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An interferon-induced protein with release factor activity is a tryptophanyl-tRNA synthetase

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Interferon gamma induces expression of a protein termed IFP 53 according to its molecular weight of 53 kDa. IFP 53 shows significant sequence homology to rabbit peptide chain release factor as well as to bovine tryptophanyl-tRNA synthetase. IFP 53 has been shown to possess release factor activity for the UGA stop codon. We demonstrate here, by using a recombinant IFP 53 fusion protein, that IFP 53 tryptophanylates tRNA. These data indicate that IFP 53 is a protein with two activities: peptide chain termination and aminoacylation.

Tryptophan; Release factor; Interferon; tRNA synthetase

1. INTRODUCTION

Aminoacylation of tRNAs is a critical step in protein biosynthesis, carried out by aminoacyl-tRNA synthetases that catalyse the esterification of a specific tRNA by the cognate amino acid [1]. Knowledge of primary structures has revealed the existence of two classes of aminoacyl-tRNA synthetases, depending on conserved sequence motifs [2]. Molecular and biochemical studies have led to the identification of several features that distinguish these enzymes from their prokaryotic homologues [1,3]. Bovine tryptophanyl-tRNA synthetase is composed of two identical subunits of molecular weight 54 kDa [4,5] compared to 37 kDa for the prokaryotic protein [6,7]. In addition to their primary role in tRNA charging, eukaryotic aminoacyl-tRNA synthetases have been implicated in splicing of mitochondrial RNAs [8–11] and regulation of translation initiation [12].

We have recently described an interferon (IFN)-induced protein termed IFP 53 according to its molecular weight [13]. IFP 53 shows high sequence homology to a rabbit peptide chain release factor [14] as well as to a previously described bovine tryptophanyl-tRNA synthetase [5], and possesses release factor activity for the UGA stop codon in vitro [13]. In this paper we show that IFP 53 is the human tryptophanyl-tRNA synthetase.

Abbreviations: IFN, interferon; eRF, eukaryotic release factor.

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2. MATERIALS AND METHODS

2.1. *Production of a fusion protein*

The recombinant λgt 10 clone 369.13.3 [13] was digested with *Eco*RI and the 1,624 bp fragment containing a full-length IFP 53 cDNA was cloned into the *Eco*RI site of the expression vector pMALc (New England Biolabs) resulting in the recombinant clone pMALc 13 Eco. pMALc 13 Eco was digested with *Stu*I and *Nsi*I, and a 7,130 bp fragment containing the complete vector and 9.90 bp of 3′ IFP 53 sequence was gel-purified. Clone λgt10 369.13.3 was digested with *Pst*I, blunt-ended using T4 DNA polymerase, ethanol-precipitated, re-digested with *Nsi*I, and the 392 bp fragment containing the 5′ IFP 53 sequence cloned into *Nsi*I/*Stu*I-restricted pMALc 13 Eco. The resulting plasmid pMALc 12.5 encodes residues 40–471 of the IFP 53 protein inserted downstream from the malE gene, which encodes the maltose binding protein. Whole cell and cytoplasmic extract of IPTG-induced cells was prepared according to the manufacturers instruction. Crude cytoplasmic extract was centrifuged for 20 min at 9,000 × *g*, the supernatant dialyzed against 50 mM Tris–HCl, pH 7.5, 1 mM EDTA, loaded onto a DEAE 52 column pre-equilibrated with 50 mM Tris–HCl, pH 7.5, 1 mM EDTA, washed with 50 mM Tris–HCl, pH 7.5, 1 mM EDTA, eluted with 300 mM KCl, 50 mM Tris–HCl, pH 7.5, 1 mM EDTA [15] and dialyzed against 100 mM Tris–HCl, pH 8.0, 1 mM EDTA. The eluted and dialyzed fraction was used for determination of aminoacylation activity. Aliquots were frozen at −70°C and protein concentration determined according to Bradford [16].

HeLa cells were grown as monolayer cultures in DMEM supplemented with 10% fetal calf serum. Crude HeLa cell extract was prepared by homogenization of the cell pellet in 50 mM Tris–HCl, pH 7.5, 1 mM EDTA using an Ultra-Turrax (Janke & Kunkel, Germany) and further processed as described for the *E. coli* cytoplasmic extract. Recombinant IFNγ (Polyferon, 2 × 10⁷ U/mg protein, Rentschler, Laubheim, Germany) was kindly provided by P. v. Wussow (Medical School Hannover).

2.2. *Determination of aminoacylation activity*

Aminoacyl-tRNA synthetases were assayed by the aminoacylation of tRNA. Unfractionated brewer's yeast tRNA (Boehringer-Mannheim) was used as substrate [5]. tRNA aminoacylation was carried out at 30°C using 25 μg of DEAE cellulose-fractionated cytoplasmic extract from *E. coli* or HeLa cells in 100 mM Tris–HCl, pH 8.0, 1 mM EDTA, 15 mM magnesium acetate, 0.05 mg/ml BSA, 7

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mg/ml total yeast tRNA, 8 mM ATP, and 0.1 mM [^{14}C]L-tryptophan (54 mCi/mmol, Amersham) or 0.1 mM [^{14}C]L-leucine (54 mCi/mmol, Amersham). At various times, 9 μl aliquots from a 60 μl reaction were removed, spotted on a Whatman GFC disk, immediately TCA-precipitated, and the radioactivity retained on the filters was counted.

3. RESULTS AND DISCUSSION

Extracts from bacterial cells transfected with the pMALc 12.5 clone and grown in the presence of IPTG were submitted to SDS-PAGE and Western blotting using anti-MBP antibodies. The fusion protein produced from pMALc 12.5 had a molecular weight of 90 kDa (see Fig. 1), which is consistent with the size of the insert-encoded protein of 48 kDa plus that of the amino-terminal vector-encoded maltose-binding protein (MBP) of 42 kDa.

After expression of the IFP 53 fusion protein, the bacterial extract was passed over a DEAE 52 column, eluted under high salt conditions, and the aminoacylation activity was determined using total yeast tRNA and [^{14}C]L-tryptophan or [^{14}C]L-leucine. Fig. 2A shows that yeast tRNA was tryptophanylated when using cytoplasmic extract from bacterial cells grown in the presence of IPTG. Omitting yeast tRNA in the aminoacylation reaction resulted in TCA-precipitable counts similar to background (data not shown). Control experiments using [^{14}C]L-leucine showed no aminoacylation of tRNA (Fig. 2B). The tryptophanyl-tRNA synthetase activity could be unambigously assigned to the IFP 53 fusion protein, as bacterial cells grown in the presence of IPTG and expressing the maltose binding protein only did not show any aminoacylation activity (Fig. 2).

Whole cell extracts of untreated and IFNγ-stimulated HeLa cells were prepared for investigating the effect of IFNγ on expression of tryptophanyl-tRNA synthetase. As shown in Fig. 3 treatment with IFNγ resulted in a significant increase of tryptophanyl-tRNA synthetase activity compared to the untreated controls. In contrast, expression of leucyl-tRNA synthetase was not affected by IFNγ (data not shown).

Fig. 4 shows a comparison of the amino acid sequences of IFP 53 [13], rabbit peptide chain release factor (RF) [14] and bovine tryptophanyl-tRNA synthetase [5]. All three proteins show sequence stretches analogous to the two consensus sequences HIGH and KMSKS found in class I tRNA synthetases: HVGH/NVGH and KMSAS, indicating, as has been noted previously [5], that the second lysine of the KMSKS sequence is not critical for tryptophanyl-tRNA synthetase activity. The HIGH signature sequence is present in IFP 53 and bovine tryptophanyl-tRNA synthetase as HVGH and as NVGH in rabbit RF. These motives play essential roles in the binding domains for ATP (HIGH) and for the 3' end of tRNAs (KMSKS) as revealed from X-ray analyses of methionyl- and tyrosyl-tRNA synthetases and the glutaminyl-tRNA synthetase/tRNA^{Gln} complex [17–19]. Considering the degree of sequence identity between the amino acid sequences, rabbit RF, IFP 53 and bovine tryptophanyl-tRNA synthetase are related at a level exceeding 90% if conserved amino acids are included in this calculation. It should be noted that in the regions of highest sequence variability IFP 53 is more similar to bovine tryptophanyl-tRNA synthetase than to rabbit RF.

The mechanisms involved in IFNγ-mediated induction of IFP 53/tryptophanyl-tRNA synthetase are yet to

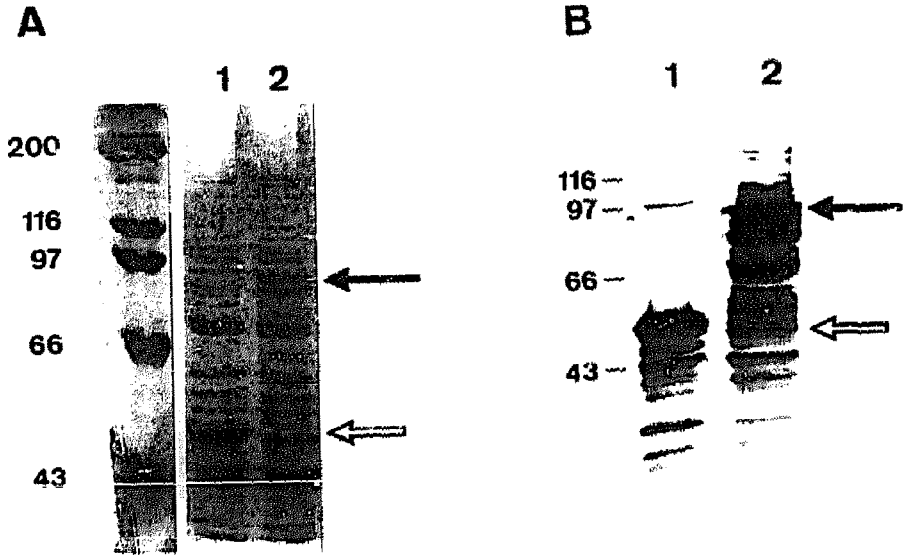

Fig. 1. SDS-PAGE stained with Coomassie Blue (A) and Western blot (B) of the protein extracts from bacterial cells after induction with IPTG. After gel electrophoresis and transfer to a nitrocellulose sheet, the proteins were detected with rabbit anti-MBP antibodies (New England Biolabs) and anti-rabbit immunoglobulin antibodies conjugated to alkaline phosphatase. (Lane 1) cells expressing the MBP-β gal protein (52 kDa open arrow); (lane 2) cells expressing the MBP-IFP 53 fusion protein (closed arrow). the molecular weight markers (kDa) are indicated.

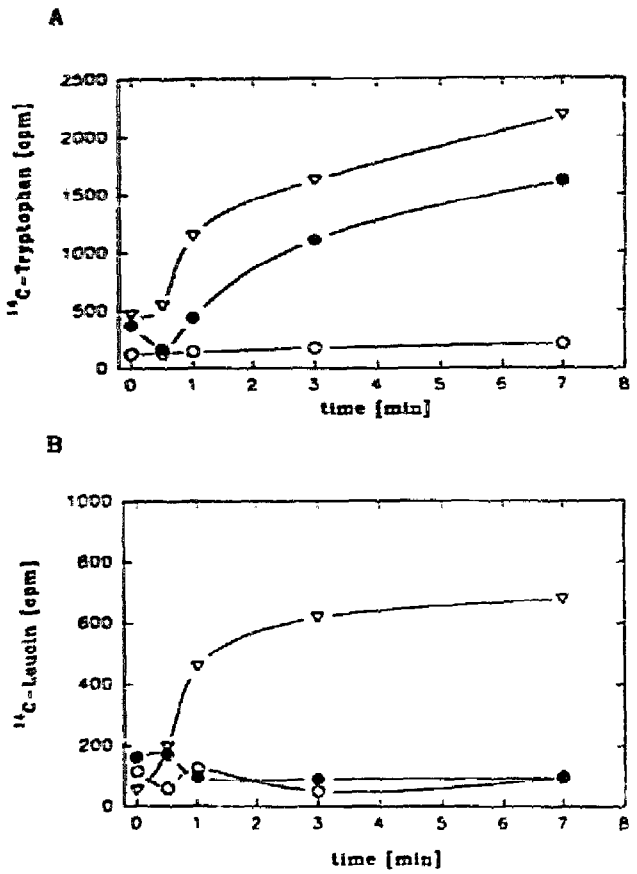

Fig. 2. Aminoacylation activity of cytoplasmic extract from bacterial cells expressing the MBP-IFP 53 fusion protein using (A) [^{14}C]L-tryptophan or (B) [^{14}C]L-leucine. (●) MBP-IFP 53 fusion protein; (○) MBP protein; (▽) reticulocyte lysate (Promega) used as positive control.

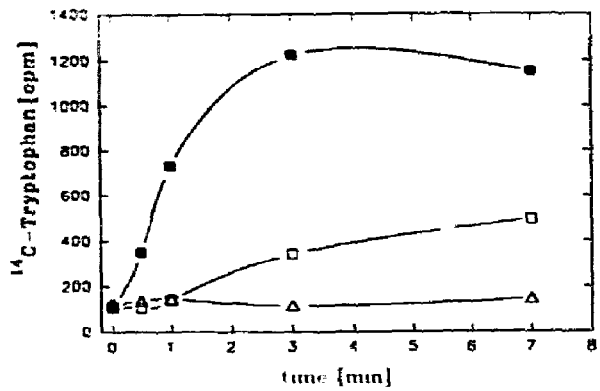

Fig. 3. Induction of tryptophanyl-tRNA synthetase in HeLa cells by IFNγ as determined by aminoacylation activity of fractionated cell extracts using [^{14}C]L-tryptophan. Cell extracts were passed over a DEAE 52 column and eluted under high salt conditions. The values shown correspond to specific enzymatic activities, corrected for protein concentration, and are expressed as cpm radioactivety retained on the filter. HeLa cells were treated for 12 h with 500 U/ml IFNγ (■) or medium (□). The background activity is given by heat-treated (10 min 95°C) cell extracts from medium-treated HeLa cells (△).

be characterized, but it is known that IFNγ induces an enzyme of tryptophan catabolism, indoleamine 2,3 dioxygenase, which is responsible for conversion of tryptophan and other indol derivatives to kynurenine, thereby leading to intracellular depletion of tryptophan [20,21]. In prokaryotes, expression of aminoacyl-tRNA synthetases is in part regulated by the cognate amino acid [22,23]. Preliminary evidence suggests that depletion of intracellular tryptophan is involved in the IFNγ-mediated induction of IFP 53.

We have shown previously that IFP 53 has release factor activity for the UGA stop codon. Here we demonstrate that IFP 53 is a tryptophanyl-tRNA synthetase. These data therefore suggest that a release factor activity is associated with the human tryptophanyl-tRNA synthetase. What could account for the two functional properties of IFP 53? The sequence homology between rabbit RF, bovine tryptophanyl-tRNA synthetase and IFP 53 implies that all three proteins possess a similar tertiary structure. The KMSKS region of class I synthetases appears to be involved in the binding of the tRNA 3' end [17–19]. The question thus arises of whether IFP 53 and rabbit RF use this region for decoding stop codons [24]. Detailed investigations of amino acid specificities in acylation of natural and modified tRNAs showed that synthetases use special proofreading mechanisms to reject non-cognate substrates [25,26]. These proofreading processes could be divided in pre- and post-transfer steps [27]. In the post-transfer step aminoacyl-tRNA esters of non-cognate amino acids are hydrolyzed before release from the enzyme to uncharged tRNAs and amino acids. This post-transfer proofreading activity is strikingly similar to the process resulting in peptide chain termination. During peptide chain elongation by aminoacyl-tRNA, peptidyl transferase catalyzes peptide bond formation when an amino group acts as a nucleophile attacking peptidyl-tRNA ester linkage. Perturbation of the peptidyltransferase by eukaryotic release factor (eRF) has been hypothesized to lead to the acceptance of water as the nucleophilic agent resulting in peptide chain termination by hydrolysis of the peptidyl-tRNA ester bond [24,28]. The sequence homology between IFP 53, eRF and bovine tryptophanyl-tRNA synthetase, as well as the similarity between post-transfer proofreading and peptide chain termination – both involve hydrolysis of the appropriate ester bonds – implies that the esterase activity leading to hydrolysis of peptidyl-tRNA may be a property of the eRF molecule.

Recent evidence suggests that eukaryotic aminoacyl-tRNA synthetases have additional functions that are unrelated to aminoacylation. The tyrosyl-tRNA synthetase of *Neurospora crassa* has been shown to be required for splicing of group I introns [8] as well as for integration of a group I intron into a ribosomal RNA sequence

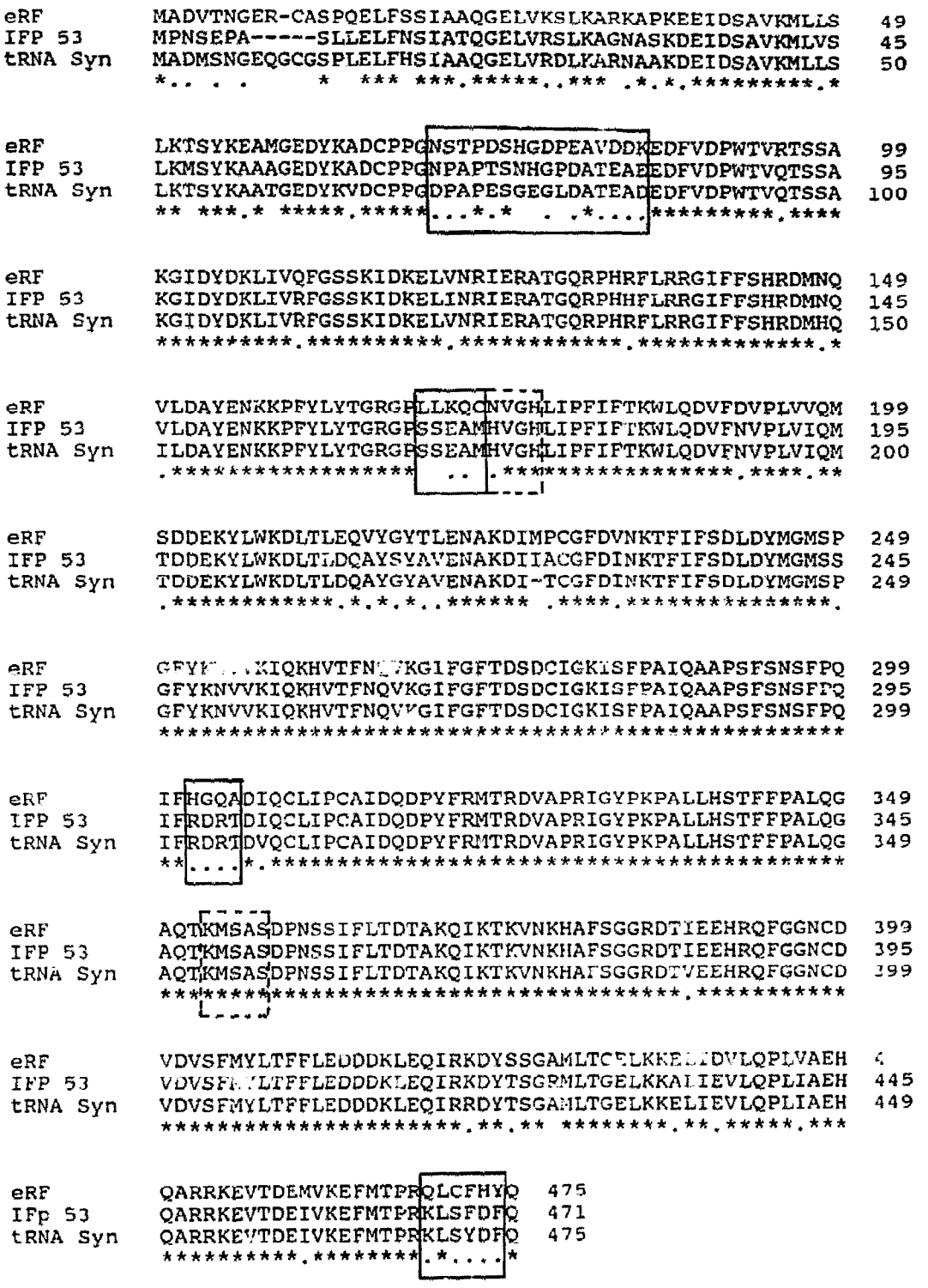

Fig. 4. Comparison of the predicted amino acid sequence of IFP 53 with rabbit peptide chain release factor (eRF) and bovine tryptophanyl-tRNA synthetase (tRNA Syn). The two consensus sequences of class I synthetases, HIGH and KMSKS, are indicated by dotted boxes; regions of greatest sequence variability are indicated by closed boxes; identical amino acids are depicted by asterisks, and similar amino acids by dots.

[11]. Similarly, evidence has been presented that in yeast mitochondrial leucyl-tRNA synthetases are involved in mRNA splicing [9]. In addition, eukaryotic synthetases have been implicated in the regulation of translation initiation [12]. Although at present we do not know whether IFP 53 represents the true eukaryotic peptide

chain termination factor, our data indicate that IFP 53 is a protein with two different activities, namely release factor activity for the UGA stop codon and tryptophanyl-tRNA synthetase activity.

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