Silencing of RNA Helicase II/Guα Inhibits Mammalian Ribosomal RNA Production*

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The intricate production of ribosomal RNA is well defined in yeast, but its complexity in higher organisms is barely understood. We recently showed that down-regulation of nucleolar protein RNA helicase II/Guα (RH-II/Guα or DDX21) in Xenopus oocytes inhibited processing of 20 S rRNA to 18 S and contributed to degradation of 28 S rRNA (Yang, H., Zhou, J., Ochs, R. L., Henning, D., Jin, R., and Valdez, B. C. (2003) J. Biol. Chem. 278, 38847–38859). Since no nucleolar RNA helicase has been functionally characterized in mammalian cells, we used short interfering RNA to search for functions for RH-II/Guα and its paralogue RH-II/Guβ in rRNA production. Silencing of RH-II/Guα by more than 80% in HeLa cells resulted in an almost 80% inhibition of 18 and 28 S rRNA production. This inhibition could be reversed by expression of wild type RH-II/Guα. A helicase-deficient mutant form having ATPase activity was able to rescue the production of 28 S but not 18 S rRNA. A phenotype exhibiting inhibition of 18 S and 28 S rRNA production was also observed when the paralogue RH-II/Guβ was overexpressed. Both down-regulation of RH-II/Guα and overexpression of RH-II/Guβ slowed cell proliferation. The opposite effects of the two paralogues suggest antagonistic functions.

The use of short interfering RNA (siRNA) has revolutionized the way in which the functions and interactions of multiple genes and their encoded proteins are deciphered. Short double-stranded RNA mediates the recognition and degradation of its homologous RNA, leading to the down-regulation of expression of the target gene; the mechanism involves a multiprotein complex (1–3). The application of siRNA facilitates the study of complex cellular processes including the biogenesis of ribosomal RNA (rRNA).

Synthesis and processing of pre-rRNA, maturation of the final products, and formation of ribosomes occur in the nucleolus. The identification of ~350 nucleolar proteins (4, 5) and the use of siRNA to silence each encoding gene provide the genetic and biochemical screenings that are necessary to elucidate the mechanism of rRNA production in mammalian cells, a process that is more defined in yeast (6–8). A number of nucleolar proteins have been functionally associated with mammalian rRNA production mostly based on in vitro experiments and indirect in vivo observations (9–13). More direct lines of evidence are limited to Bop1 (14), p19orf (15), and the DCK1 gene product dyskerin (16, 17). Although our laboratory recently showed that antisense-mediated down-regulation of RNA helicase II/Guα in Xenopus oocytes resulted in inhibition of production of 18 S rRNA and degradation of 28 S rRNA (18), we have not reported that this enzyme has a similar function in mammalian cells.

RNA helicase II/Guα (RH-II/Guα or DDX21) is a DEAD box nucleolar protein that we previously cloned and characterized (19, 20). The cDNA-derived amino acid sequence shows RNA helicase motifs at the middle region, which is responsible for its 5′ to 3′ RNA unwinding activity, and a functionally distinct domain at its C terminus that is able to introduce secondary structure to single-stranded RNA (21). Its paralogue, RNA helicase II/Guβ, has a marginal RNA helicase activity in vitro, and it does not possess an RNA folding activity (22). The high homology in the structures of the two paralogues and close proximity of their genes on human chromosome 10 suggest evolution by gene duplication (23). Their nucleolar localization suggests a functional relationship with the production of rRNA.

To gain more information about the roles of RH-II/Guα and RH-II/Guβ in rRNA biosynthesis, we employed the use of siRNA and overexpression of their wild type or negative mutant forms. Down-regulation of RH-II/Guα slowed the production of 18 and 28 S rRNA. These effects were reversed when wild type RH-II/Guα was overexpressed, whereas inducible expression of a helicase-deficient mutant reversed the inhibitory effects on 28 S rRNA but not on 18 S rRNA production. siRNA-mediated silencing of RH-II/Guβ did not inhibit rRNA biosynthesis, but overexpression of its wild type form inhibited rRNA production. In addition to affecting rRNA production, silencing of RH-II/Guα or overexpression of RH-II/Guβ resulted in inhibition of cell proliferation. The results suggest antagonistic roles of the two paralogues in rRNA production and cell proliferation.

EXPERIMENTAL PROCEDURES

Cell Culture and Transfection—HeLa cells were grown in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum, 100 IU/ml penicillin G, and 100 μg/ml streptomycin sulfate. LAP3 cells are genetically modified NIH3T3 cells that are capable of expressing exogenous genes upon IPTG induction as previously described (24). These cells were grown in similar medium containing 10% newborn calf serum.

Cells were transfected with synthetic siRNA oligoribonucleotides (Dharmacon Research, Inc.) using LipofectAMINE 2000 (Invitrogen). The siRNAs used are listed in Table I.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)—Total RNAs were isolated from monolayer cells using TRIzol reagent (Invitrogen). RT-PCR was done using the OneStep RT-PCR kit (Qiagen). A 20-μl reaction mixture contained 1× buffer, 0.4 mM dNTPs, 50 ng of
RNA. 0.8 μl of OneStep RT-PCR enzyme mix, 1.5 nm [32P]-end-labeled primer 1, and 1.5 nm primer 2. Primers for internal control were also added in the reaction. β-Actin primers were initially used as internal control, but multiple bands were obtained. Better results were obtained using primers for U1 small nuclear RNP-specific C protein mRNA (U1RNPC; GenBankTM accession number X12517). PTC-100 Programmable Thermal Controller (MJ Research, Inc.) was used to do reverse transcription at 50 °C for 30 min followed by 95 °C heating for 15 min to denature reverse transcriptase and activate HotStarTaq DNA polymerase. The succeeding 20 cycles of PCR included denaturation at 94 °C for 0.5 min, annealing at 55 °C for 1 min, and extension at 72 °C for 1 min. A final extension reaction was done at 72 °C for 10 min.

The RT-PCR product was mixed with 2 μl of Bluej ice (Invitrogen), and 6 μl of the mixture was analyzed using 5% polyacrylamide–SDS gel. After electrophoresis at 100 V, the gel was vacuum-dried and exposed to a PhosphorImager screen. All radioactive signals were measured using the Amersham Biosciences Storm 860 PhosphorImager. The primers used for RT-PCR are shown in Table II.

Western Blot—Cells were lysed in Laemmli buffer and boiled for 3 min. Protein extracts were loaded onto 10% polyacrylamide–SDS gels and blotted onto Immobilon polyvinylidene difluoride membrane (Bio-Rad). Immunoblot analysis by chemiluminescence was done using the ECL plus Western Blotting Detection System (Amersham Biosciences). Incubation at 37 °C with 5% CO2 was continued for 4 h. Cells were then washed twice with a growth medium. The chase experiment was done by incubating the labeled cells in a growth medium containing 50 mM Na2HPO4 at 0, 2, and 4 h. Total RNA was isolated using TRIzol Reagent (Invitrogen), and the RNA concentration was determined at 260 nm. Equal weight of RNA was resolved on a 1% agarose gel and blotted onto a nylon membrane (Amersham Biosciences). The membrane was dried and placed in a PhosphorImager cassette for 4 h overnight. After analysis, the RNA blot was stained with 0.2% methylene blue to visually check equal loading.

Preparation of LAP3 Stable Clones—All human and mouse RH-II/Gu cDNAs (20, 23, 26) were subcloned into the NheI and BspEI sites of pX12 vector. Both wild type and mutant forms (21) were amplified by PCR using the primers listed in Table III.

Oligonucleotides BV596 and BV597 were used to amplify human RH-II/Gu, and BV508 and BV599 were used for its mouse orthologue. The mouse RH-II/Guβ cDNA was amplified using BV777 (5’-CCACCCGCAGGCATGGCCATACAAAGACGTCGACCAGACGACGCCACAACCATCCTCTGCGGACAGCATA-3’) and BV778 (5’-AGATTAACTATCTCCGGGATGATCAAAATTCCGTTTATGAGCC-3’). BV596, BV598, and BV777 encode a FLAG epitope. Transfection, selection, and IPTG induction of LAP3 cells were as previously described (27).

Proliferation Assay—HeLa cells, which were transfected two times (24-h interval) with 10 nM siRNA for a total of 48 h, were trypsinized and counted. Equal cell numbers were replated into new 6-well plates for 72 h. Each well contained 50,000 or 100,000 cells after replating. Cells were again trypsinized, and all samples were diluted with equal volumes of medium for replating into 96-well plates, such that sample wells would have a total volume of 100 μl and 10,000 or fewer cells each.

A standard curve was generated by serial dilution of untreated HeLa cells, wherein the maximum was 20,000 cells per well and the minimum was 625. Plated cells were allowed to recover and attach for 4 h at 37 °C and 5% CO2. The CellTiter 96 Aqueous One Solution cell proliferation assay kit (Promega) was used to assay for living cells. After an incubation time of 30 min to 1 h at 37 °C, the plates were read on an enzyme-linked immunosorbent assay reader at 490 nm.

RESULTS

siRNA-mediated Down-regulation of RH-II/Guα—This study involved the use of both human and mouse cell lines. We designed siRNA to target both human and mouse RH-II/Guα as well as siRNAs that were species-specific. Down-regulation was examined at the mRNA and protein levels. Preliminary experiments indicated that 20 cycles of RT-PCR correlated linearly with the levels of mRNAs, and the use of U1RNPC as a loading control was better than β-actin.

Treatment of HeLa and LAP3 cells with 0–100 nM si-934 resulted in a decrease in the level of RH-II/Guα mRNA in both cell lines in a dose-dependent manner. When 10 nM si-934 was used, the human homologue decreased by 75%, whereas the mouse homologue decreased by 55% (Fig. 1, A and B). An siRNA that specifically targets mouse RH-II/Guα, si-935, silenced RH-II/Guα by 75% (Fig. 1B) without affecting the human orthologue (Fig. 1A). si-936, which was specific to human RH-II/Guα, decreased RH-II/Guα mRNA in HeLa cells by 45% with a minimal effect on the level of mRNA of the mouse enzyme. All three siRNAs did not significantly affect the level of RH-II/Guβ in HeLa and LAP3 cells (Fig. 1, A and B).

A significant decrease in the protein level of mouse RH-II/Guα was observed using 100 nM si-934 and 100 nM si-935, which resulted in 76 and 89% silencing, respectively (Fig. 1C). This decrease in the protein level correlates with the decrease in the mRNA level (Fig. 1B). Because of the efficacies of si-934 and si-935, we decided to use these siRNAs in the succeeding experiments.

To determine the kinetics of down-regulation of the human RH-II/Guα at the mRNA and protein levels, samples at different time points were analyzed by RT-PCR and Western blotting. Fig. 2 shows a 50% decrease in the mRNA level within 4 h after transfection without any effect on the protein level. The mRNA level continued to decrease to 10% until 72 h, and a significant decrease in the protein level was not observed until 48 h after transfection (Fig. 2B). The delayed effect of si-934 on the protein level of RH-II/Guα is attributed to a longer half-life of the protein. Determination of the time when the protein level decreased was essential in the study of its physiological function.

Down-regulation of RH-II/Guα Inhibits Production of rRNA—We hypothesized that down-regulation of RH-II/Guα would result in an aberrant processing of rRNA. After 48 h of siRNA transfection, cells were pulse-labeled with [32P]orthophosphate and then chased with unlabeled phosphate-enriched medium. As a negative control, an siRNA with scrambled si-934 sequence was included (si-934Scr). RT-PCR analysis of total RNAs taken 4 h after the chase began showed a 92%
decrease in RH-II/Gu in HeLa cells treated with si-934. Mock transfection and si-934Scr did not affect the level of RH-II/Gu mRNA (Fig. 3A).

Both mock-transfected and si-934Scr-treated cells showed normal progression in the processing of pre-rRNA within 4 h of chase (Fig. 3B). The low $^{32}$P activity at 0 h of chase would indicate that little labeled phosphate taken up by the cells had been incorporated into rRNA. Within 2 h, the $^{32}$P labeling of RNA increased, and the positions of 47 S/45 S pre-rRNA and 32 S, 28 S, and 18 S rRNA were visualized. Treatment of cells with si-934, which caused a dramatic decrease in RH-II/Gu mRNA (Fig. 3A), resulted in an inhibition of rRNA production (Fig. 3B). The decrease in the largest visible precursor, 47 S/45 S, was not as marked as the decrease in the final products, 28 and

| ID    | Sequence                                                                 | Position               |
|-------|--------------------------------------------------------------------------|------------------------|
| BV993 | 5'-CAAACTAGATCCTACAAACTAA-3'                                             | Human Gu nt 1031-1054  (sense) |
| BV994 | 5'-TGAGGTGCTACATGTTAGCTGTGTG-3'                                         | Human Gu nt 1272-1295  (antisense) |
| BV998 | 5'-TTCTTCTTGTCTACAAACTAA-3'                                             | Human Gu nt 683-689    (antisense) |
| BV459 | 5'-GAAAAGAAATTTGAGAGACAGCTGACACT-3'                                     | Mouse Gu nt 333-367    (sense) |
| BV985 | 5'-TCCCTTCTTGTCTACAAACTAA-3'                                             | Mouse Gu nt 793-819    (antisense) |
| BV986 | 5'-GGAATTTGAGAGACAGCTGACACT-3'                                          | Mouse Gu nt 329-355    (sense) |
| BV987 | 5'-GGAATTTGAGAGACAGCTGACACT-3'                                          | Mouse Gu nt 718-744    (antisense) |
| U1C'  | 5'-GCACACATGCCAAGTTTTATTGTG-3'                                           | Human U1RNPC nt 11-34  (sense) |
| U1C3' | 5'-ATCACCATCTCAGCGTGAGCT-3'                                              | Human U1RNPC nt 477-500 (antisense) |
| BV974 | 5'-AGGATCTACATTTTATTTTAGA-3'                                             | Mouse U1RNPC nt 31-56  (sense) |
| BV976 | 5'-TTCTCTCTTCAAAAATTTAATGTTA-3'                                         | Mouse U1RNPC nt 625-650 (antisense) |

**Table II**

**Primers used for RT-PCR**

| ID    | Sequence                                                                 | Position               |
|-------|--------------------------------------------------------------------------|------------------------|
| BV993 | 5'-CAAACTAGATCCTACAAACTAA-3'                                             | Human Gu nt 1031-1054  (sense) |
| BV994 | 5'-TGAGGTGCTACATGTTAGCTGTGTG-3'                                         | Human Gu nt 1272-1295  (antisense) |
| BV998 | 5'-TTCTTCTTGTCTACAAACTAA-3'                                             | Human Gu nt 683-689    (antisense) |
| BV459 | 5'-GAAAAGAAATTTGAGAGACAGCTGACACT-3'                                     | Mouse Gu nt 333-367    (sense) |
| BV985 | 5'-TCCCTTCTTGTCTACAAACTAA-3'                                             | Mouse Gu nt 793-819    (antisense) |
| BV986 | 5'-GGAATTTGAGAGACAGCTGACACT-3'                                          | Mouse Gu nt 329-355    (sense) |
| BV987 | 5'-GGAATTTGAGAGACAGCTGACACT-3'                                          | Mouse Gu nt 718-744    (antisense) |
| U1C'  | 5'-GCACACATGCCAAGTTTTATTGTG-3'                                           | Human U1RNPC nt 11-34  (sense) |
| U1C3' | 5'-ATCACCATCTCAGCGTGAGCT-3'                                              | Human U1RNPC nt 477-500 (antisense) |
| BV974 | 5'-AGGATCTACATTTTATTTTAGA-3'                                             | Mouse U1RNPC nt 31-56  (sense) |
| BV976 | 5'-TTCTCTCTTCAAAAATTTAATGTTA-3'                                         | Mouse U1RNPC nt 625-650 (antisense) |

**Table III**

**Primers used for PCR of wild type and mutant RH-II/Gu**

| ID    | Sequence                                                                 | Position               |
|-------|--------------------------------------------------------------------------|------------------------|
| BV993 | 5'-CAAACTAGATCCTACAAACTAA-3'                                             | Human Gu nt 1031-1054  (sense) |
| BV994 | 5'-TGAGGTGCTACATGTTAGCTGTGTG-3'                                         | Human Gu nt 1272-1295  (antisense) |
| BV998 | 5'-TTCTTCTTGTCTACAAACTAA-3'                                             | Human Gu nt 683-689    (antisense) |
| BV459 | 5'-GGAATTTGAGAGACAGCTGACACT-3'                                          | Mouse Gu nt 333-367    (sense) |
| BV985 | 5'-TCCCTTCTTGTCTACAAACTAA-3'                                             | Mouse Gu nt 793-819    (antisense) |
| BV986 | 5'-GGAATTTGAGAGACAGCTGACACT-3'                                          | Mouse Gu nt 329-355    (sense) |
| BV987 | 5'-GGAATTTGAGAGACAGCTGACACT-3'                                          | Mouse Gu nt 718-744    (antisense) |
| U1C'  | 5'-GCACACATGCCAAGTTTTATTGTG-3'                                           | Human U1RNPC nt 11-34  (sense) |
| U1C3' | 5'-ATCACCATCTCAGCGTGAGCT-3'                                              | Human U1RNPC nt 477-500 (antisense) |
| BV974 | 5'-AGGATCTACATTTTATTTTAGA-3'                                             | Mouse U1RNPC nt 31-56  (sense) |
| BV976 | 5'-TTCTCTCTTCAAAAATTTAATGTTA-3'                                         | Mouse U1RNPC nt 625-650 (antisense) |

**Fig. 1.** Specificity of the siRNA-mediated down-regulation of RH-II/Gu mRNA. Human HeLa (A) and mouse LAP3 (B) cells were transfected with different concentrations of siRNAs (0–100 nM). Total RNA was isolated after 48 h and analyzed by RT-PCR. Each reaction contained three pairs of primers to reverse transcribe mRNA and amplify cDNA fragments for U1 small nuclear RNP-specific C protein (U1C), RH-II/Gu (Gu), and RH-II/Gu (Gu). One primer in each pair was $^{32}$P-end-labeled. BV993, BV994, BV998, BV999, U1C', and U1C3' were used for HeLa RNA (A); BV459, BV985, BV986, BV987, BV974, and BV976 were used for LAP3 RNA (B). RT-PCR products were resolved on 5% polyacrylamide gels. A quantitative analysis of four independent experiments is shown on the right. C, a parallel experiment using LAP3 cells was conducted to analyze changes in the protein level of mouse RH-II/Gu after siRNA treatment. h, human; m, mouse.
18S rRNA. We sometimes observed an accumulation of the 47S/45S rRNA. This inconsistency could be due to inefficient transfer from gel to the membrane. Compared with mock, the use of si-934 resulted in an 83 and 82% decrease in the level of 28 and 18S rRNA, respectively, after a 2-h chase (Fig. 3B). The level of 32S rRNA decreased to a lesser extent. For example, after a 2-h chase, the level of 32S decreased by 45% (Fig. 3B, lane 5). After a 4-h chase, the level decreased by 28%, whereas 28 and 18S rRNA decreased by 74 and 76%, respectively. The results suggest that the production of 28 and 18S rRNA was affected by the down-regulation of RH-II/Gua compared with the production of 32S rRNA. The observed inhibition of rRNA production mediated by down-regulation of RH-II/Gua is similar to the effects caused by inactivation of the *DKC1* gene that encodes dyskerin, a putative pseudouridine synthase that is relevant in rRNA modification (17).

To prove equal loading of RNA, the membrane was stained with methylene blue. Only the final products, 28 and 18S rRNA, which were present in greater proportion, were stained strongly (Fig. 3B, right panel).

**Wild Type RH-II/Gua but Not Its Helicase Mutant Form Rescues Inhibition of rRNA Production**—RH-II/Gua is one of only a few RNA helicases that has RNA unwinding activity *in vitro*. This group includes, but is not limited to, p68 and p72 (28), RNA helicase A/NDH II (29, 30), eIF-4A (31), Prp22 (32), DbpA (33), NPH II (34), NS3 (35), and SV40 large T antigen (36). To determine whether or not the helicase activity of RH-II/Gua was critical for the production of rRNA, we constructed stable LAP3 clones (mouse cells) that expressed wild type and mutant forms of human RH-II/Gua upon IPTG induction. We previously showed that the RH-II/Gua SAT-M1 mutant, wherein the SAT motif was mutated to LET, did not exhibit RNA unwinding activity *in vitro* despite the presence of ATPase activity (21). Cells were first induced with IPTG for
48 h prior to treatment with si-935, a mouse RH-II/Gua-specific siRNA. Fig. 4A shows expression and nucleolar localization of the human RH-II/Gua wild type and SAT mutant upon IPTG induction.

Our purpose was to knock down the endogenous mouse RH-II/Gua while exogenously expressing the human orthologue and look for reversal of inhibition of rRNA production. We already showed in Fig. 1 that si-935 inhibits expression of mouse RH-II/Gua by 75% without a significant effect on human RH-II/Gua. Fig. 4B shows that treatment of a vector clone with si-935 resulted in 43% decrease in 28 and 18 S rRNA after a 4-h chase. This effect was reversed by exogenous expression of wild type RH-II/Gua, suggesting that the human orthologue can functionally replace the mouse enzyme (Fig. 4B, lanes 2 and 4). To our surprise expression of the human RH-II/Gua SAT-M1 mutant rescued the inhibitory effects on 28 S rRNA but not on 18 S rRNA (compare lanes 2 and 6 in Fig. 4B). This result suggests that the unwinding activity of RH-II/Gua is critical for biosynthesis of 18 S rRNA. Other mechanisms might play a role in the involvement of RH-II/Gua in the production of 28 S rRNA.

One can argue that the differences we observed might be due to unequal RNA loading on the gels, although methylene blue staining shows otherwise (Fig. 4B, right panel). To circumvent this argument, we compared the ratios of 28 to 18 S rRNA. Whereas these ratios are equal to 1.3 and 1.6 in the control and wild type human RH-II/Gua clones, the SAT-M1 mutant ratios were 2.0 and 2.4 in the absence or presence of siRNA, respectively, suggesting a decrease in 18 S rRNA in the mutant clone relative to 28 S rRNA.

The same RNA samples were analyzed for the levels of mouse and human RH-II/Gua by RT-PCR (Fig. 4C). Treatment of the vector clone with si-935 resulted in 78% decrease in mouse RH-II/Gua mRNA (lane 2). However, the efficacy of si-935 decreased when human RH-II/Gua (wild type or SAT mutant) was ectopically expressed (compare mouse Gua (mGua) in lanes 2, 4, and 6 in Fig. 4C). It is possible that human and mouse RH-II/Gua mRNAs competed for si-935 in a mouse cell line, but Fig. 1A shows that si-935 does not have an effect on human RH-II/Gua mRNA in HeLa cells. It is more likely that the ectopically expressed human RH-II/Gua stimulated the expression of the endogenous mouse RH-II/Gua as shown in lanes 3 and 5 (Fig. 4C). Apparently, the helicase activity of human RH-II/Gua is not required for this stimulation, since both wild type (WT) and mutant (SAT-M1) RH-II/Gua have similar effects.

**Down-regulation of RH-II/Gua Blocks Cell Proliferation**—After treatment of HeLa cells with si-934, we observed a slowed proliferation rate. We decided to examine the growth rate of the surviving cells. Fig. 5 shows a 55% reduction in relative growth rate of cells treated with si-934, whereas cells treated with si-934Scr showed only 10% reduction relative to the mock-transfected cells. Statistical analyses using Student’s t test showed a significant difference (p < 0.001) between si-934 treatment and mock or si-934Scr transfection. Examination of the mRNA and protein levels of RH-II/Gua shows almost com-

![Figure 4](image-url)
complete silencing (Fig. 5, B and C). The difference in magnitude between decreases in growth rate and the level of RH-II/Gua suggests a possible compensatory mechanism.

Analysis by flow cytometry of cells treated with si-934 did not reveal any specific cell cycle arrest (data not shown). The results suggest a slowing down of cellular metabolisms.

Overexpression of RH-II/Guβ Inhibits rRNA Production—It is possible that down-regulation of RH-II/Guβ could result in stimulation of expression and/or enhancement of the enzymatic activity of RH-II/Guα, compensating for the lost activity of RH-II/Guα. After treatment of HeLa cells with si-934, which specifically targets RH-II/Guα, the mRNA level of RH-II/Guβ increased 15% (Fig. 1A). We thought that this increase was significant enough to compensate for the decrease in RH-II/Guα and that this plan would be down-regulate expression of endogenous mouse RH-II/Guα with si-935 and look for rescue by expression of exogenous mouse RH-II/Guβ. To our surprise, overexpression of mouse RH-II/Guβ (Fig. 6A) inhibited the production of rRNA even without down-regulation of the α form (Fig. 6B, lane 6). The rRNA production in a clone overexpressing RH-II/Guα is similar to the vector clone treated with IPTG (Fig. 6B, lanes 2 and 4). This is consistent with the absence of effects on rRNA production when RH-II/Guβ is silenced to 20% with siRNA (data not shown). Another phenotype seen after overexpression of RH-II/Guβ is a decreased cell proliferation (data not shown), which would be expected when rRNA production is inhibited. Overall, up-regulation of the β form, or down-regulation of the α form, inhibits rRNA production and cell proliferation, suggesting an antagonistic relationship between the two paralogues.

DISCUSSION

The general mechanisms of pre-rRNA processing in mammals and frog are similar. Both involve sequential cleavages of the primary and intermediate precursors leading to the production of 28, 18, and 5.8 S rRNAs (37, 38). The general mechanism is in fact highly conserved through evolution despite minor differences as far as the functions of the factors involved are concerned.

We recently showed that antisense-mediated down-regulation of RH-II/Guα in Xenopus oocyte resulted in inhibition of production of 28 and 18 S rRNAs (18). In that study, accumulation of 20 S rRNA and the appearance of 28 S degradation products were observed, suggesting that RH-II/Guα is involved in the processing of 20 S rRNA to 18 S rRNA and helps stabilize 28 S rRNA. The present study was undertaken to address whether or not mammalian RH-II/Guα has physiological functions similar to the metazoan orthologue. siRNA-mediated down-regulation of the human and mouse RH-II/Guα resulted in a decreased 28 and 18 S rRNA production. However, we did not observe either accumulation of 20 S intermediate product or 28 S degradation in the present study.

It would not surprise us if the mode of actions of human and frog RH-II/Guα are fundamentally different. For example, mammalian nucleolin/C23 is believed to be involved in pre-rRNA processing and assembly of ribosomal proteins and rRNAs (39, 40). Its yeast homologue, Nas1p, is also required for efficient pre-rRNA processing in yeast (41). Whereas C23 has been shown to be a component of isolated ribonucleoprotein particles involved in pre-rRNA processing (42, 43), Nas1p has never been identified in yeast preribosomal complexes. Moreover, mammalian nucleolin cannot complement the yeast nsr1-delta mutant (44), suggesting differences in their mode of actions.

What, then, could be a possible mechanism for the observed correlation between down-regulation of RH-II/Guα and inhibition of mammalian rRNA production? RH-II/Guα has RNA unwinding activity, and it can also introduce secondary structure to single-stranded RNA, two opposing enzymatic activities exhibited by other RNA helicases including p68 and p72 (28). The RNA unwinding activity of RH-II/Guα, but not its ATPase,
is abolished by mutating its SAT motif to LET, which enhanced its RNA folding activity (21). The same SAT mutant did not rescue the siRNA-mediated inhibition of 18 S rRNA production (Fig. 4B), suggesting the relevance of its RNA helicase activity. On the other hand, this mutant did reverse the siRNA-mediated inhibition of 28 S, suggesting that its RNA helicase activity may not be as critical as its ATPase, RNA folding activity, and/or interaction with other nucleolar factors for the production of 28 S rRNA. The ATPase activity of RH-II/Guβ might be critical in the formation and reorganization of ribonucleoprotein complexes required for 28 S rRNA production similar to the relevance of ATPase activity in the disruption of U1A-RNA interaction by RNA helicase NPH-II (45). The enhanced RNA folding activity of SAT-M1 mutant might contribute to this reorganization. As we hypothesized in the frog oocyte studies, folding activity of SAT-M1 mutant might contribute to this interaction by RNA helicase NPH-II (45). The enhanced RNA folding activity of c-Jun, then repression of cell growth and proliferation. If the effects of si-934 on c-Jun-mediated transcriptions of genes involved in cell proliferation and death (49) remain to be shown. The effects of RH-II/Guβ on the expression of c-Jun-activated genes are also unknown.

In summary, silencing RH-II/Guα might have double-edged effects, either inclusive or exclusive. A negative effect on rRNA production would result in decreased protein synthesis and, consequently, inhibition of cell growth and proliferation. If the effects of si-934 were through inhibition of the growth-promoting activity of c-Jun, then repression of cell growth and proliferation might send stress signals to down-regulate rRNA biosynthesis and turnover of cytoplasmic ribosomes (50). Both pathways are consistent with our observations. A better understanding of the details of underlying mechanisms should provide us with knowledge of the intricate relationship between ribosome biogenesis and proliferation signals.

Acknowledgment—We thank Dr. Heidi L. Weiss for the statistical analyses.

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