The Yeast Homolog of Human PinX1 Is Involved in rRNA and Small Nucleolar RNA Maturation, Not in Telomere Elongation Inhibition*

Received for publication, June 4, 2002, and in revised form, July 1, 2002
Published, JBC Papers in Press, July 9, 2002, DOI 10.1074/jbc.M205526200

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In human cells, PinX1 protein has recently been shown to regulate telomere length by repressing the telomerase. In this work, we show that the putative yeast homolog of PinX1, encoded by the YGR280c open reading frame (ORF), is a new component of the ribosomal RNA processing machinery. The protein has a KK(E/D) C-terminal domain typical of nucleolar proteins and bears a putative RNA interacting domain widespread in eukaryotes called the G-patch. The protein was hence renamed Gno1p (G-patch nucleolar protein). GNO1 deletion results in a large growth defect due to the inhibition of the pre-ribosomal RNA processing first cleavage steps at sites Aα, Aβ, and Aγ. Furthermore, Gno1p is involved in the final 3′-end trimming of U18 and U24 small nucleolar RNAs. A mutational analysis showed that the G-patch of Gno1p is essential for both functions, whereas the KK(E/D) repeats are only required for U18 small nucleolar RNA maturation. We found that PinX1 complemented the gno1− mutation, suggesting that it has a dual function in telomere length regulation and ribosomal RNA maturation in agreement with its telomeric and nucleolar localization in human cells. Conversely, we found that Gno1p does not exhibit the in vivo telomerase inhibitor activity of PinX1.

Since Fontana discovered the nucleolus in 1790 (1), generations of scientists have studied this nucleolar structure by light microscopy. The nucleolus was described as a large and dense globular blotch in the nucleus of eukaryotic cells. Its primary function in ribosome biosynthesis was only found in the 1960s, but the precise role of proteins and RNAs known to participate in this process is still not well understood, and many other components remain to be discovered.

Ribosome biosynthesis is a complex process requiring more than a hundred different gene products. It begins with the transcription of ribosomal RNA (rRNA) by the RNA polymerases (Pol)I and III (reviewed in Ref. 2). In yeast, the 35 S pre-rRNA is transcribed by Pol I in the nucleolus and then processed into the mature RNAs 25 S, 18 S, and 5.8 S (reviewed in Ref. 3). The 5 S rRNA is independently transcribed by Pol III. The 18 S rRNA associates with ribosomal proteins to form the small 40 S subunit of the ribosome. The 25 S, 5.8 S, and 5 S rRNAs form the large 60 S ribosomal subunit with a distinct set of proteins. A complete ribosome contains ~80 ribosomal proteins, but the recent elucidation of the crystallo-graphic structure of the ribosome implies that the rRNA is the catalytic core of the translational machinery (4–6).

The various rRNA precursors and cleavage sites necessary to form the mature rRNAs from the 35 S pre-rRNA are well known (reviewed in Ref. 7). Most of our knowledge on rRNA processing comes from studies in Saccharomyces cerevisiae because of the power of genetic manipulations in this organism. The 35 S pre-rRNA undergoes excision of external transcribed spacers (ETS) and internal transcribed spacers (ITS) and numerous exonucleolytic digestions of 5′- and 3′-extremities. Additionally, more than 50 bases of the rRNAs are modified through ribose methylations and isomerization of uridines (pseudouridylation). These modifications are guided by small nucleolar RNAs (snoRNAs) associated with proteins in small nucleolar ribonucleoprotein particles (snoRNPs) (8). The sno-RNAs pinpoint the site to be modified by base pairing with the complementary sequence in the rRNA (9–11). In addition to snoRNAs, more than 70 nonribosomal proteins are involved in rRNA processing (3, 12). Some of these proteins are also involved in snoRNA maturation and/or nuclear mRNA decay.

The product of the YGR280c open reading frame was found here to be important for the first steps of 35 S pre-rRNA processing and plays a role in snoRNA maturation. The YGR280c gene product carries a domain found in more than 100 eukaryotic proteins, often in association with an RNA-binding motif called the G-patch (13). Until now the role, if any, of the G-patch was unknown. We show here that this motif is essential for the rRNA processing function of Ygr280p, which has hence been named Gno1p (G-patch nucleolar protein 1).

PinX1, the human homolog of Gno1p, was recently identified as an inhibitor of telomerase activity (14). In yeast, the deletion of GNO1 gene did not lead to telomere lengthening, suggesting that the telomere length regulatory role of PinX1 is not conserved in yeast. However, in addition to its telomeric localization, PinX1 was found in the nucleoli of human cells. This observation suggested that in addition to its telomere length regulation function, it could have a role in rRNA processing. Indeed, we found that the GNO1 deletion rRNA defect was compensated by PinX1 expression in yeast cells.

EXPERIMENTAL PROCEDURES

Media, Plasmids, and Strains—Yeast genetic techniques and media have been described by Sherman (15). Standard molecular biology techniques were as described by Sambrook and Russell (16). For localization study of Gno1p, the yeast strain was derived from MW3522 (MATa ura3–52 his3–Δ200 ade2–101 trp1–Δ63 lys2–801)

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leu2Δ Δ TPA180:3HA::HIS3MX6. The tagged strain was obtained by insertion of 13 Myc epitopes followed by a kan or TRP1 marker using the method of Longtine et al. (17). The sequence was inserted between the last codon and the stop codon of the target open reading frame (ORF). The insertion of the Myc-tagging sequences was verified by polymerase chain reaction (PCR) analysis. The expression of the tagged Gno1p, an aliquot of cells growing exponentially in 10 min at 85 °C, was analyzed with the Metamorph imaging software (Universal Imaging System).

RNA Analysis—Total RNA preparations by hot phenol extraction and Northern blot analysis were performed as described previously and analyzed by Northern blotting as described under "RNA Analysis.”

Immunoprecipitation—25 ml of cells growing exponentially in YPD medium (OD 0.6–0.8) were collected, washed twice with water, twice with Western blot buffer (20 mM Hepes, pH 7.5, 150 mM NaCl, 0.2% glycerol, 1 mM dithiothreitol, 0.1% Nonidet P-40, 0.5 mM phenylmethylsulfonyl fluoride), and resuspended in 150 μl of the same buffer with a protease inhibitor mixture prepared as recommended by the manufacturer (Roche). All subsequent steps were performed at 4 °C. The cells were broken with glass beads, and the debris was eliminated by centrifugation. 50 μl of IgG-Sepharose beads (Amersham Biosciences) were added to 150 μl of protein extract and incubated under agitation for 1.5 h. The beads were centrifuged, and the supernatant was collected. The beads were then washed five times with buffer. The RNAs present in the supernatant or the Sepharose beads were phenol extracted as described previously and analyzed by Northern blotting as described under "RNA Analysis.”

Cloning of the Human Ortholog of GNO1—A cDNA fragment encoding the human homolog of GNO1, PINXI, was cloned by PCR of a human cDNA library (28) using oligonucleotides 650 (TCCCCCGGGGATGTC-TATGCTGCGTGAAGCCTC; (37)). These boxes, GAAAATTTC and GCGATGAGATG, are located at 84 and 62 nucleotides, respectively, upstream of the ORF ATG start codon.

Sequence and Localization of Ygr280p/Gno1p—Ygr280p displayed sequence characteristics that supported the idea that it was involved in rRNA and/or snRNA metabolism (Fig. 1). First, this 271-amino acid protein has a charged C terminus bearing KK(E/D) repeats. This motif was previously found exclusively in yeast nucleolar proteins involved in rRNA processing (32). These boxes, GAAAATTTC and GCGATGAGATG, are located at 84 and 62 nucleotides, respectively, upstream of the ORF ATG start codon.

RESULTS

Expression Profile and Promoter of YGR280c/GNO1—Transcriptome analysis of yeast strains grown under various stress conditions have revealed that an important fraction of the proteins involved in transcription and maturation of rRNA are strongly inhibited with similar patterns (29, 30). Strikingly, YGR280c clustered with this group of genes. Furthermore, the YGR280c promoter contained perfect matches to two different boxes typical of genes involved in rRNA processing and the ESR (31). These boxes, GAAAATTTC and GCGATGAGATG, are located at 84 and 62 nucleotides, respectively, upstream of the ORF ATG start codon.

Immunofluorescence Analysis—For immunofluorescence visualization of the tagged Gno1p, an aliquot of cells growing exponentially in YPD were fixed and prepared as described by Kilmartin and Adams (22). The primary anti-Myc mouse antibody was 9E10. The primary anti-HA rabbit antibody was HA11 (Babco). The secondary anti-mouse IgG antibody was coupled to Alexa 488, and the anti-rabbit IgG antibody was coupled to Alexa 568 (Molecular Probes). During the final wash, DNA was stained by DAPI (4′,6-diamidino-2-phenylindole). The cells were observed with a Leica DMRXA fluorescence microscope coupled to a Roper Scientific MicroMax cooled CCD camera. The images were analyzed with the Metamorph imaging software (Universal Imaging System).

ACCGCACCT; (e) CGCGTGCGCCACCAGACTTG (23); TGGAGAA-

AATGAGGCT and GCGTTCTTGATCGATGC were used to analyze the 5′, 6, and 5.8S rRNAs (26). The following oligonucleotide was used for primer extension analysis of A3 and A4 cleavages: GGCCGCAAAATTT-

CAAGTTA (27). For snoRNA analysis, we used the following oligonucleotides: (a) UUC, TCACCTCAGAAAGGAGGC; U13, CTACTCA-

GACTCATCTGAGAAATGC; U18, CGTCGAGACTTGTGATACGAC-

CACU; U24, TTACAGATCTGTTGAGTATATTG; snR10, AGTTG-

ATTATCAATCTGCAAGCCGTC; snR30, TGGTTTTACCAAAATTA-

GACCTTGC; snR39, CAGTCGGAAGGTTGATATGTTAGC; snR41, CCCCTCTACAAGGTCTGAAACTTGCA; snR72, ATCAGAC-

TGGCTGCAAAATC.

To visualize the protein, we inserted 13 Myc epitope-encoding sequences between the last and stop codons of the GNO1 open reading frame (see “Experimental Procedures”) in a strain (MW3522) where the largest subunit of Pol I is tagged with three HA epitopes at its C terminus. Indirect immunofluorescence microscopic examination of the double-tagged strain in

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dicated that Gno1p was concentrated in the nucleolus with some staining in the nucleoplasm (Fig. 2). This localization is the one expected if Gno1p is involved in rRNA and/or snoRNA metabolism.

The G-patch of Gno1p is important for growth—In a systematic deletion analysis, Winzeler et al. (33) reported that GNO1 is an essential gene. However, when the EUROSCARF strain FVKT011 that is heterozygous for GNO1 (yggr280c-A16–769::kanMX4/YGR280c) was dissected, we found that two spores displayed wild-type growth, whereas two very small colonies appeared after 7 days of incubation at 30°C on YPD-rich medium (Fig. 3A). The kanamycin resistance phenotype segregated 2:2 together with the slow growth phenotype. Incubating a gno1Δ haploid segregant of FVKT011 at various temperatures from 16°C to 37°C did not further impair growth indicating that the phenotype was not conditional (data not shown). The generation time in YPD-rich medium of the heterozygous strain (Fig. 3A) reported by Winzeler et al. (33) because the deleted strain was not incubated long enough after dissection.

We wondered if, as in other yeast proteins bearing KK(E/D) repeats (32, 34, 35), this motif was dispensable in Gno1p for wild-type growth. As can be seen in Fig. 3B, removing the C-terminus of Gno1p beyond amino acid 150 (gno1Δ150–271) including the repeats impaired growth only very mildly. In contrast, removing the first 24 conserved residues of Gno1p produced a growth phenotype identical to that of the null mutant. Moreover, changing a conserved tryptophan of the G-patch (which is sometimes a tyrosine or phenylalanine in G-patches) to a serine strongly impaired growth (Fig. 3B). The G-patch domain can be functionally important.

Gno1p is involved in rRNA processing—The presence of the KK(E/D) repeats and the nucleolar localization, as in other proteins involved in rRNA maturation, suggested a similar role for Gno1p. During rRNA maturation, the 35 S Pol I transcript is rapidly cleaved at A0 and A1 sites to produce the 32 S precursor that is in turn cleaved at the A2 site, separating the 20 S precursor from the 27 S rRNA (Fig. 4). The 20 S precursor is then matured into 18 S rRNA. A number of endo- and exonucleolytic cleavages will produce the 25 S rRNAs and two different forms of the 5.8 S from the 27 S rRNA (see Ref. 7).

The maturation pathway was investigated by Northern blotting of total RNAs prepared from a wild-type and the gno1Δ strain (Fig. 5A), followed by probing with various oligonucleotides, the position of which is indicated in Fig. 5B. The deletion of GNO1 resulted in the accumulation of 35 S pre-rRNA, suggesting that the defect was not the consequence of a Pol I transcription defect. We observed the depletion of the 32 S pre-rRNA and the accumulation of the 23 S RNA, an aberrant processing intermediate. This phenotype was indicative of impaired cleavages at the A0, A1, and A2 sites (7). To further substantiate this diagnostic, we performed primer extension analysis with an oligonucleotide located in ITS2, between the C2 and E sites, to detect the intermediates cleaved at A2 and A3 (Fig. 5C). The 27 S A2/27 S A3 ratio decreased 3.5–4-fold as a result of the gno1Δ mutation, confirming an effect on the A2 cleavage. This observation suggested that an important fraction of the 35 S pre-rRNA was directly cleaved by the RNase MRP at the A3 site without processing at A0, A1, and A2. Many mutants in the processing of 35 S pre-rRNA produce 21 S and 22 S aberrant products, extending from A1 to A3 and from A0 to A2, respectively (7). The absence of such abnormal RNA in the gno1Δ strain indicated that A0, A1, and A2 cleavages were affected to similar levels. An increased level of 25 S rRNA relative to the 18 S rRNA was not observed in Northern blots because the amounts of these two species are those present in ribosomes of a slow but steadily growing strain.

The ratios of rRNA species relative to the tRNA Leu in the two strains were computed using the Northern blots (data not shown). In the mutant strain, the amounts of both the 25 S and the 18 S decreased 3.5-fold relative to the tRNA Leu, as did the 27 S A2/27 S A3 ratio. This observation indicated that the lower mature rRNA levels in mutant cells could be entirely accounted...
for by the pre-rRNA processing deficiency and was not the consequence of a Pol I transcription defect.

Several lines of evidence indicate that a proportion of 35 S pre-rRNA is normally processed in gno1-Δ. The presence of 27 S A2 and 20 S pre-rRNAs indicates that a fraction of the 35 S pre-rRNA is correctly cleaved at A0, A1, and A2 sites, although more slowly than in the wild-type strain as indicated by its accumulation. Previously, it was demonstrated that the 20 S pre-RNA is not the by-product of the 23 S RNA (36, 37). The downstream processing of the 20 S pre-RNA to the mature 18 S and of the 27 S pre-RNAs to mature 5.8 S and 25 S rRNAs is normal in the gno1-Δ strain.

Gno1p Is Involved in U18 and U24 snoRNA 3'-End Final Trimming—Because the A0, A1, and A2 cleavages are dependent on a number of snoRNAs (U3, U14, snR30, snR10), we tested their presence in the gno1-Δ mutant by Northern blot analysis. Deletion of GNO1 did not affect the amounts of these snoRNAs (data not shown). However, two snoRNAs, U18 and U24, were not properly processed, both being a few nucleotides longer at their 3'-end in the mutant (Fig. 6 and data not shown).

This phenotype is reminiscent of that of the rrp6-Δ exosome mutant (26) or of that of strains in which Nop5/58p or Nop1p are depleted (27, 38). Indeed, the U18 snoRNA extended form from rrp6-Δ and gno1-Δ strains co-migrated on polyacrylamide gel (Fig. 6A). It has been proposed that, in the course of intronic and polycistronic snoRNA maturation, the binding of the core factor snoRNP proteins Nop1p and Nop5/58p to the snoRNA allows the exosome complex to trim the 3'-end nucleotides through the action of Rrp6p (26). The effect on U18 and U24 snoRNAs is not due to the mislocalization or destabilization of Nop5/58p or Nop1p because the signal produced by green fluorescent protein (GFP) fusions of these proteins in the gno1-Δ mutant was as intense as in a wild-type strain and was correctly localized to the nucleolus (data not shown). We next wondered whether the deletion of GNO1 could affect the association of the snoRNP core factor proteins with the snoRNAs. Nop5/58p or Nop1p was TAP-tagged in wild-type or gno1-Δ strains. The fusions proteins were immunoprecipitated, and the presence of U18 was revealed by Northern blot. U18 immunoprecipitated with both Nop5/58p and Nop1p irrespective of the presence or absence of Gno1p (Fig. 6B). Moreover, both mature U18 snoRNA and its precursor were associated with Nop5/58p or Nop1p in gno1-Δ strain showing that Gno1p-dependent maturation of U18 snoRNA is not required for snoRNP binding.

Fig. 3. Phenotypes of gno1 deletion and point mutants. A, dissection of the GNO1/gno1-Δ FVT011 diploid strain. Slow growth of the spore always segregated with the kanamycin resistance kanR deletion marker. The spores were grown for 7 days at 30 °C on YPD medium. B, growth of wild-type and mutant gno1 strains. 10 μl of 10-fold serially diluted cultures of each strain were spotted on CAW medium and incubated for 2 days at 30 °C.

Fig. 4. Pre-rRNA processing pathway. The 35 S pre-rRNA transcript is cleaved sequentially at A0 and A1 sites in 5' ETS and then in ITS1 at the A2 site, producing the 20 S and 27 S A2 pre-rRNAs. The 20 S RNA is then cleaved at the D site yielding the mature 18 S. 85% of the 27 S A2 pre-rRNA is cleaved at the A3 site by RNase MRP and processed to give the 27 S A3. Exonucleolytic trimming of its 5'-end produces the 27 S B1L, which is processed at C1 and C2 to give the 25 S mature rRNA and the 7 S precursor of the 5.8 S rRNA. The 7 S is processed at its 3'-end by the exosome complex to yield the mature 5.8 S S1 rRNA. The remaining 15% of 27 S A2 are cleaved at B1L and then processed as the 27 S B1L producing the mature 25 S and 5.8 S S1 rRNAs.
The U18 and U24 snoRNAs incomplete 3’-end final trimming could be because of a defect in the exosome complex of which Gno1p might be a part. We thus tested whether gno1- affected the maturation of the 7S pre-rRNAs, as do bona fide exosome mutations (39). The 7S pre-rRNA extends to the processing site C2 of ITS2 at its 3’-end (Fig. 4). The exosome complex trims the 3’-extension of 7S pre-rRNA through an exonucleolytic activity. The depletion or deletion of subunits of the exosome leads to the appearance of aberrant intermediates migrating between the 7S and 6S pre-rRNAs. As seen in Fig. 6C, whereas an rrp6- mutation accumulated 7S pre-rRNA and 3’-end unprocessed intermediates between the 7S and 6S pre-rRNAs, no such precursor was observed in the gno1- strain even after long exposure of the Northern blot. Thus, Gno1p is not likely to be part of the exosome complex.

The deletion of the KK(E/D) repeats of Gno1p has a very mild effect on growth (Fig. 3B). However, the removal of the C-terminal nonessential end of Gno1p impaired the final trimming of the U18 3’-end (Fig. 6D) but not rRNA maturation (data not shown). This phenotype was similar to that of a GNO1 deletion indicating that the snoRNA and rRNA maturation defects can be separated, which suggested a role for the KK(E/D) repeats in U18 snoRNA processing. On the contrary, the G-patch is essential for all Gno1p functions because both processes are affected in the gno1-W38S mutant (Fig. 6D and data not shown).

Gno1p Is Not a Telomerase Repressor as Is Its Human Homolog, PinX1—During the course of this study, the putative human homolog of Gno1p was identified as a protein required for telomere length regulation (14). This protein, called PinX1 (GenBankTM/EBI accession number BAA91263.1; Fig. 1), was localized at telomeres, but the majority of the protein was found in the nucleoli. The telomeric role of PinX1 was investigated, and it was found to inhibit the telomerase activity in FIG. 5. Analysis of rRNA processing intermediates. A, Northern blot analysis of rRNA precursors. 10 μg of wild-type or gno1- total RNAs were separated on a denaturing agarose gel and transferred to a positively charged nylon membrane. The different pre-rRNA species were revealed by successive hybridization with 5’-32P-labeled oligonucleotides (see “Experimental Procedures” for their sequence and reference). The PhosphorImager autoradiographs revealing the different rRNA species are presented. The position of the rRNA precursors is indicated on the right side of the autoradiograph and the names of the oligonucleotide probes are shown on the left side. B, schematic positions of the hybridizing oligonucleotides used in Northern blot analysis and precursor and mature rRNA structures. Cleavage and processing sites are indicated by vertical bars. The mature rRNAs are denoted by black boxes and the hybridizing oligonucleotides by a solid bar. C, primer extension analysis of A2 and A3 cleavages. 5 μg of total RNA were used for reverse transcription analysis of wild-type and mutant strains. The reaction products were separated on an 8% denaturing polyacrylamide gel and autoradiographed. Primer extension with the oligonucleotide, hybridizing between C2 and E processing sites, reveals the 27S B1L, 27S B1M, 27S A2, and 27S A3 pre-rRNA precursors. The different elements are indicated using the same symbols as those used in A and B.

FIG. 6. Analysis of the effect of gno1 mutations on U18 and 7S to 6S pre-rRNA maturation and U18 snoRNP association. A, comparison of the U18 snoRNA maturation defect in gno1- and rrp6- strains. B, GNO1 deletion does not prevent Nop5/58p binding to U18. 2 mg of soluble proteins from strains containing Nop5/58p TAP-tagged or not, with or without gno1- were incubated with 200 μl of IgG-Sepharose beads. Total RNAs were extracted from the supernatant (S) and the bead pellets (P) and analyzed by Northern blotting. 25% of the RNAs of the supernatant and 90% of the RNAs from the pellet were loaded on the gel. C, comparison by Northern blot analysis of the 7S to 6S pre-rRNA processing in GNO1, gno1-, and rrp6- strains. A vertical bar indicates incorrectly processed forms of 7S pre-rRNA. D, effect of the gno1 mutations on U18 maturation.
vivo and in vitro. Accordingly, the depletion of endogenous PinX1 increases telomerase activity and leads to a large increase in telomere length. The PinX1 domain responsible for telomerase repression was mapped to the 74 carboxyl-terminal amino acids, a sequence which is divergent in yeast and human. We thus tested whether GNO1 mutations affected telomerase length in yeast. Genomic DNA from GNO1 mutant strains was extracted, digested, and analyzed by Southern blot hybridization (40). In contrast to PinX1 depletion, which leads to telomere elongation in human cells, the deletion of GNO1 in yeast led to a slight decrease in telomere length (Fig. 7). This mild telomere-shortening phenotype is complemented by gno1-Δ150–271, but not by the gno1-W38S nor by the gno1-Δ1–24 mutations that affect the Gno1p N-terminal conserved domain. These results indicate that the telomere length regulation function of PinX1 is not conserved in yeast Gno1p. The slight telomere length decrease is not due to lower amounts of telomerase RNA in the gno1-Δ strain because we found they were similar to those found in the wild-type strain (data not shown). This defect might result from a nonspecific defect due to the slow growth of the mutant strain. Nevertheless, these observations demonstrate that Gno1p does not act as a repressor of the telomerase activity in yeast.

**PinX1 Complements the rRNA Maturation Defect of gno1-Δ**—We designed two oligonucleotides to amplify the PINX1 open reading frame (from its start to stop codons) from a cDNA library constructed by Durfee et al. (28). The PCR fragment, whose sequence was identical to that found in the DNA data base, was cloned in the yeast multicopy expression vector pGEN (19). The construct was transformed in the gno1-Δ strain. It complemented the growth defect due to the deletion because the doubling time of the gno1-Δ strain transformed with pGEN-PINX1 was nearly identical to that of the wild-type strain (Fig. 8A). In agreement, PinX1 complemented the 25 S and 18 S rRNA defect (Fig. 8B) thus demonstrating that Gno1p rRNA processing function is conserved from yeast to human.

**DISCUSSION**

In this study, we have shown that Gno1p is required for the early processing of rRNAs. Additionally, deletion of the GNO1 gene impairs late steps of U18 and U24 snoRNA processing. Because Gno1p is related in sequence to the human PinX1 telomerase inhibitor (14), we tested the effect of the GNO1

![Fig. 7. Analysis of the effect of the gno1 mutations on telomere length.](image1)

![Fig. 8. The slow growth and rRNA processing defect of the deletion mutant is complemented by PINX1.](image2)
deletion on telomeres. A small decrease in telomere length, attributable to slow growth, was observed instead of the telomere length increase expected if Gno1p was a telomerase inhibitor in yeast. Most remarkably, the human PINX1 gene complemented the slow growth and the RNA maturation defect of the yeast GNO1 deletion. These results, together with the major localization of PinX1 in the nucleolus in human cells (14), strongly suggest that it has a dual function in telomere length regulation and rRNA processing.

Our study concluded that deletion of GNO1 impairs rRNA processing at A₀, A₁, and A₂ cleavages. This conclusion is supported by the accumulation of the 35 S pre-rRNA, the disappearance of the 32 S rRNA precursor, and the generation of the 23 S aberrant product. The 23 S RNA extends from the 5'-end of the 35 S pre-rRNA to the A₀ cleavage site, indicating that the A₀ cleavage occurs on the accumulated 35 S pre-rRNA rather than on the 27 S A₂. This conclusion is confirmed by the high ratio of the 27 S A₂ to 27 S A₀ pre-rRNAs in the mutant cells compared with that in the wild-type. We did not detect the 22 S and 21 S aberrant RNAs indicating that A₀, A₁, and A₂ cleavages are affected to similar levels. Nevertheless, approximately one-fourth of 35 S pre-rRNA follows the normal processing pathway to produce mature 5.8 S, 18 S, and 25 S rRNA allowing cell survival.

The defect in rRNA maturation caused by the deletion of GNO1 resembles that caused by the deletion of some snoRNP components. The aberrant 23 S RNA appears when U3 snoRNA or its associated proteins Nop1p and Nop5p/58p are depleted. However, the levels of the snoRNAs (U₃, U₁₄, snR₁₀, snR₃₀) or of Nop1p and Nop5p/58p implicated in the A₀, A₁, or A₂ cleavages were not affected in the gno-1Δ strain. Unexpectedly, in the gno-1Δ strain, the U₁₈ and U₂₄ snoRNAs, which are unessential guide snoRNAs implicated in base methylation of rRNAs, were extended by a few nucleotides at their 3’-end strain, as in a rpp6-Δ or in a Nop5p/58p or Nop1p-depleted strain (26). The defect in U₁₈ and U₂₄ snoRNA 3’-end trimming was not responsible for the rRNA processing defect because the deletion of the C terminus of Nop1p impaired exclusively the former process.

Several lines of evidence indicate that Gno1p acts in U₁₈ and U₂₄ snoRNA maturation after Nop5p/58p and Nop1p binding: (i) Nop5p/58p and Nop1p localized correctly in the nucleolus in the gno-1Δ strain (data not show); (ii) the snoRNAs are not depleted as is observed when Nop1p or Nop5p/58p are depleted (35); and (iii) Nop1p and Nop5p/58p did bind the premature form of U₁₈, as seen by immunoprecipitation of Nop1p-TAP and Nop5p/58p-TAP in the gno-1Δ strain. Moreover, Gno1p is probably not part of the exosome because it is not required for the trimming of the 7 S 3’-end to produce the 6 S pre-rRNA. Because we did not observe any snoRNA co-immunoprecipitation with Gno1p (U₁₈, U₁₄, U₃, and snR₄₉ were tested, data not shown), we propose that Gno1p might facilitate the action of the exosome for the final maturation of some snoRNAs by transient interactions with snoRNAs or snoRNPs.

A sequence comparison study identified a new eukaryote-specific motif that might be an RNA interacting domain (13). This domain, named the G-patch, is found in more than 100 proteins, often in association with known RNA-binding domains, suggesting an RNA binding activity or a capacity to mediate RNA-protein interaction. The most notable features of the 45-amino acid sequence of the G-patch domain are six highly conserved glycine residues and an aromatic residue most frequently a tryptophan at the position following the first conserved glycine. We have shown that the G-patch is essential in vivo for Gno1p RNA maturation activity, because the mutation of the consensus Trp-38 residue led to a phenotype identical to that of the null GNO1 allele. To date, this is the first experimental evidence that the G-patch domain is important for the function of a protein involved in RNA metabolism.

Gno1p has a C terminus containing six KK(E/D) repeats. This type of repeat is usually found in the homologous S. pombe proteins but not in those of multicellular organisms like C. elegans, D. melanogaster, or Homo sapiens where Lys-rich extensions are found instead. The repeats are shared with five other yeast proteins (Nop5p/58p, Cbf5p, Dbp3p, and A34), all nucleolar and all involved in RNA maturation except for A34, which is a Pol I subunit. In Gno1p, as in the other cases, the KK(E/D) repeats were found to be dispensable for growth and for RNA maturation. However, we found that in Gno1p the repeats were required for the final trimming of the 3’-end of some snoRNAs, a first indication for a role of these repeats.

As discussed previously, the role of Gno1p in rRNA processing and snoRNA maturation is convincingly supported by the experiments described in this work. However, recently, Zhou and Lu (14) clearly established a role for PinX1, the human homolog of Gno1p, in telomere length maintenance. PinX1 acts as an inhibitor of telomerase activity in vitro and in vivo. The depletion of PinX1 increases telomerase activity in vivo and leads to telomeres elongation. Moreover, Zhou and Lu (14) attributed all PinX1 inhibitor activity to its C terminus, which is not conserved in yeast. In yeast, the absence of Gno1p or the deletion of its nonconserved C terminus did not increase telomere length. Contrary to the phenotype expected if PinX1 and Gno1p were orthologous telomerase inhibitors, the GNO1 deletion and a null mutation of its G-patch led to a mild reduction in telomere length. This effect might be the indirect consequence of the mutant slow growth. We conclude that Gno1p does not act as a negative regulator of telomerase activity in yeast. Nonetheless, PinX1 complemented the growth and the RNA maturation defects due to the deletion of GNO1 (Fig. 8), which is consistent with its predominant nucleolar localization in human cells (14). Hence, we suggest that the RNA maturation function of Gno1p is evolutionarily conserved in PinX1, the human protein having a role both in telomerase regulation and rRNA processing.

Acknowledgments—We thank G. Peyrhoche, C. Carles M. Riva, O. Gadal, P. Thuriaux, V. van Mullem, P. Pineau, and A. Dejean for communicating unpublished results, C. Mann, M. Fromont-Racine, and B. Séraphin for providing strains and plasmids, G. Hautbergue, S. Marcand, and V. Bordas-Le Floch for help with experiments. We thank P. Thuriaux, S. Hermann-Le Dennat, S. Marcand, C. Mann, and A. Sentenac for suggestions to improve the manuscript.

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