The p43 Component of the Mammalian Multi-synthetase Complex Is Likely To Be the Precursor of the Endothelial Monocyte-activating Polypeptide II Cytokine*

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p43 is one of the three auxiliary components invariably associated with nine aminoacyl-tRNA synthetases as a multienzyme complex ubiquitous to all eukaryotic cells from flies to humans. The cDNA encoding the hamster protein was isolated by using mixed oligonucleotides deduced from peptide sequences. The 359-amino acid protein is the hamster homologue of the recently reported murine and human EMAP II cytokine implicated in a variety of inflammatory disorders. The sequence of several proEMAP II proteins suggests that the p43 component of the complex is the precursor of the active mature cytokine after cleavage at a conserved Asp residue. The COOH-terminal moiety of p43 is also homologous to polypeptide domains found in bacterial methionyl- or phenylalanyl-tRNA synthetases and in the yeast Arc1p/G4p1 protein that associates with yeast methionyl-tRNA synthetase. Our results implicate the COOH-terminal moiety of p43 as a RNA binding domain. In the native state, as a component of the multisynthetase complex, p43 may be required for tRNA channeling and, after proteolytic processing occurring in tumor cells, would acquire inflammatory properties possibly related to apoptosis. The release of a truncated p43 from the complex could be involved in mediation of the signaling of tumor cells and stimulation of an acute inflammatory response.

Aminoacyl-tRNA synthetases catalyze the activation of their cognate amino acid and transfer to the relevant tRNA. In mammals, this family of enzyme contributes two distinct high molecular mass structures: a complex made of nine synthetases and a complex involving valyl-tRNA synthetase and the cognate amino acid and transfer to the relevant tRNA. In the valyl-tRNA synthetase–EF-1H complex, this domain has been involved in protein-protein interaction between the β and γ subunits of the eukaryotic elongation factor EF-1H (10) or between valyl-tRNA synthetase and EF-1H (11). This led us to suggest that p18 is involved in anchoring the multisynthetase complex to the elongation factor EF-1H in a transient manner, thus providing the means of a vectorial transfer of aminoacylated tRNA from the synthetases to the factor.

The p38 component of the complex was tentatively identified as casein kinase I with a role in regulating synthetase activities (12), but recent cloning of its cDNA dismisses this proposed functional assignment and suggests that p38 contributes a template protein for the assembly of the complex.† The identity and role of the p43 component were not established.

To gain further insight into the function of the multisynthetase complex, we focused on the identification of its auxiliary role in the complex. In the present study, we proceed to the isolation and characterization of the p43 cDNA to investigate its functional role in the complex. We show here that p43 is a RNA binding protein that shares homology to some extent with the yeast nucleic acid binding protein G4p1/Arc1p (13, 14), which associates with methionyl- or glutamyl-tRNA synthetase. Furthermore, p43 is identical to a gene product identified during the course of this work to an inflammatory cytokine called EMAP II, elicited by tumor cells (15).

EXPERIMENTAL PROCEDURES

General Recombinant DNA Techniques—All recombinant manipulations were carried out using standard procedures (16). Nucleotide sequences were determined by the dideoxynucleotide chain termination method (17). Restriction endonucleases and DNA modification enzymes were purchased from Boehringer Mannheim, New England Biolabs, Stratagene, or Perkin-Elmer. Radionucleotides were from Amersham.

Purification of p43 and Sequencing of Peptide Fragments—The aminoacyl-tRNA synthetase complex was isolated from sheep liver (18). After fractionation of the components of the complex by SDS-polyacrylamide gel electrophoresis on a 5% polyacrylamide gel (19), polypeptides were recovered onto ProBlott membranes (Applied Biosystems) by electroblotting. Membrane pieces carrying p43 were cut and either sequenced immediately or subjected to in situ protease cleavage essentially as described (20, 21). In situ proteolytic digestion was carried out at 37 °C for 4 h, with endoproteinase Asp-N (Boehringer Mannheim) at a protease to p43 ratio of 1:50. Peptides released from the membranes

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were separated by reverse-phase HPLC\(^2\) on a C18 column. The amino acid sequence of the isolated peptides was determined by J.-P. Le Caer (Laboratoire de Physiologie Nerveuse, Gif-sur-Yvette) with a gas-phase sequencer (model 470A, Applied Biosystems).

Isolation and Sequencing of the cDNA Encoding p43 from CHO Cells—Total RNA was extracted from CHO cells as described above (22). The first strand cDNA synthesis on poly(A)\(^+\) RNA (1 µg), isolated on oligo(dT)\(_{20}\) cellulose, was primed by using an oligo(dT)\(_{20}\) (0.5 µg) and conducted at 42 °C for 90 min, with Moloney murine leukemia virus reverse transcriptase (Stratagene). PCR amplification of p43 cDNA was carried out by using a p43-specific mixed oligonucleotide as a 5\(^\prime\)-terminal primer. Amplification was allowed to proceed for 40 cycles of 1 min at 94 °C, 1 min at 62 °C, and 3 min at 72 °C with AmpliTag DNA polymerase (Perkin-Elmer). PCR products were analyzed by Southern blotting using a p43-specific mixed oligonucleotide (p43-b, Table I), end-labeled with \(\gamma\text{-}^32\text{P}ATP\) and T4 polynucleotide kinase. A 215-nucleotide-long fragment was digested with EcoRI and BamHI, corresponding to restriction sites appended to the 5\(^\prime\)-ends of the primers, and subcloned into pUC18. The nucleotide sequence of the insert was determined. It contained 130 nucleotides from the 3\(^\prime\) coding region of p43.

A longer cDNA probe was synthesized by PCR amplification between a mixed oligonucleotide (p43-c, Table I) located in the 5\(^\prime\)-upstream region of the insert and exact oligonucleotide primers deduced from the cloned insert sequence. The nucleotide sequence of the p43-cDNA was determined on both strands.

Analysis of p43 in HeLa Cells Extract—HeLa cells were grown as monolayers at 37 °C in Ham’s F-12 medium containing 5% fetal calf serum (Life Technologies, Inc.). Cells grown in ten 150-cm\(^2\) flasks were lysed by the addition of 4 ml of ice-cold lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM \(\beta\)-mercaptoethanol, 0.5% Triton X-100, 2 mM diisopropyl fluorophosphate) at 4 °C. After centrifugation at 10,000 \(\times g\) at 4 °C for 15 min to remove cell debris, the supernatant was analyzed by gel filtration on a BioGel A-5 m column (1.6 x 83 cm) equilibrated in 100 mM potassium phosphate, pH 7.5, 10% glycerol, 10 mM \(\beta\)-mercaptoethanol, and analyzed at 4 °C at a flow rate of 5 ml/h. Fractions of 2.7 ml were collected.

Elution of the aminoacyl-tRNA synthetase complex and of the p43 protein were monitored by the tRNA aminocidation assay (24) and by Western blotting using anti-sheep p43 antibodies (25).

Expression of the COOH-terminal Domain of p43 in Bacteria and Purification—The carboxy-terminal domain of the human p43 (p43Ct) was expressed in E. coli with the pET-28b expression system (Novagen). The p43 cDNA was produced by PCR between oligonucleotides 5\'-CCCATGGGCAACCTAGATTTGCCG-3' and 5\'-GAGGTTCATGCTGTCCGCA-3', which introduced a NcoI site in frame with the vector sequence encoding the His tail, respectively. The 0.5-kilobase pair (24) and by Western blotting using anti-sheep p43 antibodies (25).

Analysis of native p43 by automated Edman degradation—Analysis of native p43 by automated Edman degradation was performed starting from a 1-liter culture of BL21(DE3) \(\times pET43\text{Tct}^4\) grown at 37 °C in LB-kanamycin (50 µg/ml) broth to a cell density corresponding to \(A\text{at}^600\) of 0.5. The recombinant protein was expressed at 37 °C for 4 h after induction of 1 mM isopropyl-1-thio-\(\beta\)-D-galactosidase. All subsequent steps were conducted at 4 °C. Cells were harvested by centrifugation (6000 x g for 5 min), washed with ice-cold extraction buffer (20 mM Tris-\(\text{HCl}, pH\ 8.2, 500\) mM NaCl, 10 mM imidazole), resuspended in 20 ml of extraction buffer supplemented with 1 mM diisopropylfluorophosphate, and lyzed by sonication. After centrifugation at 10000 x g for 15 min, the lysate was loaded on a HisTrap resin (Novagen), equilibrated with 50 mM Tris-\(\text{HCl}, pH\ 8.2, 500\) mM NaCl, 10 mM imidazole, and 10% glycerol. After washing with extraction buffer, protein concentration was measured by a 200-ml linear gradient of imidazole from 20 mM to 200 mM in 20 mM Tris-\(\text{HCl}, pH\ 8.2, 500\) mM NaCl. Fractions containing p43Ct (eluted at 45 mM) were pooled (18 ml), equilibrated in 20 mM Tris-\(\text{HCl}, pH\ 7.0, 1\) mM dithioerythritol by filtration on a Sephadex G-10 column (Pharmacia Biotech Inc., 2.6 x 20 cm) and applied to a 1.6 x 20-cm column of SOURCE 15S (Pharmacia) equilibrated in the same buffer. The column was eluted with a 800 ml linear gradient of NaCl from 0 to 500 mM. Fractions containing p43Ct (eluted at 165 mM) were pooled (36 ml), concentrated, dialyzed against 20 mM potassium phosphate, pH 7.5, 1 mM dithioerythritol, and stored at −70 °C at a protein concentration of 11 mg/ml. Protein concentration was determined by using a calculated absorption coefficient of 0.41 \(A\text{at}^{280}\) unit/mg cm\(^{-1}\).

Analysis of the cDNA Encoding the p43 Component of the Multi-synthetase Complex—The strategy used to isolate the cDNA encoding the p43 component of the complex involves three major steps: 1) the identification of peptide sequences of the protein, 2) their use to design degenerate primers for PCR cloning of cDNA fragments, and 3) the screening of a cDNA library to isolate a full-length cDNA. The p43 polypeptide was isolated from the complex after denaturation by SDS and fractionation of the 11 polypeptide components by polyacrylamide gel electrophoresis. In the complexes isolated from rat or rabbit liver, the p43 and p38 components cannot be easily separated (4), leading to cross-contaminating polypeptides (27). Because the p43 component of the sheep complex migrates well apart from the p38 protein, this material was chosen to initiate the cloning process.

Analysis of native p43 by automated Edman degradation reveals the presence of a blocked NH\(_2\)-terminal residue. After transfer onto a ProBlott membrane, p43 was subjected to \(\text{i}n\ \text{sit}u\ \text{cleavage by endoproteinase Asp-N. Peptides released from the membrane were recovered and purified by reverse-phase HPLC on a C18 column. Mixed oligonucleotides were deduced from the peptide sequences (Table I) and used as primers for PCR amplification of the sheep p43-specific cDNA. After two rounds of amplification with p43-specific primers (Table I) and a poly(dT) primer, a 600-nucleotide-long cDNA of sheep origin was recovered. The 3\'-coding region of this cDNA fragment was sequenced and used to screen a \(\lambda\text{-ZAP CHO}\) cDNA library. Six independent clones were recovered and analyzed.

The 1254-nucleotide-long cDNA sequence displayed a single
Amino acid sequences of peptide fragments from the p43 component of the sheep liver complex determined in this study are indicated (pep43-). Uncertain amino acids are italicized. Three mixed sense primers, p43-b, p43-c, and p43-d, 5'- to 3'-extremity, were synthesized. For codon degeneracy, abbreviations are: Y for T or C; R for A or G; H for T, C, or A; N for T, C, A, or G; and I for inosine. Nucleotides introduced to generate noncoding sequence, in frame with the first putative ATG and 3'-end of the primers are shown with lower case letters.

Identification of Human p43 to the Precursor of the EMAP II Cytokine—The hamster p43 amino acid sequence was used to search the protein data libraries by using the Blast network facilities (29). The human and mouse endothelial monocyte-activating polypeptides (EMAP) II (15) were found to share 86 and 85% of identical amino acids with residues 47–359 of the p43 hamster protein, respectively. The mature form of EMAP II is a tumor-derived cytokine of about 20 kDa (30) released from a larger precursor after removal of the NH2-terminal moiety of the polypeptide. The cDNAs isolated for the EMAP II proteins are shorter than that obtained for the p43 protein, but amino acid sequence identities are also recovered between p43 and the translated short 5'-upstream regions of the human and mouse cDNAs. Therefore, the EMAP II cDNAs are likely to be incomplete cDNA species. The high sequence similarity between p43 and EMAP II suggested that they are the same gene product. We recently cloned the human homologue of p43 by screening a human cDNA library by the double hybrid approach using the p38 component of the complex as a bait. The sequence of human p43 is identical to that reported for EMAP II. These results clearly indicate that p43 is related to that cytokine.

As shown in Fig. 2, the p43 polypeptide sequence can be divided into six regions, according to their amino acid composition and homology to other proteins. Domain I is a basic segment, its high pi = 12.0 being essentially due to the presence of many arginine residues (9 Arg and 0 Lys, residues 1–45). This domain has not been previously isolated for the p43 protein, but amino acid sequence identities are also recovered between p43 and EMAP II proteins. Domain II is also basic (pi 9.0) but is mainly composed of lysine residues (12 Lys and 3 Arg, residues 46–119) and displays the propensity to fold into an a-helix conformation as judged from secondary structure predictions (31, 32). This region is conserved with the human and mouse proteins, which display 94 and 96% of identical residues, respectively. By contrast, domains III and IV are especially variable and the translated short 5'-upstream regions of the human and mouse cDNAs. Therefore, the EMAP II cDNAs are likely to be incomplete cDNA species. The high sequence similarity between p43 and EMAP II suggested that they are the same gene product. We recently cloned the human homologue of p43 by screening a human cDNA library by the double hybrid approach using the p38 component of the complex as a bait. The sequence of human p43 is identical to that reported for EMAP II. These results clearly indicate that p43 is related to that cytokine.

Identification of the Deducible Protein as the p43 Component of the Multi-synthetase Complex—The cDNA sequence has a coding potential for a polypeptide of 359 amino acids, with a calculated molecular mass of 39.6 kDa in agreement with the value of 43 kDa determined by SDS-polyacrylamide gel electrophoresis analysis of the complex from CHO cells (6). As previously observed by electrofocusing analysis of the complex (28), the p43 polypeptide displays a markedly basic pi, evaluated to 9.4 according to its amino acid sequence. The sequences of the three peptides obtained from the p43 component of the sheep complex (Table I) as well as some peptide sequences issued from the rat p43/p38 components (27) were recovered in the protein sequence derived from the cloned cDNA (Fig. 1). As shown below, the cDNA-encoded protein reacted with antibodies raised against the p43 component of the sheep liver complex, thereby establishing its assignment to the p43 cDNA from CHO cells.

Identification of Human p43 to the Precursor of the EMAP II Cytokine—The hamster p43 amino acid sequence was used to search the protein data libraries by using the Blast network facilities (29). The human and mouse endothelial monocyte-activating polypeptides (EMAP) II (15) were found to share 86 and 85% of identical amino acids with residues 47–359 of the p43 hamster protein, respectively. The mature form of EMAP II is a tumor-derived cytokine of about 20 kDa (30) released from a larger precursor after removal of the NH2-terminal moiety of the polypeptide. The cDNAs isolated for the EMAP II proteins are shorter than that obtained for the p43 protein, but amino acid sequence identities are also recovered between p43 and the translated short 5'-upstream regions of the human and mouse cDNAs. Therefore, the EMAP II cDNAs are likely to be incomplete cDNA species. The high sequence similarity between p43 and EMAP II suggested that they are the same gene product. We recently cloned the human homologue of p43 by screening a human cDNA library by the double hybrid approach using the p38 component of the complex as a bait. The sequence of human p43 is identical to that reported for EMAP II. These results clearly indicate that p43 is related to that cytokine.
residues and the absence of aromatic residues, a feature reminiscent of a KEKE motif identified as a possible protein-protein interaction site (33). The EMAP II cytokine starts after residue Asp193, located within the sequence Ser-(Ala/Thr)-Asp-Ser-Lys-Pro strictly and exclusively conserved in all the sequences of p43/EMAP II proteins shown in Fig. 2. Domain V, made of 102 amino acid residues (194–295), is conserved between the hamster, mouse, sheep, and human p43 proteins (90% identity) and is also recovered with 60% identity in a yeast protein identified for its affinity for quadruplex nucleic acids (G4p1; Ref. 13) or for its ability to interact genetically with a pore-associated protein involved in tRNA biogenesis (Arc1p; Ref. 14). This yeast protein interacts with cytoplasmic methionyl- and glutamyl-tRNA synthetases, enzymes associated, in mammalian cells, into the multisynthetase complex. Domain V is also recovered within the carboxyl-terminal polypeptide extension of methionyl-tRNA synthetase from the nematode Caenorhabditis elegans (73% identity) or from all the eubacterial or archae methionyl-tRNA synthetases (36 and 33% identity with Thermus thermophilus and Methanococcus jannaschii methionyl-tRNA synthetase, respectively). A hypothetical 12.3-kDa protein from the ileX-eggR intergenic region of E. coli exactly coincides with this domain and displays 39% identity. Finally, a similar domain (36% identity) is also recovered within the amino-terminal segment of the large subunit of the tetrameric (α2β2) bacterial phenylalanyl-tRNA synthetase.

Fig. 2. Alignment of the amino acid sequences of p43 and related proteins. Top panel, the p43 protein from hamster (Cricetulus griseus; p43-Cg) is schematized according to the six functional or structural domains referred to in the text. Related proteins sharing one or several of these domains are represented below. When homologous proteins have been described from different species, only one member of each family is indicated. This includes the precursor of the EMAP II cytokine from Homo sapiens (proEMAPII-Hs; accession number U10117); a quadruplex nucleic acids binding protein G4p1 from S. cerevisiae (G4p1-Sc; accession number U31348); methionyl-tRNA synthetase from C. elegans (MetRS-Ce; accession number Z73427) or T. thermophilus (MetRS-Tt; accession number M64273), taken as a representative of all eubacterial and archae methionyl-tRNA synthetases; the large β subunit of phenylalanyl-tRNA synthetase from Synechococcus sp. (βPheRS-Ssp; accession number X94345) and an unknown open reading frame from E. coli (ORFX-Ec; accession number U18997). The segment of p43 identified with the EMAP II cytokine is indicated by an arrow. The numbering refers to the position of the corresponding amino acid boundaries in the sequence of the complete proteins. Bottom panel, a detailed sequence alignment is shown in the block region corresponding to the β-barrel domain of phenylalanyl-tRNA synthetase. Only amino acids from homology blocks are indicated. Numbers refer to insertions. Additional sequences are the EMAP II precursor from Mus musculus (proEMAPII-Mm; accession number U10118); the partial sequence of p43 from Ovis aries (p43-Oa; this study); an unknown open reading frame from the sequence of a cDNA fragment isolated from Oriza sativa (ORFX-Os; accession number D23020); methionyl-tRNA synthetase from E. coli (MetRS-Ec; accession number X57925), H. influenzae (MRS-Hi; accession number U32753), B. stearothermophilus (MRS-Bst; accession number X57925) or B. subtilis (MRS-Bsu; accession number V00291); and the β subunit of phenylalanyl-tRNA synthetase from E. coli (βPheRS-Ec; accession number W02291), B. subtilis (βPheRS-Bsu; accession number X53057), or T. thermophilus (βPheRS-Tt; accession number X65609). For each sequence, the position of the first amino acid is indicated. Numbers in parentheses refer to partial amino acid sequences. The secondary structure elements, indicated as arrows for β-strands and rectangles for α-helices, are based on the crystal structure of the large subunit of phenylalanyl-tRNA synthetase from T. thermophilus (34).
It corresponds to the domain B2 from the crystal structure of phenylalanyl-tRNA synthetase from *T. thermophilus* (34). The particular feature of this domain is that it forms a discrete six-stranded β-barrel structure. The carboxyl-terminal domain of hamster p43 constitutes domain VI, which shares more than 95% identical residues with human and mouse EMAP II, 41% with *C. elegans* methionyl-tRNA synthetase, and 40% with yeast G4p1 in a more restricted segment.

Therefore, p43 is made of several distinct protein domains, one of which, domain V, is a recurrent protein module spread over a wide range of organisms, including archaea, eubacterial, and eukaryote species, as a fusion protein. A common feature of these proteins is their ability to bind nucleic acids.

**Structural Behavior of Human p43**—Because two distinct protein species related to p43, a component of the multisynthetase complex and a precursor of the EMAP II cytokine, have been described, we considered the possibility that two discrete polypeptides expressed from the same gene coexist within the cell. The oligomeric state of the p43 protein from cultured human HeLa cell lines was investigated. A crude extract from exponentially growing HeLa cells, obtained after lysis in the presence of 150 mM NaCl and 0.5% of Triton X-100 to thoroughly solubilize the cellular proteins, was subjected to size fractionation on a BioGel A-5 m column (Fig. 3). Fractions 24–60 were analyzed by Western blotting using anti-p43 antibodies. Lane C corresponds to a control sample containing 1 μg of homogeneous rat liver complex.

**Accessory Components of Aminoacyl-tRNA Synthetase Complex**

![Gel filtration behavior of p43 from a HeLa cell extract](image)

A crude extract from exponentially growing HeLa cells was subjected to fractionation on a BioGel A-5 m column as described under “Experimental Procedures.” Initial rates of lysyl-tRNA (C) and arginyl-tRNA (♀) synthesis were measured. Inset, fractions 24–60 were analyzed by Western blotting using anti-p43 antibodies. Lane C corresponds to a control sample containing 1 μg of homogeneous rat liver complex.

**Superose 12 chromatography of p43Ct**

Gel filtration was conducted as described under “Experimental Procedures,” in 50 mM potassium phosphate, pH 7.5, 10 mM 2-mercaptoethanol, 10% glycerol. The peak of elution of thyroglobulin (669 kDa) and dithioerythritol (154 Da) mark out the void volume of the column (Vv) and the total bed volume (Vt), respectively. The elution volumes of chymotrypsinogen A, p43Ct, and cytochrome c are 14.04, 14.16, and 14.33 ml, respectively. The protein concentration of p43Ct in the loaded sample was 2.2 mg/ml (~0.12 mM).

**Affinity of the COOH-terminal Domain of p43 for Nucleic Acids**—Because domains V and VI of p43 display sequence homology with a yeast protein independently isolated as G4p1 or Arc1p for its ability to bind to quadruplex nucleic acids or tRNA, respectively, we analyzed the nucleic acid affinity of the COOH-terminal moiety of p43. Homogeneous p43Ct was used in a band shift assay to measure its propensity to form protein-RNA complexes. When in vitro transcribed tRNAs were used in this assay, a shift of the labeled tRNA was detected (Fig. 5). The same pattern was observed with several yeast tRNA transcripts: tRNA<sup>Phe</sup> and tRNA<sup>Asp</sup> (Fig. 5), tRNA<sup>Ala</sup> and tRNA<sup>Met</sup> (not shown). The deduced K<sub>d</sub> dissociation constant was estimated to be about 40 μM in the presence of a high salt concentration (150 mM NaCl) in the incubation mixture. Two sorts of complexes are formed when increasing the protein concentration, suggesting that a 1:1 and a 1:2 tRNA-protein complex are produced (Fig. 5). Because identical shifts were observed with different tRNA molecules, this tRNA-protein interaction is likely to involve structural rather than sequence recognition.
domain, and the possibility that its removal destabilizes the actual dimer interface cannot be excluded. The finding that the isolated COOH-terminal domain of p43 (Fig. 4) behaves as a monomer is at odds with a putative role of a dimerization domain.

A protein domain related to domain V of p43 is also recovered from the NH₂-terminal region of bacterial phenylalanyl-tRNA synthetase. The x-ray structure of the αβ T. thermophilus enzyme complexed with two tRNA₃Phe molecules does not provide any functional role for this domain (38). However, the β-barrel-like structure of this domain, called the B2 domain, suggested that it could be involved in protein-RNA interactions. Several RNA binding proteins display this type of structural organization: the anticodon binding domain of aspartyl-tRNA synthetase (39), the major cold-shock protein CspB (40), the staphylococcal nuclease (41), and the ribosomal protein L14 (42). Therefore, we tested the possibility that the isolated COOH-terminal region of p43 could be a RNA binding domain. As shown in Fig. 5, gel shift experiments revealed its propensity to associate with tRNA molecules in a structure- rather than sequence-dependent manner. This result corroborates the data previously described for the yeast G4p1 and Arc1p proteins, where it was shown that the intact proteins are nucleic acids binding proteins, and attributes this property to the COOH-terminal moiety of these proteins. The yeast p43 homologues and the p43 protein described in this study differ in their NH₂-terminal regions. Whereas Arc1p/G4p1 interacts with methionyl-tRNA synthetase to form a binary complex, mammalian p43 associates within the multisynthetase complex. This suggests that the protein domain involved in these interactions is located within the variable NH₂-terminal domain of these proteins.

What could be the function of p43 within the multisynthetase complex? The ARC1 gene, encoding the yeast homologue of p43, was cloned by a genetic complementation assay for LOS1, a gene involved in general tRNA export, and related to the nuclear pore complex (14). ARC1 could complement LOS1 by virtue of its ability to bind tRNA, to interact with methionyl-tRNA synthetase and to stimulate its activity. A decreased nuclear export of tRNA could be compensated by an increased synthetase activity. However, because the Arc1p-tRNA interaction seems to be a feature not restricted to tRNA₃Met, it must be envisioned that the stimulation of synthetase activity by Arc1p is not restrained to methionyl-tRNA synthetase. Another possibility is to consider that Arc1p is involved in the direct delivery of tRNA from the nuclear pore complex to the synthetase and participates in the channeling of tRNA within the cytoplasm. According to the tRNA channeling model developed by Deutscher and co-workers (43, 44), in mammalian cells, tRNA molecules would be channeled from the synthetases to elongation factor 1 and to ribosome and back to the synthetase without mixing with other components from the cellular fluid. In that connection, newly synthesized tRNAs entering the cytoplasm through the nuclear pore complex could require association with another protein to be incorporated into the tRNA cycle. If p43 is involved in the channeling of tRNA from the nucleus to the synthetases, the finding that no free p43 was revealed in a HeLa cell crude extract (Fig. 3) suggests that p43 triggers the multisynthetase complex in the vicinity of the nuclear pore complexes.

The cloning of the p43 component of the multisynthetase complex also revealed an unexpected link between protein synthesis and the cytokine network. A tumor-derived cytokine, called EMAP II, was identified for its propensity to activate endothelial cells, leucocytes and monocytes, and to potentiate the effect of tumor necrosis factor (30). The mature EMAP II is...
a 22-kDa polypeptide generated by cleavage of a precursor (15). Little is known about the molecular mechanisms implicated in controlling the synthesis and processing of the precursor into a mature cytokine. The murine and human EMAP II cDNAs (15) encode a protein of 34 kDa, which proves to be identical to p43. In the present study, we were unable to identify any free or and/or truncated form of p43 in extracts from exponentially growing HeLa cells. Therefore, one likely hypothesis concerns a possible coupling between the breakdown of protein synthesis in tumor cells and the release of an active EMAP II cytokine. This release could involve a specific degradation pathway. It is of particular interest to notice that the site of cleavage occurs after the Asp residue in the sequence Ser-Ala-Asp-Ser corresponding to a cysteine protease called interleukin-1 convertase. This protease, which is a family of intracellular cysteine proteases, has been found in many cells. It is known to cleave cytokines at the cytoplasmic cleavage site. The presence of a KEKE motif upstream of the cleavage site also suggests that degradation of p43 could occur via the multicatalytic protease (33). Partial proteolysis of the multisynthetase complex with yeast tRNAMet, tRNAAsp, tRNAAla, and tRNAPhe, respectively. We thank Drs. F. Fasiolo (IBMC, Strasbourg, France), R. Giege (IBMC, Strasbourg, France), H. Grosjean (LEBS, Gif-sur-Yvette, France), and O. Uhlenbeck (University of Colorado, Boulder, CO) for the gifts of plasmids for transcription of yeast tRNA^Met^, tRNA^Asp^, tRNA^Ala^, and tRNA^Phe^, respectively. We thank Jean-Pierre Le Caer for performing the amino acid sequence analyses. The excellent technical assistance of Martine Tricotnet and Marie-Thérèse Latreille during part of this work is gratefully acknowledged.

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