c-Jun Supports Ribosomal RNA Processing and Nucleolar Localization of RNA Helicase DDX21*³

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The molecular mechanisms by which the AP-1 transcription factor c-Jun exerts its biological functions are not clearly understood. In addition to its well established role in transcriptional regulation of gene expression, several reports have suggested that c-Jun may also regulate cell behavior by non-transcriptional mechanisms. Here, we report that small interfering RNA-mediated depletion of c-Jun from mammalian cells results in inhibition of 28 S and 18 S rRNA accumulation. Moreover, we show that c-Jun depletion results in partial translocation of RNA helicase DDX21, implicated in rRNA processing, from the nucleolus to the nucleoplasm. We demonstrate that DDX21 translocation is rescued by exogenous c-Jun expression and that c-Jun depletion inhibits rRNA binding of DDX21. Furthermore, the direct interaction between c-Jun and DDX21 regulates nucleolar localization of DDX21. These results demonstrate that in addition to its transcriptional effects, c-Jun regulates rRNA processing and nucleolar compartmentalization of the rRNA processing protein DDX21. Thus, our results demonstrate a nucleolar mechanism through which c-Jun can regulate cell behavior. Moreover, these results suggest that the phenotypes observed previously in c-Jun-depleted mouse models and cell lines could be partly due to the effects of c-Jun on rRNA processing.

The AP-1 transcription factor c-Jun has an important role in the positive regulation of G₁/S cell cycle progression, cancer cell proliferation, and survival (1, 2). However, recent studies have indicated that some of the cellular functions of c-Jun could be mediated through non-conventional mechanisms distinct from its function in transcriptional regulation of AP-1-mediated gene expression. For example, it was demonstrated that c-Jun mutants incapable of inducing AP-1-mediated gene expression potently protect PC12 cells from apoptosis (3). Also, recent work regarding the pathogenesis of human cancer showed no evidence for the regulation of c-Jun target genes in tumors with c-Jun overexpression, due to an amplification of the c-jun locus (4).

To search for molecular mechanisms by which c-Jun regulates cell behavior, we previously purified a c-Jun associating protein complex from human 293 cells. Interestingly, this protein complex consists of several nucleolar proteins (5, 6). On the other hand, Wagner and co-workers (7) reported recently that the G₁/S cell cycle arrest observed in c-Jun-deficient mouse liver cells is similar to that observed in 40 S ribosomal protein S6-deficient mice (8), which display compromised ribosome biogenesis. In addition, c-Jun protein has been shown to localize to the perinucleolar area of cells where it colocalizes with the nucleolar protein DNA topoisomerase I (5, 9, 10). Taken together, these results suggest that in addition to its well established effects on gene transcription, c-Jun might have as of yet unidentified nucleolar functions.

In this study we demonstrate that in addition to its function as a prototypical transcription factor, c-Jun regulates nucleolar compartmentalization and rRNA binding of DDX21. We show that c-Jun depletion results in partial nucleoplasmic translocation of DDX21, decreased binding of DDX21 to rRNA, and inhibition of 28 S and 18 S rRNA accumulation. Moreover we show that depletion of DDX21 inhibits G₁/S cell cycle progression, suggesting that the G₁/S cell cycle effects of c-Jun depletion may be partly due to interference with DDX21 function.

EXPERIMENTAL PROCEDURES

Cell Culture and Transfections—HeLa and HT-1080 cells were obtained from ATCC. Two independent strains of
c-Jun and DDX21 were depleted by pooling the respective siRNA oligos to a final concentration of 200 nM. Cells were transfected with EGFP-DDX21, PS-CFP2-DDX21, EGFP-B23, and HA-c-Jun expression constructs using FuGENE-assisted transfection (Roche Applied Science). 24 h after transfection, cells were processed for either immunofluorescence or Western blotting. Transfection of HT-1080 and HeLa cells with siRNA duplex oligonucleotides was performed using Oligo-fectamine (Invitrogen). The following sense siRNA sequences were used for targeting human c-Jun: DDX21, 5′-GAUCCUG-AACAGAGCAUGTT-3′ and 5′-AAGCAGCACGUUGCACACATT-3′; and si-934, 5′-AAGACAGCGUGCAGGAGATT-3′ and 5′-GUGUGCAAGAAGAAGAATT-3′ (13). c-Jun and DDX21 were depleted by pooling the respective siRNA oligos to a final concentration of 200 nM. In all experiments, a scrambled duplex (5′-GUAAACAUUGAGACACGGCTT-3′) was used as a control. Cells were processed 48 or 72 h after transfection. Information about cloning of mammalian expression constructs used in this study is available upon request.

**Immunofluorescence Microscopy and Photoswitch Experiments**—Immunostaining of cells grown on coverslips was performed by simultaneous fixation and permeabilization in 20 mM PIPES, pH 6.8, 4% formaldehyde, 0.2% Triton X-100, 10 mM EGTA, and 1 mM MgCl₂ for 10 min at room temperature. All fixed cells were incubated for 30 min at room temperature in blocking solution (phosphate-buffered saline, 3% fetal calf serum). Primary antibodies used were rabbit monoclonal c-Jun (Signal Transduction), human polyclonal DDX21 (14), and mouse monoclonal B23 (Santa Cruz Biotechnology). Primary antibodies were detected with Cy2- or Cy3-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories). All antibody incubations were carried out for 90 min at room temperature. Wide-field fluorescence microscope images were collected using a Leica DMRE microscope equipped with a high resolution Hamamatsu Photonics ORCA C4742-95 CCD camera. Fluorescence intensities were determined using MetaMorph imaging software (Universal Imaging). Relative nucleoplasm/nucleolar distribution of endogenous nucleolus or nucleoplasm of 30–50 cells/group.

For colocalization studies, confocal microscope images were collected using a Zeiss LSM 510 META laser scanning confocal microscope configured on an inverted Axiovert 200M stand (Zeiss) equipped with a Plan-Apochromat 63×/1.4 oil DIC objective. Cy2 fluorescence was excited at 488 nm with an argon ion laser, and emission was recorded through a 500–530-nm infrared band-pass filter. Cy3 fluorescence was excited at 543 nm with a helium-neon laser, and the emitted light was recorded through a 560-nm long-pass filter. Single z-sections were collected (0.8 μm thick) using Zeiss LSM 510 version 3.2 software.

For photoswitch and fluorescence recovery after photobleaching (FRAP) studies, cells were transfected with PS-CFP2-DDX21, EGFP-DDX21 (15), or EGFP-DDX21 mut and grown on glass-bottom dishes (MatTek). The size and location of the photobleach and photoswitching area were adjusted with the Zeiss LSM 510 version 3.2 software. Fluorescence intensities were measured using photomultiplier tubes connected to the microscope and analyzed using the Zeiss LSM 510 version 3.2 software through EGFP (excitation, 405 nm; emission, 480 nm) and FITC channels (excitation, 490 nm; emission, 528 nm). Digital images were superimposed and assembled using Adobe Photoshop software.

**Sequence Alignment and Structural Modeling of the C-terminal End of Human DDX21**—Based on a BLAST-PHIL search, the C-terminal end of human DDX21 protein (amino acids 563–783) was aligned against seven related proteins in vertebrates that shared the conserved RNA binding C termini. The sequence alignment was performed using MALIGN and MALFORM within the Bodil visualization and modeling package. Secondary structure prediction was performed by aligning the C-terminal part of human DDX21 against three proteins that share sequence similarity with it (Protein Data Bank codes 2NRT, 1B2R, and 1UB1). Ten different structural models were generated based on these results using the program Modeler. The model with the lowest value for objective function, given by Modeler, was chosen. Sequence alignments were generated using the program ALSCRIPT, and the figure was created with the PYMOL Molecular Graphics System (DeLano Scientific).

**Peptide Synthesis and Microinjection Experiments**—Peptides were synthesized with t-butoxycarbonyl chemistry on an Applied Biosystems 433A stepwise synthesizer. Peptide 1 corresponds to DDX21 amino acids 744–758 (FRGQREGSRG-ATT-3) and 5′-AACGCAGACGUUGCACACATT-3′ and 5′-GUGUGCAAGAAGAAGAATT-3′ (13). c-Jun and DDX21 were depleted by pooling the respective siRNA oligos to a final concentration of 200 nM. In all experiments, a scrambled duplex (5′-GUAAACAUUGAGACACGGCTT-3′) was used as a control. Cells were processed 48 or 72 h after transfection. Information about cloning of mammalian expression constructs used in this study is available upon request.

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**DDX21-rRNA Immunoprecipitation Analysis and Northern Blotting**—To study the ability of DDX21 to bind rRNA, GFP-DDX21 was immunoprecipitated from HeLa cells treated with scrambled or c-Jun siRNA, and the rRNA subunits that coimmunoprecipitated with DDX21 were subsequently identified by Northern blot analysis using rRNA subunit-specific probes (16). The total RNA was isolated using RNeasy (Qiagen). RNA was separated on a 1% agarose-formaldehyde gel and blotted on Hybond-N membranes (GE Healthcare). The following 32P end-labeled DNA oligonucleotides were used to visualize rRNA precursors: 45/47S, 5′-CCTCCCGCGGAAACCGCTAGCTTG-ACCTGGACGCCGGGGGGCGAGC-3′; 32 S, 5′-GGCG-GGCAGACGGGCCCAGCCTCGCCGCTTAGGGGA-3′; 18 S rRNA, 5′-ACACCGTGGTGCCCATG

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3 The abbreviations used are: MEF, mouse embryonic fibroblast; siRNA, small interfering RNA; WT, wild type; mut, mutant; CFP, cyan fluorescent protein; GFP, green fluorescent protein; EGFP, enhanced GFP; HA, hemagglutinin; ER, estrogen receptor; FITC, fluorescein isothiocyanate; GST, glutathione S-transferase; PS, photoswitchable; PIPES, 1,4-piperazinediethanesulfonic acid; JNK, c-Jun NH2-terminal kinase.
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GTAGGGCAGGCGACCTACCATCGAAAGTTGATAG-3'; and 28 S, 5'-CCAGCTATCTGAGGAAACTTCGGGAG-GAACCAGCTACTAGATGTCCG-3'. RNA was separated on a 1% agarose-formaldehyde gel and blotted on Hybond-N membranes. The autoradiogram was exposed and visualized using a PhosphorImager (Fuji).

RESULTS

c-Jun Depletion Inhibits rRNA Processing—We recently reported increased expression of 28 S and 18 S rRNA in cells transformed with the viral oncogenic v-Jun protein (17). As the nucleolus is the site where rRNA processing occurs, identification of several nucleolar proteins associated with c-Jun (5, 6) prompted us to test the hypothesis that c-Jun might be involved in the regulation of rRNA processing. To this end, the steady-state abundance of 45 S, 32 S, 20 S, and 18 S rRNAs in c-Jun siRNA-transfected HeLa cells was quantified by Northern blot analysis. In concert with our previous results (17), we found that c-Jun depletion (Fig. 1A) results in the inhibition of 28 S and 18 S accumulation, whereas no inhibition in either 45 S or 32 S rRNA levels is observed (Fig. 1B). The -fold difference in rRNA subunit expression levels, in comparison with 45 S, is shown below the radiographs (Fig. 1B). To obtain a quantitatively and dynamic view of the role of c-Jun in rRNA processing, the effect of c-Jun depletion on the accumulation of in vivo 32P-labeled rRNA subunits was measured. In this assay, the effect of c-Jun depletion on rRNA accumulation was assessed by comparing the intensity of 32P-labeled rRNA species 2 and 4 h after 32P addition to samples harvested immediately after the 32P pulse (0 h). Consistent with the results above, a clear decrease in 28 S and 18 S rRNA accumulation was observed in c-Jun-depleted cells at both the 2- and 4-h time points (Fig. 1C). The decrease in c-Jun-depleted cells was ~3–4-fold for 28 S and 2–3-fold for 18 S for both time points (Fig. 1C). No marked effect on 45 S, 32 S, or 20 S rRNA accumulation was observed in response to c-Jun depletion (Fig. 1C). Taken together, these results demonstrate that c-Jun depletion reduces 28 S and 18 S rRNA subunit accumulation in human cancer cells.

c-Jun Regulates DDX21 Nuclear Compartmentalization—Among the nucleolar proteins identified to associate with c-Jun (5, 6), RNA helicase DDX21 (RHII/Gu) has a physiological role in rRNA processing. Inhibition of DDX21, whether by siRNA-mediated depletion or by antibody microinjection, results in a decrease in both 28 S and 18 S rRNA levels in human and Xenopus models (13, 18). We demonstrated previously that endogenous c-Jun and DDX21 coimmunoprecipitate from human cancer cells in culture (6). Interestingly, we found that both exogenous and endogenous c-Jun and DDX21 colocalize at the perinucleolar rim of HeLa cells used in this study (supplemental Fig. S1). Considering the established role of DDX21 in rRNA processing (13, 18) and the physical association between c-Jun and DDX21, we investigated whether the rRNA processing defect observed in c-Jun-depleted cells is reflected in the expression or subcellular localization of DDX21. Western blot analysis of DDX21 protein expression in either c-Jun siRNA-transfected cells or c-Jun−/− mouse embryonic fibroblasts (c-Jun−/−) did not reveal any obvious differences in DDX21 expression as compared with c-Jun-expressing cells (Fig. 2, A and B). In contrast, when endogenous DDX21 staining in immortalized MEFs derived from c-Jun-deficient mice (c-Jun−/−) (11) was compared with wild-type cells (WT MEF), a clear increase in nucleoplasmic DDX21 staining was observed (Fig. 2C). Nucleoplasmic localization of DDX21 in these c-Jun−/− cells was not due to any clonal effect, as a similar phenotype was observed in c-Jun siRNA-transfected HeLa cells (Fig. 2D) and in an independently generated c-Jun−/− cell line (Fig. 2F) (12). Large-field images of the cells displayed in Fig. 2, C and D, are shown in supplemental Fig. S2. Quantification of the ratio between nucleoplasmic and nucleolar DDX21 staining

FIGURE 1. c-Jun depletion inhibits 28 S and 18 S rRNA accumulation. A, Western blot analysis of c-Jun protein expression levels in cells used for rRNA analysis in B. B, Northern blot analysis of rRNA abundance in c-Jun and scrambled (Scr.) control siRNA-transfected cells 48 h after siRNA transfection. C, pulse-chase analysis of rRNA subunit abundance in c-Jun-depleted HeLa cells 48 h after siRNA transfection. An autoradiograph of 32P-labeled RNA is shown. A and B, quantitation of relative abundance of rRNA subunits as compared with 45 S intensity in the same sample is shown below the autoradiograms. C, -fold differences in rRNA accumulation are depicted relative to the intensity at the 0-h time point, set as 1. B and C, each experiment was repeated three times with similar results.
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Taken together, the above results demonstrate that c-Jun depletion from cells in culture, in addition to an rRNA processing defect (Fig. 1), results in nucleoplasmic translocation of the rRNA processing protein DDX21. Moreover, the results of the rescue experiments confirm that the effects seen in DDX21 localization arise due to a loss of c-Jun. Furthermore, as c-Jun depletion does not influence B23 localization, we conclude that DDX21 translocation is likely not due to general effects of c-Jun depletion on nucleolar integrity.

c-Jun Depletion Inhibits DDX21 Binding to 45 S and 32 S rRNA—It has been proposed that the mobility of nuclear proteins indirectly reflects the affinity of proteins for their substrates (20). To study whether increased nucleoplasmic localization of DDX21 observed in c-Jun-depleted cells is reflected in the nucleoplasmic/nucleolar mobility of DDX21, both the inward and outward nucleolar movements of DDX21 in wild-type and c-Jun−/− cells were measured using a novel photoswitchable variant of cyan fluorescent protein (PS-CFP2). This CFP protein changes its emission spectra from 460 nm (CFP) to 515 nm (FITC) after photoactivation by a 405-nm laser line (21). Fig. 3 shows a representative time series of photoactivated nucleoli in WT MEF (Fig. 3A) and c-Jun−/− cells (Fig. 3B). Quantification of the DDX21 half-life (t1/2) for nucleolar recovery revealed a significantly shorter recovery time for PS-CFP2-DDX21 proteins in c-Jun−/− cells. The mean t1/2 recovery time was 100 s in c-Jun−/− cells compared with 235 s in WT cells (Fig. 3C). Similarly, the mean t1/2 exit rate for PS-CFP2-DDX21 from the nucleolus of c-Jun−/− cells (45 s) was significantly shorter than the 100 s seen for WT cells (Fig. 3D). These results confirm that the observed effects of c-Jun depletion on DDX21 compartmentalization (Fig. 2) can be recapitulated by a photoswitching methodology that is not susceptible to subjective criteria. Furthermore, these results strongly suggest that c-Jun depletion decreases the affinity of DDX21 for nucleolar rRNA substrates.

To directly study the effects of c-Jun depletion on the rRNA binding affinity of DDX21, rRNA subunits associated with DDX21 were analyzed by immunoprecipitation and Northern
blotting. We found that in scrambled siRNA-transfected cells, DDX21 associated with 45 S and 32 S pre-rRNA subunits, whereas no significant association of DDX21 with 28 S or 18 S rRNA was detected (Fig. 3E). In support of the role for c-Jun in the regulation of rRNA binding of DDX21, we found a clear decrease in the binding of DDX21 to both 45 S and 32 S rRNAs in c-Jun-depleted cells (Fig. 3E). As c-Jun depletion did not reduce cellular 45 S or 32 S amounts, but instead inhibited the accumulation of 28 S and 18 S (Fig. 1B and C), these results together indicate that c-Jun depletion reduces DDX21 binding to 45 S and 32 S rRNAs and inhibits their processing to 28 S and 18 S. To our knowledge, this is the first demonstration that a transcription factor regulates rRNA binding of an rRNA processing protein and thereby affects rRNA processing.

Interaction with c-Jun Supports Nucleolar Localization of DDX21—Even though we demonstrated previously that c-Jun binds directly to DDX21 (6), it is unknown whether this direct interaction is required for c-Jun-elicited support of nucleolar localization of DDX21. Using truncated recombinant GST-DDX21 proteins, we found that amino acids 731–740 at the C-terminus of DDX21 are required for direct interaction between c-Jun and DDX21 (Fig. 4A). Accordingly, random mutations in the 734–738 region (FRGQR/YEGIQ) abolished the binding of the GST-DDX21 628–783 fragment to His-c-Jun (Fig. 4B). Interestingly, the c-Jun interaction domain of DDX21, between amino acids 734 and 738 on the C-terminal tail, maps to one of four Arg-Gly repeats (repeat 2) found in the vicinity of the RNA binding domain of DDX21 (supplemental Fig. S3, A and B). Moreover, sequence similarity analysis revealed that the c-Jun binding domain and the RNA binding domain are the most conserved areas of the C-terminal tail of DDX21 throughout the animal kingdom (supplemental Fig. S3A). This suggests that both of these domains are important for DDX21 function.

In accordance with the requirement for direct c-Jun-DDX21 interaction supporting nucleolar localization of DDX21, we found that mutation of repeat 2 (EGFP-DDX21 mut) resulted in significantly increased nucleoplasmic localization when compared with WT DDX21 (Fig. 4C, as well as higher mobility of the protein as measured by FRAP (supplemental Fig. S4). Interestingly, our previous work demonstrated that a corresponding...
To obtain additional evidence demonstrating that direct interaction between c-Jun and DDX21 is required for c-Jun-mediated nucleolar localization of DDX21, two recombinant peptides were synthesized, one spanning the putative interaction site, amino acids 729–743 (peptide 2), and another spanning the adjacent Arg-Gly repeats, amino acids 744–758 (peptide 1) (Fig. 4D and supplemental Fig. S3A). Preincubation of c-Jun with peptide 2 prevented the direct interaction between c-Jun and DDX21 in vitro, whereas peptide 1 had no apparent effects on the interaction (Fig. 4D). Next, peptides 1 and 2 were microinjected, together with FITC-labeled dextran, into the nuclei of HeLa cells, and the cells were stained for endogenous DDX21. Importantly, in line with the results above (Fig. 4C), peptide 2 caused a significant relocation of DDX21 from the nucleolus to the nucleoplasm 3 h after microinjection (Fig. 4, D, E, and F). These results provide important independent evidence that direct c-Jun-DDX21 interaction regulates nucleolar compartmentalization of DDX21.

To demonstrate in greater detail that DDX21 nucleolar localization in c-Jun−/− cells (Fig. 2) is not due to long-term transcriptional effects related to c-Jun depletion, but that c-Jun directly regulates DDX21 localization, c-Jun protein was conditionally re-expressed in Jun−/− MEF nuclei. To this end, Jun−/− MEFs were transiently transfected with a cDNA construct encoding HA-c-Jun and fused to the hormone binding domain of the estrogen receptor (HA-JunER) (6), and nuclear translocation of HA-JunER was induced by tamoxifen treatment. Importantly, we found that addition of tamoxifen to transfected cells provoked nuclear translocation of the HA-JunER fusion protein and rescued the nucleolar localization of endogenous DDX21 1 h after tamoxifen treatment (Fig. 4G).

Because immediate rescue of the nucleolar localization of DDX21 by nuclear translocation of HA-JunER is unlikely to be mediated by c-Jun-elicted gene expression, these data further indicate that its direct association with c-Jun supports nucleolar localization of DDX21.

Finally, as nucleolar localization of DDX21 appears to be dependent on direct c-Jun-DDX21 interaction, we investigated...
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Whether the increased rRNA binding activity of DDX21 in the presence of c-Jun (Fig. 3E) is also due to direct binding of c-Jun to DDX21. To this end, we performed an in vitro RNA binding assay with recombinant His-c-Jun and the GST-DDX21 C-terminal tail containing the RNA binding domain (Ref. 22 and supplemental Fig. S3). As shown in Fig. 4H, we found that recombinant His-c-Jun repeatedly increased GST-DDX21 binding (amino acids 628–783) to fluorescently labeled RNA, which was previously demonstrated to bind DDX21 (23). In contrast, His-Akt, used as a negative control, did not increase DDX21 binding to RNA (Fig. 4H), demonstrating the specificity of c-Jun-mediated effects on DDX21 RNA binding activity. Along with increased nucleolar shuttling and decreased rRNA immunoprecipitation observed upon c-Jun depletion (Fig. 3, A–E), these results together strongly indicate that c-Jun has a direct effect on stimulating DDX21 rRNA binding capacity.

**FIGURE 5.** DDX21 and c-Jun depletion results in G1/S cell cycle blocking. A, the HeLa cell proliferation rate was measured by a bromodeoxyuridine (BrdU) incorporation assay 72 h after siRNA transfection with scrambled (Scr), c-Jun, DDX21, or c-Jun + DDX21 siRNAs. B, flow cytometric cell cycle analysis of propidium iodide-stained cells 72 h after transfection with scrambled, c-Jun, DDX21, or c-Jun + DDX21 siRNA oligos is shown. A and B, mean value ± S.D. of three independent experiments is shown. *, p < 0.05 by Student’s t test compared with scrambled siRNA-transfected cells; **, p > 0.05 by Student’s t test compared with c-Jun siRNA-transfected cells.

**A**

**B**

**DISCUSSION**

The molecular mechanisms by which c-Jun exerts its biological functions are not thoroughly established. Even though c-Jun has been shown to regulate expression of several target genes, recent studies have suggested that regulation of AP-1-mediated transcription might not be required for all of the functions of c-Jun in cell proliferation and survival (3, 4). This view is also supported by results demonstrating that the N-terminal phosphorylation of c-Jun by JNK kinases is not absolutely required for the G1/S cell cycle and the proliferation-promoting activity of c-Jun, although it is critical for the full transcriptional activity of the protein (7, 24).

Here, we demonstrate that c-Jun depletion results in the inhibition of rRNA processing, increased nucleolar shuttling of DDX21, and decreased DDX21 binding to 45 S and 32 S rRNAs. Altogether, these results identify a potential novel mechanism by which c-Jun regulates cell behavior. Importantly, our peptide microinjection (Fig. 4, E and F) and c-JunER (Fig. 4G) experiments strongly suggest that the mechanism by which c-Jun regulates DDX21 localization is not mediated by transcription. However, the precise molecular mechanism by which c-Jun depletion causes rRNA processing defects and decreased DDX21 rRNA binding affinity remains to be explored fully. Our results suggest that a physical interaction between c-Jun and DDX21 is important for proper nucleolar localization of DDX21. Moreover, it seems evident that direct binding of c-Jun to the C-terminal tail of DDX21 enhances the intrinsic RNA binding affinity of DDX21. Importantly, a similar “hit-and-run” mechanism resulting in conformational changes at the binding surface has been suggested recently to regulate protein-protein interactions (25, 26).

Wagner and co-workers (7) reported previously that the G1/S cell cycle arrest observed in c-Jun-deficient murine liver cells is reminiscent of that reported for a mouse model displaying compromised ribosome biogenesis (8). These results, together with our observations that c-Jun depletion results in inhibition of 28 S and 18 S rRNA accumulation, suggest that the phenotypes previously observed in c-Jun-depleted mouse models and cell lines may stem not only from defects in transcriptional regulation of gene expression. Instead, these phenotypes could be partly due to the effects of c-Jun on rRNA processing. Moreover, based on our results it is clear that c-Jun-deficient cells are aberrant as compared with their wild-type counterparts in many aspects (rRNA processing, protein trafficking, and protein compartmentalization) in addition to their gene expression profiles. This observation should have importance when assessing the cell biological role of c-Jun in its entirety. It is worth noting that depletion of c-Jun-activating kinases JNK1 and JNK2 also results in decreased c-Jun expression and increased nucleolar localization of DDX21. More generally, our results could open new avenues for understanding the mechanisms by which transcription factors that have nucleolar interaction partners (e.g. c-Myc (27)) regulate cell behavior.

The molecular data presented in this study indicate that c-Jun has a role in supporting rRNA processing. To our knowledge, this is the first report to clearly suggest an alternative mechanism by which c-Jun may regulate cell behavior. Our results do not confront the established role of c-Jun as a transcription factor involved in the regulation of several cellular

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processes, but rather are in agreement with the emerging view that cellular behavior is regulated by the synchronous and complementary action of different cellular processes, such as transcription and ribosome biogenesis.

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