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

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Abbreviation: MTR-1, zinc finger metal response element-binding transcription factor; La, RNA-binding phosphoprotein antigen; p53, tumor suppressor protein; RNP, ribonucleoprotein; hnRNP, heterogeneous nuclear RNP; RpS25, ribosomal protein S25; pgpB, p-glycoprotein; SELDI, surface enhanced laser desorption/ionization mass spectrometry

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SUMMARY:
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 ribosomal protein S25 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 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 which allows cells to respond to changes in their nutritional environment. There are a number of genes for which transcription is enhanced following nutrient deprivation by amino acid starvation, among these include asparagine synthetase (AS) and ribosomal protein S25 (2). The initial cellular response to amino acid limitation results in increased AS mRNA and thereby increased functional AS and asparagine proteins (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 induction of apoptosis (9). Moreover, 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 export of the increased S25 mRNA and thus making it unavailable for translation. Preliminary analysis in response to the S25 up-regulation and nuclear retention of its mRNA suggested that 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 a 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 Eagle’s medium (MEM) (pH 7.4) whereas, H5 hepatoma cells were grown in modified Ham’s F12 in which 5 % CO2 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 h or 24 h in either amino acid-starved or fed medium. Cytosolic and nuclear extract fractions were then separated and isolated using a step wise lysis of cells that gives functional nuclear and cytoplasmic protein fractions (NER-PER, Pierce) following the manufacturers instructions. Protein content was determined using BCA Protein Assay (Pierce) and samples stored at -80 °C.

RNA Synthesis and Labeling- Biotinylated RNA was synthesized by invitro transcription using T7 or SP6 RNA polymerase following a modified Maxiscript kit protocol (Ambion). The rCTP was replaced with 32PαCTP or biotinylated rCTP containing an 11-carbon linker to achieve a labeling of approximately 4-6 biotin groups per 100 nucleotides (nt) of RNA (Roche 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. P. americanus p-glycoprotein (pgpB) DNA template was used for the synthesis of an unrelated RNA control.
Electromobility Shift Assay- EMSA method was followed as described with minor modifications (14). Biotin-labeled RNA (diluted by 50-100 fold) was incubated in 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-Horse Radish Peroxidase conjugate using North2South chemiluminescent nucleic acid hybridization and detection kit (Pierce) and evaluated by Fluor-S multiimager (Biorad).

Polyacrylamide Gel Electrophoresis and Immunoblotting- Cytoplasmic and nuclear protein extracts (50 µg) were separated by 3-8% SDS-polyacrylamide gel electrophoresis and transferred to PVDF 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 µg) were size separated and electroblotted as mentioned above. Membrane bound proteins were renatured overnight as described (37) and washed twice after incubation with either radiolabeled S25 or pgpB RNA for 2 hours at room temperature. The dried membranes then were exposed overnight to PhosphorImager (Molecular Dynamics, Inc) screens.

Surface Enhanced Laser Desorption/Ionization (SELDI)- SELDI-time of flight mass spectrometry was performed using (Ciphergen biosystems) ProteinChip Array analysis. The epoxy group substrate of the PS2 chip features were initially coupled with streptavidin to allow subsequent binding of 150 ng biotin-labeled invitro transcribed S25 RNA. Following a wash step, 25 µg nuclear extract protein in total volume 50 µl was incubated with each target feature using bio-processor aspect of these chips which allows 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-base buffer supplemented with 1 mM MgCl₂, 0.05 % Nonidet P-40, and 5 % BSA. A 50 µl 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 µg 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.

**Immunoprecipitated RNA Analysis**– First strand cDNA was prepared from the immunoprecipitated RNA using Superscript II kit (Life Technologies) 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 Apoptosis Detection Kit recommendation (R&D Systems). The resultant lysate was treated with DNA extraction buffer prior to DNA precipitation and resuspension in Tris-base buffer. DNA was resolved by 2 % agarose gel electrophoresis and the resulting gel was stained with ethidium bromide and visualized on an UV transilluminator. DNA laddering of 180-200 basepairs 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 and thus causing a shift in the probe’s mobility, compared to the fed samples (Fig. 1A). Addition of 100-fold molar excess unlabeled probe to the reaction mixture eliminated visibility of shifted complex (data not shown). Also, addition of 10-fold molar excess of an unrelated, unlabeled mRNA (the 1 Kb 3’-end fragment of Pleuronectes americanus p-glycoprotein B (pgpB), 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 to the fed or control nuclear extract samples (Fig. 1B, lane 1 and 2). Protein bands with relative mobilities of approximately 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. 1B, 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 surface enhanced laser desorption/ionization (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. SELDI analysis of four different pairs of fed/starved extracts identified two distinctive protein peaks (43,537 and 72,830 Da) in the starved compared to the fed nuclear extracts that approximated the relative mobility of polypeptides observed in Northwestern evaluation of starved nuclear samples (Fig. 1C). Search of the protein database 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 (personnel communication, Dr. Daniel J. Kenan, Duke University) 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, immunoprecipitation (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. 2A). 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. 2B). 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. 2C, lane 2 and 3). Since 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. 2C, lane 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 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.

*hnRNP A and C Interaction with S25 mRNA-protein Complex*- The random diffusion-based transport model to transport RNA from the nucleus suggests that heterogeneous nuclear protein(s) (hnRNP) 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). Others, such as hnRNP C1 and C2 are restricted to the nucleus and are thought to, in conjunction with their nuclear/cytoplasm shuttling partners, perform an important post-transcriptional regulatory step in the pathway of gene expression (18). RT-PCR analysis indicated that both hnRNP 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. 2C, lanes 6-9, and Fig. 2D). Thus, at least for this situation, the nuclear retention of S25 under starved conditions was not dependent or a result of interaction with nucleus restricted hnRNP C1. Instead, the S25 mRNA nuclear retention is unique by the lack of involvement of hnRNP A1 and thereby its shuttle mechanism of mRNA export. The hnRNP literature suggests 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, 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 to 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, methylguanine-DNA methyltransferase (MGMT) and 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 morphology, in overall protein or RNA synthesis rates or in the levels of p53 expression compared to fed Fao cells (Fig. 2A). Examination of S25 expression in these cells compared to 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 to 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. 2C). 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). Apoptosis is a programmed process and is thought to involve orderly changes in gene expression (21); therefore, 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 25 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 (MT) gene expression in which MTF-1 serves as an intracellular zinc sensor to activate MT gene expression (23, 24). Activation of the latent cytosolic MTF-1 with zinc results in nuclear translocation of MTF-1 followed by increased binding to metal response elements (MREs) in the MT promoter (25). Furthermore, numerous signal transduction
pathways apparently interact with MTF-1’s activities which 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. 2E). The implication would be that the signal for MTF-1 to bind the nuclear retained S25 mRNA under conditions of amino acid deprivation is neither metal ion- nor MTF-1 nuclear translocation-dependent, and therefore governed by a mechanism independent of its transcriptional regulation of MT that is associated with heavy metal homeostasis or oxidative stress (24, 27). To our knowledge MTF-1 has not previously been described to have RNA-binding properties nor 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 ribosomal 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, 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 expression of the S25 protein to become coordinated with expression levels of the other ribosomal proteins and thus restore 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 release of the nuclear accumulated S25 mRNA, leading to elevated levels of S25 protein, which we did observe (Fig. 4B) and initiation of yet 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 p53’s role 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 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 below).
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FIGURE LEGENDS:

Fig. 1. Analyses to detect S25 mRNA binding proteins in Fao hepatoma cells. (A) Representative gel-mobility shift detection assay showing interactions between starved (S) or fed (F) cellular protein extracts from nuclear (N) or cytosolic (C) fractions 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, likely MTF-1) binding to S25 RNA probe from starved (S) 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 immunoprecipitation (IP) reactions in amino acid-starved (S) or -fed (F) cells using indicated IP and Probe antibodies (anti-P53, Sigma; anti-MTF-1 (acknowledgements); anti-La (acknowledgements)). 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 presence of AS or S25 mRNA. (D) Immunoblot analysis of (IP) reactions in amino acid-starved (S) cells using indicated Probe antibodies to confirm presence or absence of hnRNP A and C in nuclear retained complex. (E) Immunoblot analysis using MTF-1 antisera against equal amounts of cytosolic (C) and nuclear (N) extracts from amino acid-starved (S) and -fed (F) cells to compare MTF-1 cellular compartment protein levels.
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 is 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 etopside (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 cytosol (C) fraction.

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 up-regulation of S25 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. Replenishment of nutrients or elimination of the stressor at this point will result in S25 mRNA becoming nuclear export competent, loss of p53, MTF-1 and La binding, initiation of hnRNP A binding, and 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.
Stressor (amino acid starvation)

Increased S25 transcription (p53 dependent)
(Increased S25 mRNA levels)

S25 mRNA

other proteins MTF-1 p53

S25 mRNA retained in nucleus

Survival mode

Prolonged stress

Apoptotic Trigger

S25 mRNA nuclear export competent

S25 assists/involved in programed cell death

No stressor (amino acid rich)
or p53-deficiency

S25 transcription coordinately controlled along with other ribosome components

S25 mRNA

other proteins

hnRNP A

S25 mRNA nuclear export competent

Stressor removed

S25 mRNA

other proteins

hnRNP A

S25 protein synthesis coordinately controlled along with other ribosome components

S25 participates in biogenesis of functional ribosomal subunit
Ribosomal protein S25 mRNA partners with MTF-1 and La to provide a p53-mediated mechanism for survival or death
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