THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 277, No. 6, Issue of February 8, pp. 4147–4151, 2002
Printed in U.S.A.


# Ribosomal Protein S25 mRNA Partners with MTF-1 and La to Provide a p53-mediated Mechanism for Survival or Death*

Received for publication, October 10, 2001, and in revised form, November 19, 2001
Published, JBC Papers in Press, December 7, 2001, DOI 10.1074/jbc.M109785200

**Tadepalli Adilakshmi and Roney O. Laine‡**

*From the Whitney Laboratory, University of Florida, St. Augustine, Florida 32080*

**Coordinate regulation of the ribosomal protein genes is entrusted to a number of signal transduction pathways that can abruptly induce or silence the ribosomal genes. We have uncovered a cellular model system, which selectively induces the ribosomal protein S25 gene in hepatoma cells that are stressed by nutrient deprivation. Our results indicate that p53 along with two other identified proteins, MTF-1 and La, post-transcriptionally regulate the synthesis of the S25 protein by controlling the nuclear export of the stress-induced S25 mRNA. This system is unique in that the nuclear-retained S25 mRNA is exported to the cytosol only upon replenishment or alternatively after prolonged starvation to participate in a p53-mediated apoptotic sequence of events. This p53-dependent survival or death pathway involves a previously unreported protein relationship among these three actors, one of which, MTF-1, has not yet been shown to have RNA-binding characteristics.**

Eukaryotic cells expend much of their energy on synthetic components of protein biosynthesis, and as a result, the cell has evolved mechanisms that tightly control the synthesis of the components of the ribosomes. Ribosome biogenesis is coordinately controlled by a variety of mechanisms including transcriptional and post-transcriptional regulation in response to changes within or outside the cell such as carbon source and nutrient availability (1, 2). Control of transcription within the cell by metabolites is a mechanism that allows cells to respond to changes in their nutritional environment. There is a number of genes for which transcription is enhanced following nutrient deprivation by amino acid starvation, among these include asparagine synthetase (*ASNS*)[1] and ribosomal protein S25 (*RPS25*) (2). The initial cellular response to amino acid limitation results in increased AS mRNA and thereby increased functional AS and asparagine protein (3, 4). Cells lacking in AS activity and thus in asparagine exhibit cell cycle arrest (5) and induction of apoptosis (6, 7). S25 mRNA levels also are increased in the initial response to amino acid deprivation (8), but unlike AS, the selective uncoordinated increase in S25 expression signals the induction of apoptosis (9). Moreover, the studies using ribosomal protein S6-deficient *versus* wild-type liver cells suggest that a defect in ribosome biogenesis can cause activation of a p53-mediated checkpoint, leading to cell cycle block and potential DNA damage (10). However, the mechanism(s) of cellular arrest and programmed cell death in response to cellular stress such as oncogenic signals and nutrient deprivation remain poorly understood. Interestingly, in our experimental model, the cells have devised a mechanism in the early stages of starvation to coordinate the levels of the S25 protein with those of the other ribosomal protein genes by preventing the export of the increased S25 mRNA, thus making it unavailable for translation. Preliminary analysis in response to the *RPS25* up-regulation and nuclear retention of its mRNA suggested that the storage of mRNA could permit an elevated rate of synthesis for this particular ribosomal protein to be initiated immediately upon cellular replenishment (8, 11). Stress conditions in the form of salt, heat, and ethanol have been reported to induce nuclear retention of the bulk of poly(A) RNA but not mRNAs encoding heat shock proteins. The export competence of the heat shock protein mRNAs seems to result from differences in nature of the ribonucleoprotein (RNP) package (12, 13). Thus, we explored the RNA-trafficking portion of this sequence of events to determine whether specific protein(s) or protein complexes might be associated with and/or responsible for the up-regulation and nuclear retention of the S25 mRNA and thus to better characterize this phenomenon.

## EXPERIMENTAL PROCEDURES

*Cell Culture and Preparation of Cellular Extracts*—Fao and H4IIE hepatoma cells were grown in modified minimum Eagle's medium, pH 7.4, whereas H5 hepatoma cells were grown in modified Ham's F-12 medium in which 5% $CO_2$ was added to the atmosphere as described previously (5). The cells were grown to 70–80% confluence and were either transferred to Hanks'-buffered saline solution or MEM lacking serum as the amino acid-starved and fed conditions, respectively. Monolayers of Fao, H4IIE, and H5 rat hepatoma cells were harvested after growth for 9 or 24 h in either amino acid-starved or fed medium. Cytosolic and nuclear extract fractions were then separated and isolated using a stepwise lysis of cells that gives functional nuclear and cytoplasmic protein fractions (NE-PER, Pierce) following the manufacturer instructions. Protein content was determined using BCA protein assay (Pierce), and samples were stored at −80 °C.

*RNA Synthesis and Labeling*—Biotinylated RNA was synthesized by *in vitro* transcription using T7 or SP6 RNA polymerase following a modified Maxiscript kit protocol (Ambion). The rCTP was replaced with [$^{32}$P]$\alpha$CTP or biotinylated rCTP containing an 11-carbon linker to achieve a labeling of ~4–6 biotin groups/100 nucleotides of RNA (Roche Molecular Biochemicals). The DNA templates corresponded to the complete transcript of rat ribosomal protein S25 (466 nucleotides in length) and a 900-nucleotide fragment of rat asparagine synthetase. *Pleuronectes americanus* p-glycoprotein (pgpB) DNA template was used for the synthesis of an unrelated RNA control.

*Electromobility Shift Assay*—Electromobility shift assay method was followed as described with minor modifications (14). Biotin-labeled

* This work was supported in part by the National Institutes of Health Grant DK49644. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: The Whitney Laboratory, University of Florida, 9505 Ocean Shore Blvd., St. Augustine, FL 32080. Tel.: 904-461-4031; Fax: 904-461-4008; E-mail: laine@whitney.ufl.edu.

[1] The abbreviations used are: *ASNS*, asparagine synthetase gene; *RPS25*, ribosomal protein S25 gene; AS, asparagine synthetase; MTF-1, zinc finger metal response element-binding transcription factor; La, RNA-binding phosphoprotein antigen; p53, tumor suppressor protein; RNP, ribonucleoprotein; hnRNP, heterogeneous nuclear RNP; mRNP, messenger RNP; S25, ribosomal protein S25; pgpB, *p*-glycoprotein; SELDI, surface-enhanced laser desorption/ionization mass spectrometry; IP, immunoprecipitation; RT, reverse transcription.

RNA (diluted by 50–100-fold) was incubated in the presence or absence of nuclear or cytoplasmic protein extracts pre-treated with RNase T1 and from either amino acid-starved or fed cells. Potential RNA-protein complexes were resolved by agarose electrophoresis and then electroblotted to positively charged nylon membrane (Hybond N). RNA was detected with streptavidin-horseradish peroxidase conjugate using North2South chemiluminescent nucleic acid hybridization and detection kit (Pierce) and evaluated by Fluor-S multi-imager (Bio-Rad).

*Polyacrylamide Gel Electrophoresis and Immunoblotting*—Cytoplasmic and nuclear protein extracts (50 $\mu$g) were separated by 3–8% SDS-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes. Proteins of interest were detected using appropriate antibodies and West femto chemiluminescent detection system (Pierce).

*Northwestern Analysis*—Cytoplasmic and nuclear protein extracts from amino acid-starved or fed cells (20 $\mu$g) were size separated and electroblotted as mentioned above. Membrane bound proteins were renatured overnight as described previously (37) and washed twice after incubation with either radiolabeled S25 or pgpB RNA for 2 h at room temperature. The dried membranes then were exposed overnight to PhosphorImager screens (Molecular Dynamics, Inc).

*Surface Enhanced Laser Desorption/Ionization (SELDI)*—SELDI-time-of-flight mass spectometry was performed using ProteinChip array analysis (Ciphergen Biosystems). The epoxy group substrate of the PS2 chip features were initially coupled with streptavidin to allow subsequent binding of 150 ng of biotin-labeled *in vitro* transcribed S25 RNA. Following a wash step, 25 $\mu$g of nuclear extract protein in a total volume of 50 $\mu$l was incubated with each target feature using a bioprocessor aspect of these chips that allows the incubation of larger volumes. The targets are then fixed with sinapinic acid and then transferred into the chip reader for analysis. All PS2 protein chip arrays were analyzed according to an automated data collection protocol. Data interpretation was augmented by the use of the ProteinChip software, version 2.0.1.

*Immunoprecipitation of Endogenous mRNP Complexes from Cellular Extracts*—Protein A-Sepharose beads were swollen in Tris-based buffer supplemented with 1 mM $MgCl_2$, 0.05% Nonidet P-40, and 5% bovine serum albumin. A 50-$\mu$l aliquot of the protein A-bead slurry was incubated overnight at 4 °C with excess immunoprecipitating antibody. The antibody-coated beads were split into two aliquots, each containing 100 $\mu$g of nuclear extract from either amino acid-starved or fed Fao cells. The immunoprecipitation reactions were incubated at room temperature for 2 h followed by a 1 M urea wash. For RNA extraction, these washed beads were resuspended in Tris-based buffer supplemented with 0.1% SDS and proteinase K, and the RNA was then phenol-chloroform-isoamylalcohol-extracted and ethanol-precipitated. For protein, the washed beads were incubated in sample buffer followed by SDS-PAGE and immunoblot analysis using appropriate antibody.

*Immunoprecipated RNA Analysis*—First strand cDNA was prepared from the immunoprecipitated RNA using Superscript II kit (Invitrogen) following the instructions of the supplier. S25 and AS messages were amplified with gene-specific primers. For relative quantitation, the number of amplification cycles was restricted to the exponential phase of the PCR reaction. The respective products were resolved on 1.2% agarose gel and positively confirmed by nucleotide sequencing.

*Analysis of DNA Fragmentation*—Monolayers of Fao, H4IIE, and H5 rat hepatoma cells were harvested after growth for 24 h in either amino acid-starved or fed medium and treated with lysis buffer per the recommendations of the apoptosis detection kit (R&D Systems). The resultant lysate was treated with DNA extraction buffer prior to DNA precipitation and resuspension in Tris-based buffer. DNA was resolved by 2% agarose gel electrophoresis, and the resulting gel was stained with ethidium bromide and visualized on a UV transilluminator. DNA laddering of 180–200 base pairs is characteristic of apoptosis.

## RESULTS

*S25 mRNA-Protein Interactions in the Nucleus*—Potential RNA-protein interactions were evaluated by agarose-based electromobility shift assays using biotin-labeled full-length S25 RNA as a probe (14). Reconstitution of messenger ribonucleoprotein complexes (mRNP) was performed using the S25 riboprobe incubated in the presence of nuclear extracts pre-treated with RNase T1 to digest the endogenous RNA. At least three proteins are bound to the S25 mRNA from the starved nuclear extracts, thus causing a shift in the mobility of the probe compared with the fed samples (Fig. 1*A*). The addition of 100-fold molar excess unlabeled probe to the reaction mixture eliminated the visibility of the shifted complex (data not shown). Also, the addition of 10-fold molar excess of an unrelated and unlabeled mRNA (the 1 kb of 3′-end fragment of pgpB (GenBank™ accession number AY053461) to the mixture had no effect on the shifted complex (data not shown). These observations suggest that the S25-protein interactions are sequence-specific. To confirm the formation of RNA-protein complexes and to estimate the molecular mass of the RNA-binding proteins Northwestern analysis was performed, again using the full-length S25 RNA as a probe. Results from this assay support the gel-shift data and demonstrate that this probe interacts more strongly with 4–5 different protein bands in starved nuclear extracts when compared with the fed or control nuclear extract samples (Fig. 1*B*, *lanes 1* and *2*). Protein bands with relative mobilities of ~73, 53, 43, and 36 kDa were detected in the nuclear extracts assayed from stressed cells. The visible binding by the probe was significantly reduced in the presence of excess unlabeled S25 RNA (Fig. 1*B*, *lane 3*), and no probe-binding differences were seen when substituting the S25 RNA probe for the unrelated pgpB RNA probe (data not shown). In addition, no S25 probe-binding differences were seen between fed and starved samples using cytosolic extracts (data not shown). A novel application of the ProteinChip SELDI mass spectrometry (Ciphergen Biosystems) was used to confirm the findings from the Northwestern analysis and to more precisely identify the molecular mass of the potential protein candidates in amino acid-starved nuclear extracts that interacted with the S25 RNA probe. The biotin-labeled RNA probe was bound to the streptavidin-coated chip surface to which nuclear extracts were applied. A SELDI analysis of four different pairs of fed/starved extracts identified two distinctive protein peaks (43,537 and 72,830 Da) in the starved extracts compared with the fed nuclear extracts that approximated the relative mobility of polypeptides observed in Northwestern evaluation of

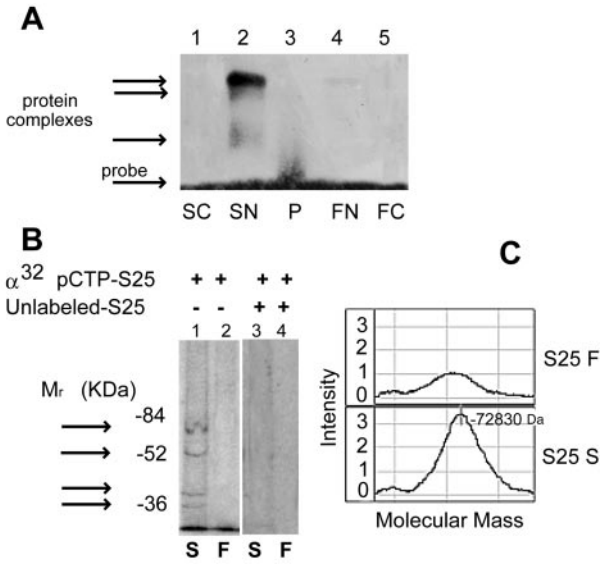


FIG. 1. **Analyses to detect S25 mRNA-binding proteins in Fao hepatoma cells.** *A*, representative gel-mobility shift detection assay showing interactions between starved or fed cellular protein extracts from nuclear (*SN* or *FN*) or cytosolic (*SC* or *FC*) fractions, respectively, and biotin-labeled S25 RNA probe. *Lane 3* shows reaction containing probe (*P*) only. *B*, Northwestern blot showing interaction between amino acid-starved (*S*) and fed (*F*) nuclear extracts and labeled S25 RNA in presence and absence of 100-fold excess of unlabeled probe. *Arrows* depict relative mobility ($M_r$) of most prominent bands. *C*, representative SELDI time-of-flight mass spectroscopy analysis showing molecular mass of protein (72,830 Da, MTF-1) binding to S25 RNA probe from starved nuclear extracts.

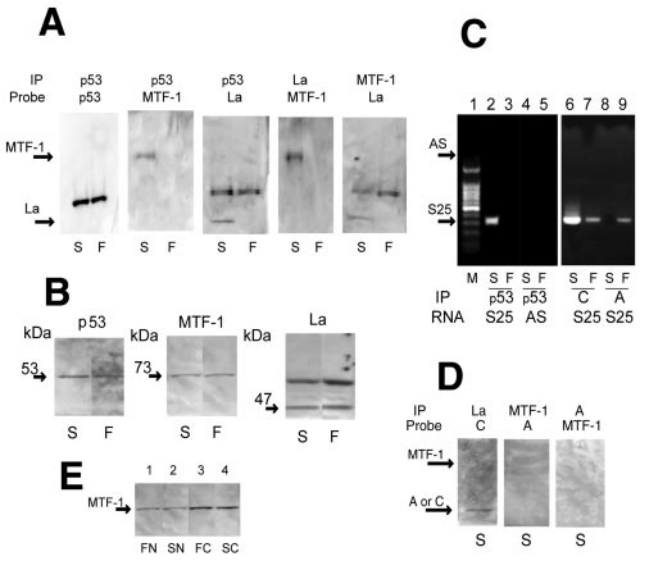


FIG. 2. **Analyses to determine protein make-up of the nuclear-retained S25 mRNA-protein complex in starved Fao hepatoma cells.** *A*, immunoblot analysis of IP reactions in amino acid-starved (*S*) or fed (*F*) cells using indicated IP and probe (*Probe*) antibodies (anti-P53 (Sigma), anti-MTF-1 (from Dr. Glen Andrews, University of Kansas Medical Center); anti-La (from Dr. Daniel Kenan, Duke University Medical Center)). Higher molecular mass bands detected with La antibody probe and observed in both fed and starved extracts were not identified. *B*, immunoblot analysis using equal amounts of fed and starved nuclear protein extracts. *C*, RT-PCR analysis of S25 or AS mRNA associated with mRNP complexes from amino acid-starved (*S*) or fed (*F*) nuclear extracts isolated by IP. *C*, anti-hnRNP C1; *A*, anti-hnRNP A1. *Arrows* indicate base pair size that represents the presence of AS or S25 mRNA. *D*, immunoblot analysis of IP reactions in amino acid-starved cells using indicated Probe antibodies to confirm the presence or absence of hnRNP A and hnRNP C in nuclear-retained complex. *E*, immunoblot analysis using MTF-1 antisera against equal amounts of cytosolic (*SC* and *FC*) and nuclear (*SN* and *FN*) extracts from amino acid-starved and fed cells, respectively, to compare MTF-1 cellular compartment protein levels.

starved nuclear samples (Fig. 1*C*). A search of the protein data base to identify proteins within a small window of these molecular masses gave two potential candidates, tumor suppressor protein p53 (43,451 Da) and zinc finger metal response element-binding transcription factor MTF-1 (72,633 Da, mouse). A third candidate, the RNA-binding phosphoprotein La antigen (47,777 Da) was identified from studies performed in another laboratory[2] that analyzed mRNA subsets in ribonucleoprotein complexes using cDNA arrays (15).

*Starvation-induced RNP Complex*—The above *in vitro* reconstitution techniques established a framework for examining S25 mRNA-protein interactions. To faithfully reflect the structure of the native complexes, IP experiments were performed to detect messenger ribonucleoprotein (mRNP) complexes involved in the post-transcriptional regulation/retention of S25 mRNA. IP experiments performed on the nuclear extracts and subsequent immunoblot assays indicated that p53, MTF-1, and the La antigen form or are included in a complex associated specifically with the nuclear extracts in amino acid-starved but not fed Fao cells (Fig. 2*A*). The immunoblots prepared using nuclear and cytosolic extracts from both fed and starved cells showed no obvious increase in protein expression or any apparent translocation of protein levels from one compartment to another for any of these three proteins in response to the starvation (Fig. 2*B*). RNase treatment of starved nuclear extracts prior to the immunoprecipitation step eliminated co-precipitation of the other two proteins, demonstrating the RNA dependence of the complex (data not shown). mRNA associated with the mRNP complexes isolated by IP were amplified by gene-specific primers by RT-PCR techniques. S25 transcripts could be amplified from the IP reactions from starved but not fed nuclear extracts (Fig. 2*C*, *lanes 2* and *3*). Because AS is also increased in Fao cells in response to amino acid starvation (3), the presence of As mRNA in the complex was analyzed as a control. We checked for the presence of AS mRNA, but after 40 cycles of amplification, there was no indication of this message included with the starvation-induced protein complex (Fig. 2*C*, *lanes 4* and *5*). This finding fits with the known regulation of the AS message, which unlike S25 mRNA is exported to the cytoplasm where it is translated into the functional AS protein that is necessary for cell survival. Therefore, the three proteins are included in a RNA-dependent complex associated specifically with the nuclear-retained S25 mRNA under starved conditions.

*Heterogeneous Nuclear Protein(s) (hnRNP) A and C Interaction with S25 mRNA-Protein Complex*—The random diffusion-based transport model to transport RNA from the nucleus suggests that hnRNPs bind to all transcripts and escort the pre-mRNAs through the maturation process and allow these messages to become nuclear export-competent (16). Some of these proteins, such as hnRNP A1, K, and E, actually shuttle between the nucleus and cytoplasm accompanied by their respective mRNAs (17). Other proteins, such as hnRNPs C1 and C2, are restricted to the nucleus and are thought to perform, in conjunction with their nuclear/cytoplasm shuttling partners, an important post-transcriptional regulatory step in the pathway of gene expression (18). RT-PCR analysis indicated that both hnRNPs A1 and C1 were found associated with normal or fed S25 nuclear mRNAs, whereas only hnRNP C1 co-precipitated with the starvation-dependent retained complex (Fig. 2, *C*, *lanes 6–9*, and *D*). Thus, at least for this situation, the nuclear retention of S25 under starved conditions was not dependent or a result of the interaction with nucleus-restricted hnRNP C1. Instead, the S25 mRNA nuclear retention is unique by the lack of involvement of hnRNP A1 and by its shuttle mechanism of mRNA export. The hnRNP literature suggest that there is a struggle between RNA-binding proteins that are nuclear-restricted and nuclear export-competent (18). Both protein forms bind transcripts in which the nuclear-retained form is released from the mRNP prior to export, and the shuttling form remains part of the complex during export through the pore and association with the polysomes. The mechanism leading to the retention of S25 mRNA within the nucleus could be a result of the starvation-induced binding of p53, MTF-1, and La proteins along with the hnRNP C to make the S25 mRNA export incompetent. Alternatively, the addition of hnRNP A to the mRNP complex might be essential to signal the export competency and/or to remove the nuclear retention signal, namely hnRNP C from the complex. Under starvation conditions, the association of p53, MTF-1, and La with the S25 mRNA might interfere with the binding of hnRNP A, thus obstructing the export signal.

*S25 mRNA Nuclear Retention Depends on Functional p53*—To further investigate the role of p53 in our model system, the rat hepatoma cell line H5, which contains mutant p53, was evaluated for changes in S25 expression under conditions of nutrient deprivation. The H5 cells compared with the p53 wild-type H4IIE rat hepatoma parental line do not express functional p53 as demonstrated by the absence of p53-dependent induction of p21 and methylguanine-DNA methyltransferase and by the failure of p53-dependent cell cycle blockage upon ionizing radiation (19). Within our 9-h time period of amino acid starvation, Fao cells undergo little or no change in

[2] D. J. Kenan, personal communication.

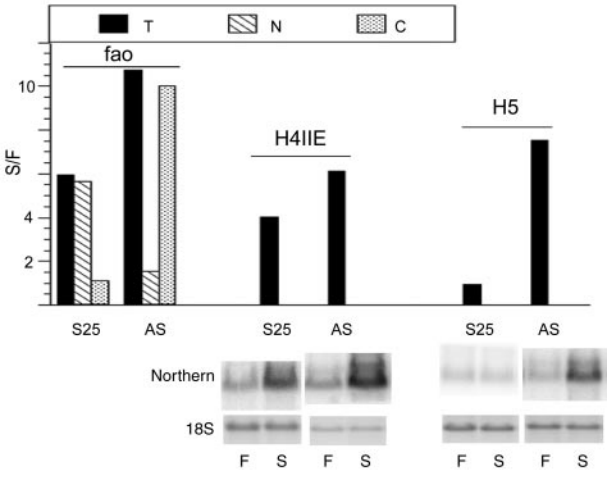


FIG. 3. **S25 and AS mRNA levels in Fao (3, 8), H4IIE, and H5 rat hepatoma cells during amino acid starvation.** Cells cultured under either amino acid-starved (*S*) or fed (*F*) conditions for 9 h were used to obtain cellular fractions for isolation of RNA: total cell (*T*), cytoplasm (*C*), or nucleus (*N*) and Northern analysis performed on these samples. The starved/fed ratio of the Northern data is presented and representative of two or more analyses for AS and S25.

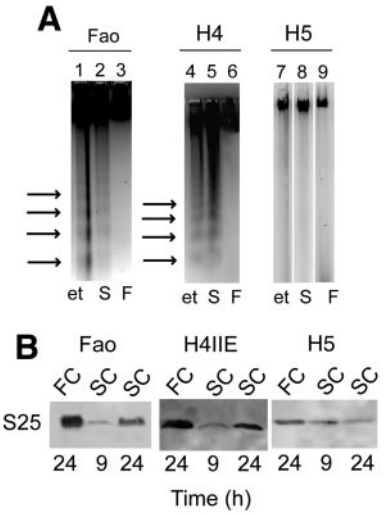


FIG. 4. **Effects of prolonged amino acid starvation (24 h) on DNA fragmentation and S25 protein in Fao, H4IIE, and H5 rat hepatoma cells.** *A*, DNA fragmentation was evaluated on amino acid-starved (*S*), fed (*F*), and fed cells treated with etoposide (*et*), which induces apoptosis. *Arrows* depict DNA bands representative of apoptotic DNA fragmentation. *B*, effect of prolonged (24 h) *versus* short term (9 h) amino acid starvation on S25 cellular protein levels in fed (*FC*) and starved (*SC*) cytosol fractions.

morphology in overall protein or RNA synthesis rates or in the levels of p53 expression compared with fed Fao cells (Fig. 2*A*). Examination of S25 expression in these cells compared with the parent H4IIE showed that the RNA level was not increased upon starvation (Fig. 3), and consequently no nuclear retention of the mRNA is realized. Therefore, a functional p53 protein is not only implicated in the nuclear retention of the S25 mRNA but very well may be involved in its initial transcriptional up-regulation observed with the stress condition. There is no change in the AS expression characteristics in the H5 compared with Fao or H4IIE cells in which increased AS mRNA levels are observed in amino acid-starved *versus* fed cells (Fig. 3). In addition, no AS mRNA is associated with the nuclear-retained RNA-protein complex in starved Fao cells (Fig. 2*C*). These observations taken together suggest that the signaling for AS up-regulation in response to amino acid starvation is p53-independent and by a different mechanism than observed in S25 regulation.

Ribosomal protein S25 has been found to play a growth-suppressive role in the nonproliferating liver, but its expression is reduced during liver regeneration (20). Thus, the suggestion was that S25 expression is reduced during cell growth and increased during cell death. Recent studies looking at increased gene expression associated with the apoptotic process identified ribosomal protein S25 as one of the up-regulated genes in the apoptotic pathway leading to primary spermatocyte cell death (9, 20). Apoptosis is a programmed process and is thought to involve orderly changes in gene expression (21), therefore, any involvement of S25 at the early stage of this process might suggest some pivotal role. In an attempt to understand the relationship between S25 expression and the induction of apoptosis, DNA fragmentation and S25 protein levels were determined throughout the starvation process. DNA fragmentation analysis confirmed that prolonged amino acid starvation induces apoptosis in Fao and H4IIE cells, which also now exhibit increased levels of S25 protein (Fig. 4). The p53-deficient H5 cells show no induction of apoptosis or increase in S25 protein levels under the prolonged starvation (Fig. 4). These observations strongly suggest that the induced apoptosis and increased S25 protein levels are p53-mediated.

## DISCUSSION

The overall logic for the selection of factors p53, MTF-1, and La by the cell to exert this stringent control of the expression of S25 protein remains to be elucidated. MTF-1 has been shown to be impacted by oxidative stress (22), hypoxia (23), and essential for metal ion regulation of metallothionein gene expression in which MTF-1 serves as an intracellular zinc sensor to activate metallothionein gene expression (23, 24). The activation of the latent cytosolic MTF-1 with zinc results in nuclear translocation of MTF-1 followed by increased binding to metal response elements in the metallothionein promoter (25). Furthermore, numerous signal transduction pathways apparently interact with the activities of MTF-1 that have resulted in MTF-1 to be considered an essential gene (26). Under amino acid-starvation conditions, there is no obvious translocation of MTF-1 from the cytosol to the nucleus of the cell (Fig. 2*E*). The implication would be that the signal for MTF-1 to bind the nuclear-retained S25 mRNA under the conditions of amino acid deprivation is neither metal ion-dependent nor MTF-1 nuclear translocation-dependent and therefore governed by a mechanism independent of its transcriptional regulation of metallothionein that is associated with heavy metal homeostasis or oxidative stress (24, 27). To our knowledge, MTF-1 has not been described previously to have RNA-binding properties or any function that directly involves p53 or La proteins. Our observations preview a new and novel biological function for MTF-1 and increase speculation on its overall significance on gene regulation.

The role for the La phosphoprotein in transcription remains somewhat obscure, particularly for binding polymerase II transcripts (28). Yet, there are reports of La interacting with ribososmal protein mRNAs mediating the transport of RNAs as a chaperone in post-transcriptional processing, processing transcripts into specific ribonucleoproteins, and a proteolysis-mediated mechanism of La relocation to the cytoplasm during apoptosis (28–30). At this point we have no other biochemical information to elucidate the purpose or role of La in the up-regulation and/or nuclear retention of S25 mRNA in response to amino acid starvation.

If uncoordinated increased levels of ribosomal protein S25 are involved in the signaling process for initiating apoptosis,

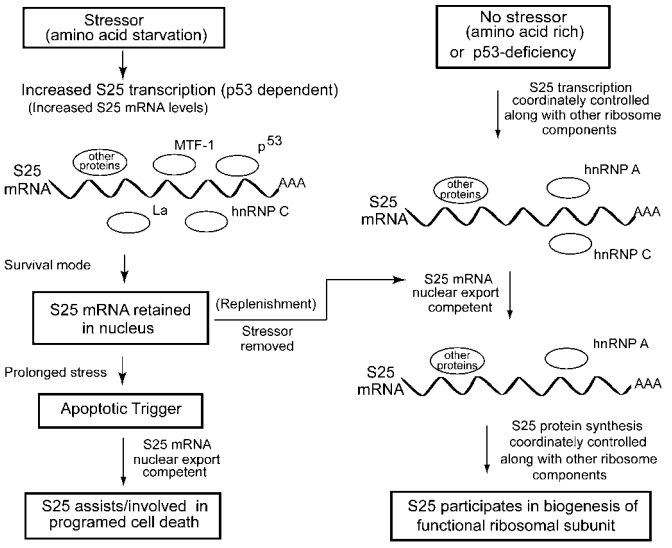


FIG. 5. **Mechanistic model of stressor-induced survival or programmed-death pathway (*left*) *versus* normal unstressed amino acid-rich pathway (*right*) in cells.** Amino acid starvation results in the up-regulation of *RPS25* and its increased mRNA levels are retained within the cellular nucleus and associated with numerous proteins including p53, MTF-1, and La. This RNA-protein complex is unique by the absence of hnRNP *A*, a replenishment of nutrients or elimination of the stressor at this point will result in S25 mRNA becoming nuclear export competent, a loss of p53, MTF-1, and La binding, the initiation of hnRNP A binding, and a return to cellular metabolic balance. Conversely, prolonged starvation or stress will activate an apoptotic trigger or signal and result in selective translation of S25, which then assists or is involved in the apoptotic process.

exacting control of the increased levels of S25 mRNA would be required to regulate S25 translation and thus precisely determine the ultimate fate of the cell. Replenishment of the cells would reduce S25 mRNA levels and associated retention and allow the expression of the S25 protein to become coordinated with expression levels of the other ribosomal proteins, thus restoring the balance for the translation machinery (8). Conversely, prolonged deprivation of nutrients would result in further deterioration of cellular metabolism, eventually exceeding some critical metabolic threshold for survival and then followed by initiation of pathway(s) leading to programmed cell death. In the proposed mechanistic model, this phase would signal the release of the nuclear accumulated S25 mRNA leading to elevated levels of S25 protein, which we did observe (Fig. 4*B*), and initiation of yet an undefined role of ribosomal protein S25 in apoptosis (Fig. 5). Increased ribosomal protein S25 mRNA levels were reported in adriamycin-resistant HL60 cells without concurrent S25 protein increase (31), and common nuclear- and nucleolar-targeting features were observed between S25 protein and the HIV-1 REV protein (32), both examples supporting the premise of a more complex and encompassing regulatory role for this ribosomal protein. Our observations illustrate the biochemical flexibility of p53 and that it engages in a multitude of pathways to mediate metabolic control. Although there is evidence for p53-dependent cross-talk between ribosome biogenesis and the cell cycle (33), there are limited examples of the role p53 in gene and cellular growth regulation resulting from its RNA-binding properties or of p53-mediated transcription-independent apoptosis (21, 34–36). The relationship of p53 and S25 mRNA gives an example fulfilling both of these roles and illuminates an unique pathway of cellular regulation involving both survival and programmed death. Therefore, in addition to the previously described S25 nutrient anticipatory-response, we are proposing the identification of a transcription-independent p53-mediated apoptotic process as the second phase of cellular response to the mRNA nuclear retention from nutrient deprivation (See Fig. 5).

*Acknowledgments*—We thank Dr. Glen Andrews (University of Kansas Medical Center, Kansas City, KS) for generously donating antisera to MTF-1; Dr. Daniel Kenan (Duke University Medical Center, Durham, NC) for generously providing antisera to the La antigen; Dr. Gideon Dreyfuss of (University of Pennsylvania School of Medicine, Philadelphia, PA) for kindly providing antibodies to hnRNP A and C1; Dr. Melvin Center of Kansas State University, Manhattan, KS) for kindly providing antisera to the S25 ribosomal protein; Dr. Michael Campa of (Duke University Medical Center, Durham, NC) for assistance in SELDI; and Dr. Michael Kilberg of (University of Florida, Gainesville, FL) for comments on the manuscript.

REFERENCES

1. Mager, W. H. (1988) *Biochim. Biophys. Acta* **949,** 1–8
2. Laine, R. O., Hutson, R. G., and Kilberg M. S. (1996) *Prog. Nucleic Acid Res.* **53,** 219–245
3. Hutson, R. G., and Kilberg, M. S. (1994) *Biochem. J.* **304,** 745–750
4. Barbosa-Tessmann, I. P., Chen, C., Zhong, C., Siu, F., Schuster, S. M., Nick, H., and Kilberg, M. S. (2000) *J. Biol. Chem.* **275,** 26976–26985
5. Gong, S. S., Guerrini, L., and Basilico, C. (1991) *Mol. Cell. Biol.* **11,** 6059–6066
6. Story, M. D., Voehringer, D. W., Stephens, L. C., and Meyn, R. E. (1993) *Cancer Chemother. Pharmacol.* **32,** 129–133
7. Bussolati, O., Belletti, S., Uggeri, J., Gatti, R., Orlandini, G., Dall'Asta, V., and Gazzola, G. C. (1995) *Exp. Cell Res.* **220,** 283–291
8. Laine, R. O., Shay, N. F., and Kilberg, M. S. (1994) *J. Biol. Chem.* **269,** 9693–9697
9. Wang, W., and Chapin, R. E. (2000) *Toxicol. Sci.* **56,** 165–174
10. Volarevic, S., Stewart, M. J., Ledermann, F. Z., Terracciano, L., Montini, E., Grompe, M., Kozma, S. C., and Thomas, G. (2000) *Science* **288,** 2045–2047
11. Kilberg, M. S., Hutson, R. G., and Laine, R. O. (1994) *FASEB J.* **8,** 13–19
12. Saavedra, C., Tung, K. S., Amberg, D. C., Hopper, A. K., and Cole, C. N. (1996) *Genes Dev.* **10,** 1608–1620
13. Gallouzi, I., Brennan, C. M., Stenberg, M. G., Swanson, M. S., Eversole, A., Maizels, N., and Steitz, J. A. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97,** 3073–3078
14. Rodgers, J. T., Patel, P., Hennes, J. L., Bolognia, S. L., and Mascotti, D. P. (2000) *Anal. Biochem.* **277,** 254–259
15. Tenenbaum, S. A., Carson, C. C., Lager, P. J., and Keene, J. D. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97,** 14085–14090
16. Politz, J. C., and Pederson T. (2000) *J. Struct. Biol.* **129,** 252–257
17. Pinol-Roma, S., and Dreyfuss, G. (1993) *Trends Cell Biol.* **3,** 151–155
18. Nakielny, S., and Dreyfuss. G. (1996) *J. Cell Biol.* **134,** 1365–1373
19. Grombacher, R., Eichhorn, U., and Kaina, B. (1998) *Oncogene* **17,** 845–851
20. Sun, D., Kar, S., and Carr, B. I. (1995) *Cancer Lett.* **89,** 73–79
21. Woods, D. B., and Vousden, K. H. (2001) *Exp. Cell Res.* **264,** 56–66
22. Dalton, T. P., Li, Q., Bittel, D., Liang, L. C., and Andrews, G. K. (1996) *J. Biol. Chem.* **271,** 26233–26241
23. Murphy, B. J., Andrews, G. K., Bittel, D., Discher, D. J., McCue, J., Gren, C. J., Yanovsky, M., Giaccia, A., Sutherland, R. M., Laderoute, K. R., and Webster, K. A. (1999) *Cancer Res.* **59,** 1315–1322
24. Heuchel, R., Radtke, F., Georgiev, O., Stark, G., Aguet, M., and Schaffner, W. (1994) *EMBO J.* **13,** 2870–2875
25. Smirnova, I. V., Bittel, D. C., Ravindra, R., Jiang, H., and Andrews, G. K. (2000) *J. Biol. Chem.* **275,** 9377–9384
26. Gunes, C. Heuchel, R., Georgiev, O., Muller, K. H., Lichtlen, P., Bluthmann, H., Marino, S., Aguzzi, A., and Schaffner, W. (1998) *EMBO J.* **17,** 2846–2854
27. Dalton, T. P., Bittel, D., and Andrews, G. K. (1997) *Mol. Cell. Biol.* **17,** 2781–2789
28. Maraia, R. J., and Intine, R. V. A. (2001) *Mol. Cell. Biol.* **21,** 367–379
29. Carter, M. S., and Sarnow, P. (2000) *J. Biol. Chem.* **275,** 28301–28307
30. Croslo, C., Boyl, P. P., Loreni, F., Pierandrei-Amaldi, P., and Amaldi, F. (2000) *Nucleic Acids Res.* **28,** 2927–2934
31. Li, M., and Center, M. S. (1992) *FEBS Lett.* **298,** 142–144
32. Kubota, S., Copeland, T. D., and Pomeantz, R. J. (1999) *Oncogene* **18,** 1503–1514
33. Pestov, D. G., Strezoska, A., and Lau, L. F. (2001) *Mol. Cell. Biol.* **21,** 4246–4255
34. Moll, U. M., and Zaika, A. (2001) *FEBS Lett.* **493,** 65–69
35. Miller, S. J., Suthiphongchai, T., Zambetti, G. O., and Ewen, M. E. (2000) *Mol. Cell. Biol.* **20,** 8420–8431
36. Del Sal, G., Koaro, E. M., Utrera, R., Cole, C. N., Levine, A. J., and Schneider, C. (1995) *Mol. Cell. Biol.* **15,** 7152–7160
37. Bichsel, V. E., Walz, A., and Bickel, M. (1997) *Nucleic Acids Res.* **25,** 2417–2423