Caspase-3-dependent and -independent Degradation of 28 S Ribosomal RNA May Be Involved in the Inhibition of Protein Synthesis during Apoptosis Initiated by Death Receptor Engagement

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Activation of death receptors initiates intrinsic apoptosis programs in various parts of the cell. To explore the possibility that ribosomal RNA (rRNA), essential for translation in ribosomes, is a target of pro-apoptotic proteins, rRNA was analyzed by electrophoresis in two apoptosis systems: human Jurkat cells treated with anti-Fas antibody and human U937 cells treated with tumor necrosis factor-α. In both systems, bands in addition to those of unmodified rRNA were detected a few hours after death receptor engagement. In both systems, the primary additional band was identical and comprised the 3'-terminal region of 28 S rRNA. The degradation of 28 S rRNA was simultaneous with protein synthesis inhibition in both systems. The caspase-3 inhibitor Z-DEVD-FMK suppressed rRNA degradation and protein synthesis inhibition in Jurkat cells but not in U937 cells. Together, our data suggest that different pathways are activated in the two systems we studied, and the final steps in these pathways use very similar or identical ribonucleases to cleave 28 S rRNA. These data suggest a physiological link between rRNA degradation and inhibition of protein synthesis. In general, apoptosis execution initiated by death receptor engagement is promoted by protein synthesis inhibition. Triggered by rRNA degradation, malfunction of the protein synthesis machinery may prompt death execution.

Death receptors are a unique group of transmembrane proteins that directly mediate cell death that is triggered by extracellular stimuli (1). Fas (CD95), tumor necrosis factor receptor 1 (TNFR1), CD120a, and p75 neurotrophin receptor are death receptors. These receptors have a homologous cytoplasmic sequence called the death domain and very similar cysteine-rich extracellular regions.

Recently, the signal transducing molecules that are downstream from death receptors and trigger the cell’s intrinsic apoptosis machinery have been studied. Two signaling pathways involving Fas have been reported: mitochondria-dependent pathway and mitochondria-independent pathway (2, 3). In either pathway, downstream effector caspases such as caspase-3 are activated to initiate apoptosis. The activity of these two pathways varies between cell types and tissues. The pathway downstream of TNFR1 is more complicated than the pathway downstream of Fas because, depending on the intracellular environment, activation of this receptor can lead to apoptosis, necrosis-like cell death or a pro-inflammatory response. In addition, FAP-1 associated with death receptors modulates death signaling (4, 5), and Fas-associated death domain protein (FADD) can initiate necrosis-like organized cell death (6). Analysis at the molecular level of cell death caused by death receptors improves our understanding of development, homeostasis, diseases, and cancer because apoptosis through death receptors plays a pivotal role in these phenomena (7–9).

At a particular point after an apoptotic insult, cells reach a decisive point, past which rescue from apoptosis is impossible (10). Once this point is passed, apoptosis enters the period termed the execution phase. One of the characteristics of the execution phase is irreversible degradation of cellular components, during which caspase-3-activated deoxyribonuclease (CAD) digests genomic DNA (11) and caspase-6 digests nuclear cytoskeleton lamin (12). These events contribute to the final morphologic change in the nucleus that is characteristic of apoptosis. In addition, caspase-3 digests some signaling proteins (12). Such irreversible degradations accelerate cell death. Therefore, to fully decipher the story of apoptosis, delineating the events at the onset of the execution phase is important.

Protein synthesis is a characteristic of cell viability. In fact, inhibition of protein synthesis by drugs such as actinomycin-D causes apoptosis in some cell types (13). The ribosome is a large protein-RNA complex essential for protein synthesis. In humans, the ribosome (80 S) composes a large subunit, involving 46 proteins and 28 S, 5.8 S, and 5 S RNA species and a small subunit including 33 proteins and 18 S RNA (14). Although ribosomal regulation in de novo protein synthesis has been the focus of many studies, work addressing its role in apoptosis had not been published previously. Ultrastructural analysis has shown that ribosomes dissociate from the rough endoplasmic reticulum during apoptosis of some tissues (15, 16). Such morphologic studies encouraged us to pursue the molecular analysis of the ribosome during apoptosis.

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¶ The abbreviations used are: TNFR, tumor necrosis factor receptor; CAD, caspase-3-activated deoxyribonuclease; RACE, rapid amplification of cDNA ends; RNase, ribonuclease; rRNA, ribosomal RNA; TEMED, N,N,N',N'-tetramethylethylenediamine.
Because ribosomal RNA (rRNA) is an essential component for protein synthesis (17), we have examined in the present study the role of rRNA in apoptosis caused by activation of death receptors. Apoptosis induced by cAMP analogs features the degradation of rRNA (18). Although some small drugs including cAMP analogs strongly induce apoptosis, their pleiotropic effects can complicate attempts to separate physiologic death pathways from unusual secondary pathways that are stimulated by side effects of the drugs. The molecular mechanisms and biologic significance of alterations in rRNA had not been investigated previously. Here we show that 28 S rRNA is selectively degraded by the same (or a very similar) ribonuclease (RNase) in the Jurkat and U937 systems. In addition, examination of the association between rRNA degradation and protein synthesis revealed the potential involvement of this degradation in the malfunction of protein synthesis machinery.

EXPERIMENTAL PROCEDURES

Cell Lines and Culture—The human T-cell leukemia cell line Jurkat (ATCC, Manassas, VA) and human monoclonal leukemia cell line U937 (DE-4; RIKEN Cell Bank, Tsukuba, Ibaraki, Japan) were maintained in RPMI 1640 (Sigma) supplemented with 10% heat-inactivated fetal calf serum (Sanou, Japan, Tokyo, Japan), 100 units/ml penicillin, and 100 μg/ml streptomycin and grown in an atmosphere of 5% CO2 at 37°C.

Apoptosis by Death Receptor Activation—In the Fas-Jurkat system, Jurkat cells were washed once with serum-free RPMI 1640 and resuspended in the same medium. To induce apoptosis, a mouse monoclonal anti-Fas antibody (CH-11; MBL, Nagoya, Japan) was added at a concentration of 30 ng/ml, and the cells were incubated for various periods of time. Control cells were incubated under the same conditions but without CH-11. Cells incubated with 100 ng/ml unrelated mouse IgM (No. 02–6800; Zymed Laboratories Inc., San Francisco, CA) were an additional control.

In the TNFR-U937 system, U937 cells were washed once with serum-free RPMI 1640 and resuspended in the same medium. To induce apoptosis, recombinant human TNF-α (R & D Systems, Minneapolis, MN) was added at a concentration of 30 ng/ml, and the cells were incubated for various periods of time. Control cells were incubated under the same conditions but without TNF-α.

To examine the effect of caspase-3 inhibition on apoptosis, cells were incubated for 1.5 h at 37°C in serum-free RPMI 1640 containing a cell-permeable form of Z-DEVD-FMK (50 μM; caspase-3 inhibitor II; Calbiochem) that had been dissolved in dimethyl sulfoxide to create a 25 mM stock solution (2). Polyacrylamide-agarose composite gel electrophoresis. Bands in addition to those of unmodified rRNA appeared in the apoptotic Jurkat cells. Inhibitors of the caspase-3 pathway produced only partial inhibition of apoptosis. This observation agrees with the suggestion (2) that caspase-3 is only a late event in the apoptotic process.

DNA Fragmentation Assay—Fragmented DNA was extracted with Triton X-100-containing buffer (19) and subjected to 1.4% agarose gel electrophoresis followed by ethidium bromide staining.

RNA Fragmentation Assay and Northern Blotting—Total RNA was isolated from cells by using the Trizol reagent (Life Technologies, Inc.). The concentration of the solution was determined by measuring the absorbance at 260 nm.

Polyacrylamide (2.5%)–agarose (0.5%) composite gel electrophoresis was modified from the method of Peacock and Dingman (20). Distilled water (13 ml) was added to 75 mg of electrophoresis-grade agarose (Iwai Techno) and cool the composite gel, it then was kept at 4°C for 1 h. Polyacrylamide-water (2.5 μl of RNA solution) were mixed with an equal volume of 90°C (37°C) formamide, 10% (37°C) glycerol, incubated for 3 min at 65°C, cooled for 5 min at room temperature, and then loaded onto the gel. Gels were run for 30 to 40 min at 200 V in 1× TAE buffer, which was precooled on ice; the gels then were stained with ethidium bromide.

For Northern blotting after composite gel electrophoresis, RNA was electroblotted onto Hybond-N+ membrane (Amersham Pharmacia Bio-tech) in 25 mM Tris containing 0.19 μM glycine by using the Mini Trans-Blot Cell (Bio-Rad) at 0.3 A for 1 h and fixed by using the UV Stratalinker (Stratagene, La Jolla, CA). Oligodeoxynucleotide acid probes were synthesized by ESPEC-OILIGO Service Corp. (Tsukuba, Ibaraki, Japan) and terminally labeled by using γ[-32P]ATP (Amersham Pharmacia Bio-tech) and T4 polynucleotide kinase (Takara, Otsu, Shiga, Japan). The blots were prehybridized for 15 min in QuickHybrid hybridization solution (Stratagene) and hybridized for 1 h with a 32P-labeled oligonucleotide probe. The blots were washed with 0.1% SDS, 2× SSC (1× SSC: 15 mM sodium citrate containing 0.15 μM NaCl) followed by 0.1% SDS, 0.1× SSC and subjected to autoradiography with Kodak X-OMAT AR films.

Rapid Amplification of 5′ cDNA Ends (5′-RACE)—Apoptotic RNA fragments were isolated by combining agarose gel electrophoresis with a “crush and soak” method involving phenol. 5′-RACE was performed with the 5′-RACE kit version 2.0 (Life Technologies, Inc.) according to the manufacturer’s instructions. The following reverse primers recognizing the human 28 S rRNA sequence were used: 5′-GCTCAA-CAGGGTCCTC-3′ (antisense of nucleotides 3873–3888) for first-strand cDNA synthesis and 5′-CAGGGTCTTCGAAATC-3′ (antisense of nucleotides 3655–3674) for the subsequent polymerase chain reaction.

The products were analyzed by agarose gel electrophoresis. Amplified cDNA was isolated from the gels with the QIAQuick gel extraction kit (Qiagen, Hilden, Germany) and directly sequenced by using the reverse primer for polymerase chain reaction and the Big Dye terminator cycle sequencing kit (PE Biosystems, CA) with confirmation of the sequence results, cDNA from apoptotic Jurkat cells was cloned into pBluescript II KS (Stratagene) and sequenced with M13 universal primers.

Collection of Polymersomes—To prevent disassembly of polymersomes, cells (2×107) were cultured for 10 min in the presence of cycloheximide (5 μg/ml; Sigma) before harvesting (22). Harvested cells were washed with phosphate-buffered saline, lysed for 15 min on ice with 2.5 ml of 50 mM Tris-HCl buffer (pH 7.5) containing 1% Triton X-100, 1% sodium deoxycholate, 0.25 mM sucrose, 25 mM KCl, 5 mM MgCl2, 10 μg/ml cycloheximide, 10 mM 2-mercaptoethanol, and 1 mM phenylmethylsulfonyl fluoride, and centrifuged for 10 min at 15,000×g to collect the post-mitochondrial fraction (the supernatant). Ultracentrifuge tubes (No. 341682; Beckman Instrument Co.) were filled with 0.75 ml of a 2.0 M sucrose solution with 50 mM Tris-HCl buffer (pH 7.5) containing 25 mM KCl, 5 mM MgCl2, and 10 mM 2-mercaptoethanol and 0.63 ml of a 0.5 M sucrose solution. The post-mitochondrial supernatant (0.9 ml/tube) was added as the top zone, and the tubes were centrifuged for 3 h at 2°C and 105,000×g. Polymersomes compose more than 90% of the resulting pellet (23). Polymersomes were harvested from the pellets by using 50 mM Tris-HCl buffer (pH 7.5) containing 1% Triton X-100, 0.15 M NaCl, 5 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride, and centrifuged for 5 min at 15,000×g. The supernatant was subjected to 10% SDS-polyacrylamide gel electrophoresis and autoradiography. Autoradiograms were scanned by using an image analyzer (Model ES-8000; Epson, Tokyo, Japan), and the intensity of the bands was evaluated with the NIH Image software (National Institutes of Health, Bethesda, MD).

RESULTS

Stimulation of Death Receptors Caused Degradation of rRNA in Cytoplasmic Polysomes—We selected two apoptosis systems: the human T-cell leukemia cell line Jurkat, treated with the anti-Fas antibody CH-11 to activate Fas (the Fas–Jurkat system), and the human monoclonal leukemia cell line U937, treated with TNF-α to activate TNFR1 (the TNFR-U937 system). These systems including leukemia-type cell lines seem reasonable because Fas and TNFRII have been shown to be important in the development and function of leukocytes in mice lacking these receptors (3, 24).

To examine the alteration of rRNA in these apoptosis systems, total RNA was isolated by using Trizol and analyzed by polyacrylamide-agarose composite gel electrophoresis. Bands in addition to those of unmodified rRNA appeared in the apo-
apoptotic Jurkat cells; electrophoresed. RNA from the post-mitochondrial fractions obtained during the polysome isolation also was analyzed.

Lane 1, electrophoresis of RNA isolated from polysomes. Polysome fractions were collected by ultracentrifugation, and RNA from these fractions was treated with TNF-α; unrelated mouse IgM (100 ng/ml); the same conditions but without an apoptosis inducer. Were treated for 12 h with anti-Fas antibody (100 ng/ml). U937 cells were treated for 6 h with TNF-α, lanes 1-4, polysomes of control Jurkat cells; lanes 5-7, polysomes of control Jurkat cells; lanes 2-3, post-mitochondrial fraction from apoptotic Jurkat cells; lane 6, post-mitochondrial fraction from control Jurkat cells. Additional RNA bands (due to cleavage of 28 S rRNA) are indicated by the asterisks and arrowheads.

Ribosomes are generated in the nucleoli and are exported from the nucleus as mature forms (25). To examine whether the extra RNA molecules we identified occurred in cytoplasmic ribosomes, cytoplasmic polysome fractions from apoptotic Jurkat cells were collected by ultracentrifugation. RNAs collected from the fractions were analyzed by using composite gel electrophoresis to identify smaller RNA molecules (data not shown).

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A Putative Cutting Site of 28 S rRNA in Variable Region Eight Is Identical in the Fas-Jurkat and TNFR-U937 Systems—Although these two systems stimulate different death receptors, the patterns of extra RNA were very similar (Fig. 1A). In particular, both systems yielded a prominent band (indicated by the arrowhead in Fig. 1A) just smaller than 18 S rRNA; the similar mobility of this band from the two cell types suggests an identical structure. We then sought the molecular identity of this RNA species. After composite gel electrophoresis, RNA from apoptotic and control Jurkat cells were electrophoresed onto nylon membranes, and the blots were hybridized with probes for 28 S and 18 S rRNA. This prominent band hybridized with the 28 S rRNA probe (Fig. 2A, left panel), but no extra bands were detected with the probe for 18 S rRNA (Fig. 2A, right panel). This finding suggests that this RNA species derived from RNase-degraded 28 S rRNA.

To precisely map the cutting site, we performed Northern blotting analysis by using oligonucleotide probes for various partial sequences of 28 S rRNA. The prominent RNA band identified previously comprised the 3′-terminal region of 28 S rRNA (Table I), and the putative cleavage site presumably was somewhere between nucleotides 3251 and 3710. Next, this RNA fragment was isolated from Jurkat and U-937 cells undergoing apoptosis and assessed carefully by using 5′-RACE. The analysis of amplified cDNA fragments revealed a single band with identical mobility from both systems (Fig. 2B). The diffuse nature of the bands mainly is due to the various lengths of the dC-tail of the template cDNAs. Sequencing of these cDNA samples revealed that in both systems, the 5′-end of the RNA molecule was nucleotide 3536; by definition (26, 27), the cutting site maps to variable region eight (Fig. 2C). This result suggests that although these systems stimulate different death receptors, the cutting mechanism may be very similar, possibly executed by the same RNase. This result further suggests that the extra RNA species identified by the asterisks in Fig. 1 is the 5′-terminal region of 28 S rRNA (see also Fig. 2A, middle panel).

Dependence of rRNA Degradation on Caspase-3-like Activity Differed Between the Fas-Jurkat and TNFR-U937 Systems—Caspases are a family of proteases that are specifically activated by apoptotic stimuli (12). During the execution phase of apoptosis, effector caspases are activated, and the various substrates are digested to promote cell death. In particular, caspase-3 is a key player involved in degradation of many components in cells. If the execution phase leads to rRNA degradation, caspase-3 may be upstream of its degradation. To investigate this possibility, Jurkat cells were pretreated with a caspase-3 inhibitor, a cell-permeable form of Z-DEVD-FMK, which is executed by caspase-3-activated CAD (Fig. 3A, lower panel). Interestingly, degradation of 28 S rRNA was suppressed by the caspase-3 inhibitor (Fig. 3A, upper panel), suggesting that caspase-3 or a caspase-3-like protease is involved in the degradation of 28 S rRNA.
involved in the degradation of 28 S rRNA in the Fas-Jurkat system.

We next examined the involvement of caspase-3 in rRNA degradation in the TNFR-U937 system. In this system, the caspase-3 inhibitor had no effect on rRNA degradation (Fig. 3B, upper panel). This result was not because the inhibitor failed to

![Fig. 2. Structure of the primary rRNA fragment in the Fas-Jurkat and TNFR-U937 systems. A, Northern blotting of total RNA with probes for 28 S rRNA (left and middle panels) and 18 S rRNA (right panel). Total RNA was isolated from Jurkat cells treated with anti-Fas antibody, electrophoresed, and subjected to Northern blotting with the labeled probes: 5'-ACAAACCCCTTGTGTCGAGGGCTGA-3' (antisense of nucleotides 5001–5024 of human 28 S RNA), 5'-ACGTGTTAGACTCTTCCGCTGGTG-3' (antisense of nucleotides 1305–1328 of human 28 S RNA), and 5'-TGGATCTTCGCGAGGCTCCACCTA-3' (antisense of nucleotides 1843–1866 of human 18 S RNA). The primary rRNA fragment is indicated by the arrowhead. B, electrophoresis of 5'-ends of cDNAs from 28 S rRNA fragments. Induction of apoptosis is described under “Experimental Procedures.” cDNA was amplified from the primary rRNA fragment by using reverse transcriptase and terminal deoxynucleotidyl transferase (TdT) followed by polymerase chain reaction. Controls were prepared under the same conditions but without terminal deoxynucleotidyl transferase. All samples were electrophoresed in 1.8% agarose gels and stained with ethidium bromide. Lane 1, the Fas-Jurkat system; lane 2, control for the Fas-Jurkat system; lane 3, the TNFR-U937 system; lane 4, control for the TNFR-U937 system. C, schematic representation of human 28 S rRNA and the putative cutting site in our apoptosis systems. The structure of 28 S rRNA has been defined in previous reports (26, 27). Striped box, constant region; open box, variable region; S/R, a-sarcin/rin loop (29, 43); V8, variable region 8; arrow, putative cutting site. nt, nucleotides.

![Table 1. Summary of hybridization with 28 S rRNA-derived probes](image)

| Probe sequence | Position in human 28 S rRNA | Hybridization |
|----------------|-----------------------------|--------------|
|                |                             | Extra RNA    | 28 S rRNA | 18 S rRNA |
| 5'-GCCCTTAGGCAATCTCCCTACCC-3' | 2853–2876 | – | + | – |
| 5'-ACGTGGGCCGATCCGCGGAAG-3' | 3229–3250 | – | – | – |
| 5'-GATCTTATCCCCCGCTCC-3' | 3711–3724 | + | – | – |
| 5'-ACGATCGAGTAGTGATGATTTCCAC-3' | 4010–4034 | + | – | – |
| 5'-ACAAACCCCTTGTGTCGAGGGCTGA-3' | 5001–5024 | + | – | – |

* Structure of human 28 S rRNA (5025 nucleotides) was determined by Gonzalez et al. (21).
work in this cell line: Z-DEVD-FMK suppressed DNA ladder formation in U937 cells (Fig. 3, lower panel) just as it had in Jurkat cells. In light of the identical structure of the main RNA fragment, different pathways, caspase-3-dependent and -independent pathways, likely activate the same or very similar RNases in these systems; these pathways ultimately converge in the cleaving of 28 S rRNA.

Degradation of 28 S rRNA Caused by Death Receptors Was Concomitant with Protein Synthesis Inhibition—We then sought the role of rRNA degradation in apoptosis. Our hypothesis was that irreversible fragmentation of rRNA compromised the function of ribosomes, that is, protein synthesis. To confirm this hypothesis, the kinetics of 28 S rRNA fragmentation and protein synthesis were examined. In the Fas-Jurkat system, a prominent 28 S rRNA-derived fragment, which gradually intensified, reproducibly was detected beginning 4 h after the addition of the monoclonal anti-Fas antibody CH-11 (Fig. 4A). In the same system, protein synthesis began to decrease at the onset of rRNA fragmentation (Fig. 4B).

To examine whether this coincidence was observed in another apoptosis system caused by death receptor engagement, the kinetics analysis was performed in the TNFR-U937 system, in which RNA fragmentation began to be detected 2 h after receptor stimulation (Fig. 5A). This cell line seemed especially sensitive to the low serum environment, and this stress caused a moderate decrease in protein synthesis in the absence of apoptotic stimulation (Fig. 5B, right panel). However, apoptosis caused a more dramatic shutdown of protein synthesis (Fig. 5B, left panel). Again, fragmentation of rRNA was concomitant with protein synthesis inhibition. These results in the two different systems suggested that fragmentation of rRNA occurs during the early execution phase of apoptosis (fragmentation occurred just a few hours after stimulation). Furthermore, rRNA fragmentation seems to be involved in the apoptosis-associated inhibition of protein synthesis in the cell.

Dependence of Protein Synthesis Inhibition on Caspase-3-like Activity Supported the Involvement of rRNA Fragmentation in Protein Synthesis Inhibition during Apoptosis—The close transient relationship between rRNA degradation and inhibition of protein synthesis (Figs. 4 and 5) suggests that this degradation is involved in the inhibition. To test this possibility, the effect of the caspase-3 inhibitor Z-DEVD-FMK on protein synthesis was examined in the two apoptosis systems. The caspase-3-inhibitor suppressed protein synthesis inhibition (Fig. 6A) and rRNA degradation (Fig. 3A) in Jurkat cells. In contrast, Z-DEVD-
FMK failed to recover protein synthesis in U937 cells (Fig. 6B), in which rRNA degradation was caspase-3-independent (Fig. 3B). Therefore, degradation of rRNA may be a physiologically important cellular signal mediator for the malfunction of the protein synthesis machinery in our apoptosis systems.

**DISCUSSION**

Malfunction of the ribosome causes death of cells and organisms. Until now, various small chemicals that specifically attack the ribosome have widely been used as antibiotics (28). Furthermore some natural toxins are protein ribotoxins. For example, ricin and α-sarcin catalyze covalent modifications in adjacent nucleotides of 28 S rRNA, leading to malfunction of the ribosome in vitro and in vivo (29). Colicin E3 specifically cleaves a single site in 16 S rRNA in the small subunit of the bacterial ribosome, resulting in inactivation of the ribosome and death of the host bacteria (30). Here we propose the existence of a new RNase that is cryptic in normal cells but is activated to cleave 28 S rRNA in the polysome during apoptosis triggered by either of two death receptors.

The putative cleavage site was identical in the 28 S rRNAs from the Fas-Jurkat and TNFR-U937 systems (Fig. 2). According to phylogenic studies of rRNA, the cleavage site is in variable region eight, which is absent in bacteria. This finding contrasts with the observation that α-sarcin and ricin attack an α-sarcin/ricin loop in a constant region (Fig. 2C) that is involved in the binding of aminoacyl-tRNA and in GTP hydrolysis (29). The function of variable regions currently is unknown. Variable region eight is conserved in vertebrates and contains a unique double-loop structure (27). The apoptosis-associated cleavage might induce a change in the higher order structure of the rRNA and ribosome. The three-dimensional structure of the yeast ribosome has been published recently (31). Future structural analysis of eukaryotic ribosomes will provide insight into the possible function(s) of this variable region.

From the comparison of the band intensities of intact 28 S rRNA and the main RNA fragment (Fig. 1B), most ribosomes in the polysomes seem to be intact. Even though destructive digestion occurs in some ribosomes, how can degradation of so few ribosomes cause the inhibition of protein synthesis at the cellular level? This paradox was reported more than 20 years ago in studies of protein ribotoxins. In intact cells treated with ribotoxins such as ricin (32) and colicin E3 (33), protein synthesis stopped when only a fraction of the ribosomes was inactivated. Two mechanisms that are not mutually exclusive have been proposed. In the first, impaired ribosomes are a dominant negative factor in polysomes. In this situation, one or a few ribosomes with damaged rRNA occur per polysome, attach to
the mRNA, and block further elongation of the peptide chains. This block especially seems to inhibit synthesis of housekeeping proteins, which are constitutively synthesized in large quantities and are important for cell viability. In the second proposed mechanism, cleavage of rRNA generates signals for the inhibition of protein synthesis. The ricin- and α-sarcin-induced modification of rRNA activates stress-activated protein kinase (34). Therefore, this pathway might be activated downstream of rRNA degradation in our systems. A link between the pathway of stress-activated protein kinase and inhibition of protein synthesis has been reported very recently (35).

Another candidate of the signaling molecules activated by rRNA degradation is a double-stranded RNA-activated protein kinase. Activated by double-stranded RNA, RNA-activated protein kinase autophosphorylates, phosphorylates eukaryotic translation initiation factor-2, and eventually inhibits protein synthesis (36). RNA-activated protein kinase is a ribosome-binding protein that associates with the large subunit via the ribosomal protein L18 (37). rRNA has a complex secondary structure involving many loops of double-stranded RNA (26). Cleavage of 28 S rRNA might expose part of such a structure in a large subunit, thereby stimulating RNA-activated protein kinase. RNA-activated protein kinase is activated in U937 cells that have been treated with TNF-α (38). Our culture cell systems may help to resolve the apparent paradox.

Determining the structure of an RNA fragment (Table I and Fig. 2) provides a key piece of information needed to identify RNase responsible for cleaving 28 S rRNA. So what kind of RNase is involved in this cleavage? Explaining the selective cleavage of 28 S rRNA in light of current knowledge of RNases is impossible. The base on the nucleotide at the 3′ side of the phosphodiester bond preferentially broken by human pancreatic-type RNase is cytosine (39). In human cells, 2–5A-dependent RNase is involved in this cleavage? Explaining the selective cleavage of 28 S rRNA might expose part of such a structure in a large subunit, thereby stimulating RNA-activated protein kinase.

rRNA Degradation Caused by Death Receptor Engagement

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