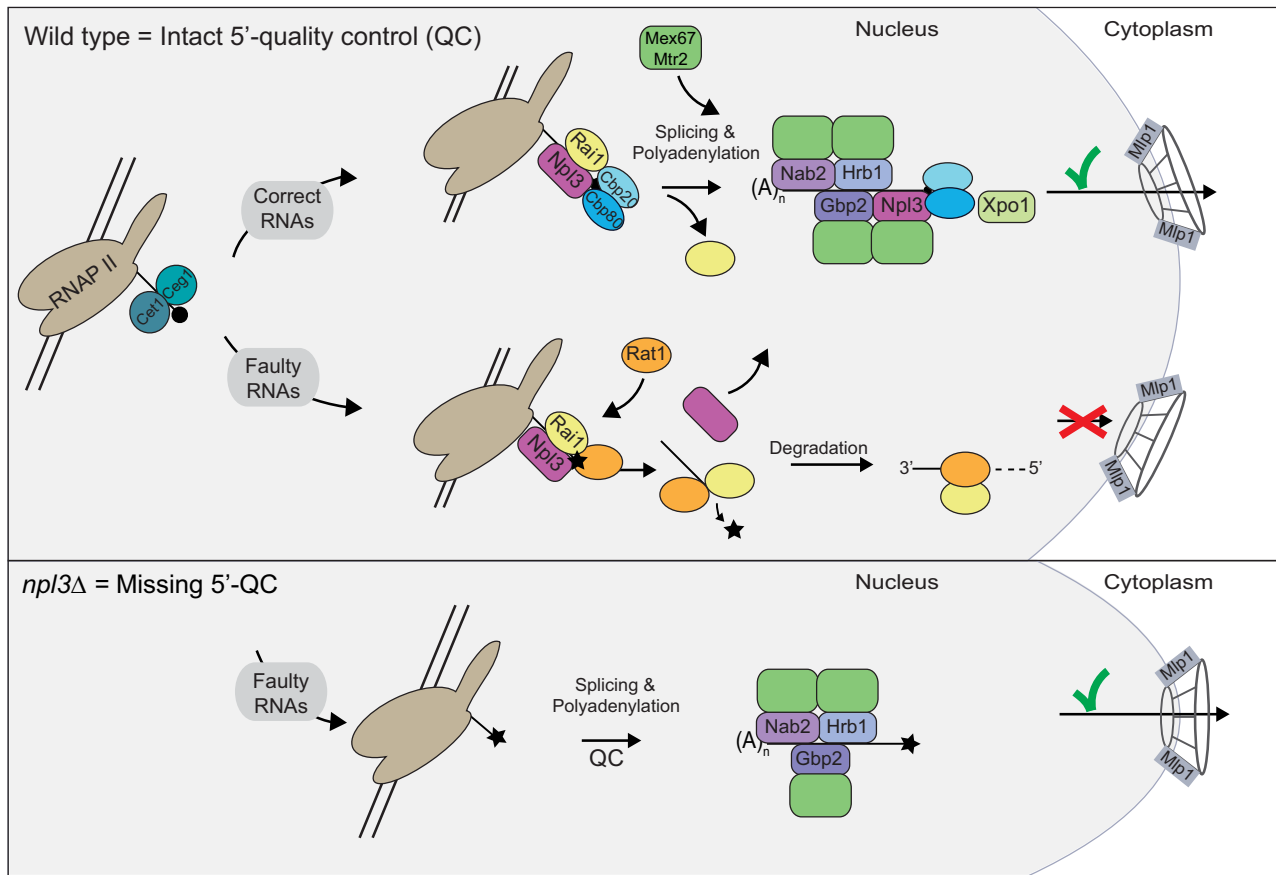
Figure 6. Model for the Npl3-mediated nuclear mRNA quality control at 5'-caps. RNA-polymerase II (RNAP II) synthesized transcripts are capped by the capping machinery Cet1 and Ceg1. Upon correct capping, CBC associates. Npl3 and Rai1 bind to the 5'-end of the emerging transcript. The interaction of Npl3 with CBC results in the dissociation of Rai1 and allows the recruitment of Mex67. Upon completion of splicing and polyadenylation, signalled by the other guard proteins Gbp2, Hrb1 and Nab2, the mature mRNA is exported to the cytoplasm. Proper Mex67 coverage of the guard proteins is monitored by Mlp1 at the nuclear pore complex (NPC) and passage is allowed. On transcripts that have a defective 5'-cap, CBC cannot bind. The missing interaction of Npl3 and CBC prevents Mex67 binding and Rai1 can interact with Rat1. Npl3 dissociates and the Rat1–Rai1 complex degrades the pre-mRNA. In the absence of Npl3, incorrect 5'-capping is not detected and transcripts are not retained and degraded in the nucleus.

dation when CBC is missing. Although it was shown that Rat1–Rai1 eliminate uncapped or cap-unmethylated transcripts, as those accumulate in cells deleted for *RAI1* (43), it remained unclear how improper capping is monitored. In particular, because it is difficult for a cell to distinguish whether an mRNA is not yet capped or whether there had been difficulties in the capping process and the transcript was not capped at all and is therefore faulty. Furthermore, it was unknown how the Rat1–Rai1 degradation machinery is recruited to faulty transcripts and how and when their stimulatory activity on each other is initiated. Without their partner neither of the two enzymes works efficiently. Moreover, the Rai1 activity to convert uncapped pre-mRNA ends into monophosphorylated 5'-ends was shown to be stimulated by Rat1 and vice versa, and the Rat1 exoribonuclease activity is stimulated by Rai1 (29,43). Here we show that Npl3 interacts with Rai1 and this physical contact occurs on the mRNA before Rat1 joining (Figure 3C). In our model we propose that this Npl3–Rai1 complex may persist on an mRNA that was not properly capped, while a CBC successfully placed onto the 5'-cap could potentially disrupt their interaction, as binding of both proteins

to Npl3 in the same complex was not detectable (Figure 5C, E). Thus, on correctly capped mRNAs, detection of the CBC by Npl3 might be the signal for accurate capping and might lead to a destabilization of the Npl3–Rai1 complex. The interaction of Npl3 with CBC furthermore seems to promote its interaction with Mex67 resulting in the receipt of the first export 'ticket'. In support of such a model, we show that on mRNAs on which Npl3 is Mex67-bound, it does not interact with the degradation machinery and vice versa. On pre-mRNAs on which Npl3 interacts with Rai1 and Rat1, the Mex67 binding is not detectable (Figure 5A–E).

Our model in which we propose that Npl3 represents a switch that selects either degradation or export has similarities to the guard protein mediated quality control of splicing. Here, the surveillance factors Gbp2 and Hrb1 interact with the late spliceosome and if difficulties in splicing arise, possibly when their interaction with the spliceosome is not released, they recruit the TRAMP-complex onto faulty transcripts, resulting in mRNA degradation. On correctly processed mRNAs however, they are free to interact with Mex67 allowing export (8).

Due to the fact that all maturation steps of a pre-mRNA are initiated sequentially, starting with 5'-capping, followed by splicing and ending with 3'-processing and polyadenylation, it seems conceivable that unsuccessful quality control steps might be communicated to the subsequent events. Npl3 binds close to the 5'-end and its binding additionally peaks at the 3'-end of the transcribed gene (17,20). This is consistent with a function of this guard protein in the surveillance of 5'-capping and it could have an additional role in 3'-processing. In fact, Npl3 was already suggested to be an antagonist of 3'-processing events (44). Moreover, the deletion of *NPL3* was shown to delay splicing and it was suggested that Npl3 might be involved in recruitment of the early splicing machinery (45). In line with this, it was shown that also CBC promotes the presence of Npl3 on transcribed genes and in turn pre-mRNA splicing and export (46). Thus, it is possible that this guard protein might communicate potential difficulties to subsequent events so that ideally no additional energy is wasted for further processing and transport of less- or non-functional transcripts. But since Npl3 is also a highly abundant protein, it is equally well possible that it participates as a surveillance factor in several different processing events. Future studies need to address potential downstream quality controlling roles of this guard protein and the crosstalk of the processing events.

In multicellular organisms CBC was shown to interact with the NEXT-complex that functions as an exosome co-factor and furthermore, a protein termed ARS2 has been shown to interact with CBC and stimulate its 3'-end maturation (12). These findings placed ARS2 and CBC in the center of RNA-surveillance. However, how defective capping is recognized and how ARS2 stimulates further 3'-processing events in multicellular organisms is still unclear. Generally, transcripts are retained in the nuclei when they are faulty, but several examples exist, in which a transcript can also be retained for regulatory means. Thus, several different retention mechanisms have been developed, for instance retention in nuclear speckles, paraspeckles, at the nuclear matrix or on chromatin, or at the NPC through the Mlp1 homologue TPR (47). However, all retention mechanisms have in common that the protein-context of an RNA is not optimal for export. While TAP-p15 promotes export, serine/arginine-rich (SR) proteins, that are homologues to the guards Npl3, Gbp2 and Hrb1, participate in transcript retention. Some of the human SR-proteins shuttle like the yeast guards in association with TAP-p15 to the cytoplasm and some of them have been shown to interact with the nuclear RNA-degradation machinery (5,48). Interestingly, defects in some of the guard protein homologues were shown to be involved in the development of mostly neurodegenerative diseases and some of them have oncogenic potential (5,49). Npl3 shows highest homology to Serine/Arginine Splicing Factor 1 (SRSF1), which is an oncoprotein frequently overexpressed in human cancers (49). But so far in human cells, no SR-protein mediated quality control system has been reported for the surveillance of the 5'-cap.

In this study we have uncovered that the guard protein Npl3 is central in the 5'-capping surveillance. Generally, quality control systems were suggested to include two elements: a *sensor* that identifies the defect of the mRNA and a *degrader* that eliminates the RNA (39). In case of

the 5'-capping we identified Npl3 as the guard and *sensor* for 5'-capping defects that delivers the *degrader*, Rai1 to faulty transcripts. We can even broaden this view for mRNA quality control by adding a third player, the *label* of conducted proper quality control, in this case the export receptor Mex67, which resembles the first 'export ticket'. That Npl3 stays in the center of this nuclear quality control event as sensor and switch that decides for one or the other fate of the transcript is an important step in transcript maturation.

DATA AVAILABILITY

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

ACKNOWLEDGEMENTS

FUNDING

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