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Human cytoplasmic isoleucyl-tRNA synthetase: Selective divergence of the anticodon-binding domain and acquisition of a new structural unit

(aminoacyl-tRNA synthetases/structural relationships/evolution/alignment guided cloning by PCR)

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ABSTRACT **We show here that the class I human cytoplasmic isoleucyl-tRNA synthetase is an exceptionally large polypeptide (1266 aa) which, unlike its homologues in lower eukaryotes and prokaryotes, has a third domain of two repeats of an ≈90-aa sequence appended to its C-terminal end. While extracts of *Escherichia coli* do not aminoacylate mammalian tRNA with isoleucine, expression of the cloned human gene in *E. coli* results in charging of the mammalian tRNA substrate. The appended third domain is dispensable for detection of this aminoacylation activity and may be needed for assembly of a multisynthetase complex in mammalian cells. Alignment of the sequences of the remaining two domains shared by isoleucyl-tRNA synthetases from *E. coli* to human reveals a much greater selective pressure on the domain needed for tRNA acceptor helix interactions and catalysis than on the domain needed for interactions with the anticodon. This result may have implications for the historical development of an operational RNA code for amino acids.**

The relationship between trinucleotides and amino acids spelled out by the genetic code is determined by the specificity of aminoacyl-tRNA synthetases, which catalyze attachment of amino acids to the cognate tRNAs bearing anticodon trinucleotides (1–4). The enzymes are among the most ancient proteins and, in their earliest versions, probably consisted of polypeptides that catalyzed formation of aminoacyladenylates (5–8). The sequences and structures of the adenylate-formation domain of tRNA synthetases are the basis for dividing them into two distinct classes of 10 enzymes each (1, 9–11). Several (at least 8) of the enzymes aminoacylate small RNA oligonucleotides that recapitulate part or all of the 7-bp acceptor stem of their cognate tRNAs (12). The specific sequence/structures in small RNA helices that confer aminoacylation constitute an operational RNA code for amino acids which presumably has a historical relationship to the genetic code (12).

The class-defining catalytic domain which also encodes determinants for tRNA acceptor helix interactions is the functional unit needed for aminoacylation of RNA oligonucleotides. Joined to the class-defining domain is a second domain, idiosyncratic to the synthetase, which provides interactions with the parts of the tRNA which are distal to the amino acid attachment site. In some synthetases, this second domain interacts directly with the anticodon (13, 14), while in other enzymes no contact is made between the second domain and the anticodon (15, 16). Thus, to a first approximation, the two domains in tRNA synthetases interact with the two distinct domains of the L-shaped tRNA structure so that the parts of the synthetase and of the tRNA which are needed for the operational RNA code are segregated into discrete protein and RNA domains. In recent experiments, a fragment containing the conserved active-site domain of a tRNA synthetase has been shown to have the same activity as the full-length enzyme for aminoacylation of an RNA oligonucleotide corresponding to the cognate tRNA acceptor stem (17).

As a prototypical example of a member of a subgroup of the most recently evolved class I enzymes, we are particularly interested in the isoleucine enzyme. The five members of this subgroup, like all class I enzymes, have an N-terminal nucleotide-binding fold consisting of alternating β-strands and α-helices and a C-terminal domain that is rich in α-helices and that contains residues needed for interactions with the parts of the tRNA distal to the amino acid attachment site (18, 19). These enzymes, the cysteinyl-, isoleucyl-, leucyl-, methionyl-, and valyl-tRNA synthetases, are grouped together because they are more closely related in sequence to each other than to the other five members of class I (19, 20). In *Escherichia coli* these five enzymes vary in size from 461 to 951 aa and are active as monomers. The size variation is in large part explained by the variability in the lengths of the two insertions designated connective polypeptide 1 (CP1), which is inserted between the third and fourth β-strands of the nucleotide-binding fold, and CP2, which is placed between the fourth β-strand and the third α-helix (21). In all of these enzymes, CP1 is the largest of the two insertions and varies in *E. coli* from 61 aa in cysteinyl-tRNA synthetase to 277 aa in isoleucyl-tRNA synthetase (19). While a portion of CP1 may be deleted from isoleucyl-tRNA synthetase without loss of function (21), this insertion is known to facilitate acceptor helix interactions in the related glutaminyl-tRNA synthetase, whose three-dimensional structure in complex with $tRNA^{Gln}$ has been determined (13).

The variable size of the CP1 insertion in class I enzymes possibly reflects the different origins of the motifs recruited into the catalytic domain for acceptor helix interactions (22). We wondered whether this insertion, once acquired, was under the same relative selective pressure as the class-defining catalytic core with which it is combined and, in addition, whether a similar selective pressure was exerted on the C-terminal anticodon-binding domain. Because isoleucyl-tRNA synthetase has the largest CP1 insertion, in addition to a large C-terminal anticodon-binding domain, we thought that the human cytoplasmic enzyme offered an opportunity to explore these questions in more depth.‖

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Abbreviations: RACE, rapid amplification of cDNA ends; CP, connecting polypeptide.
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‖The sequence data reported in this paper has been deposited in the GenBank data base (accession no. D28473).

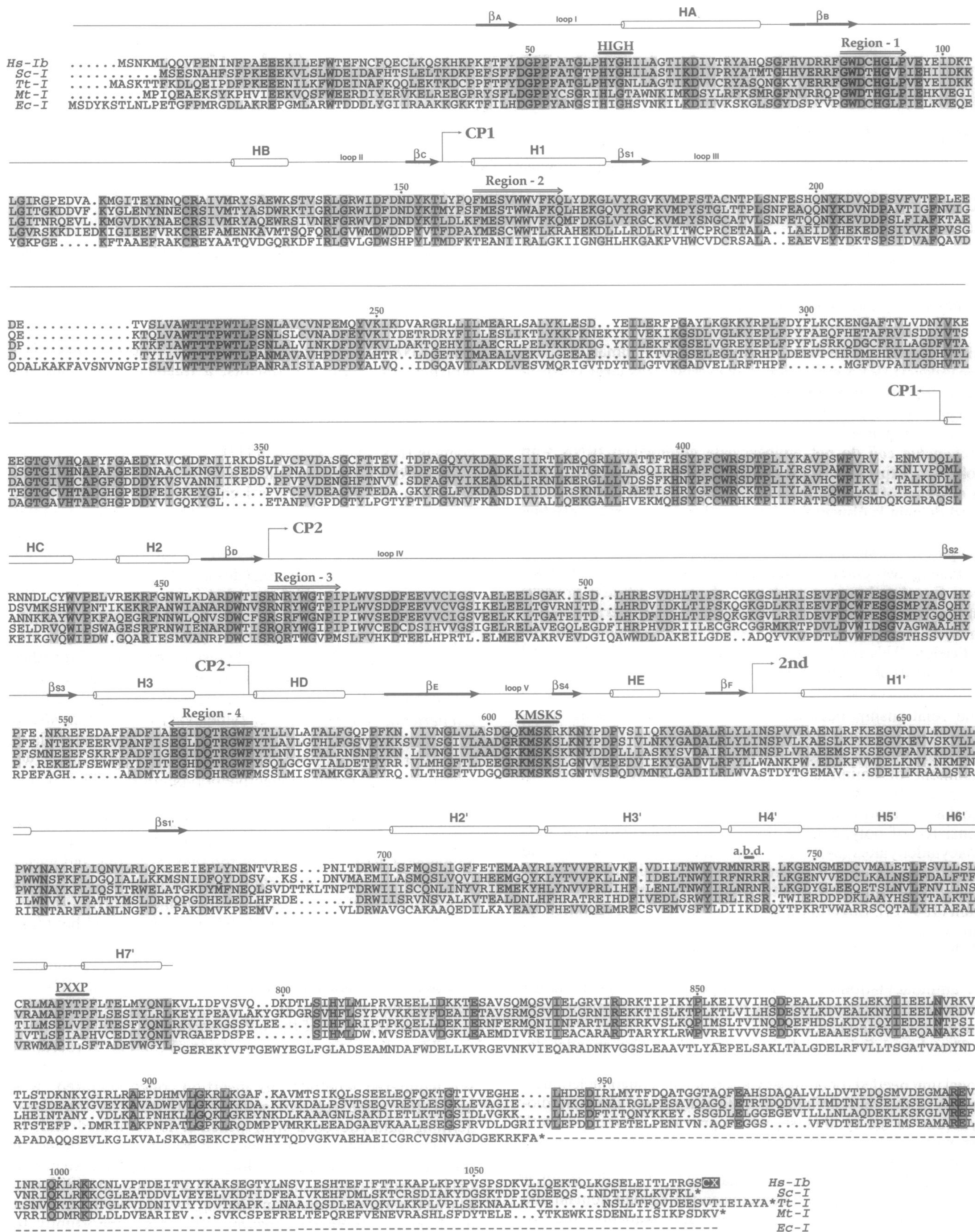

FIG. 1. Sequence alignment of isoleucyl-tRNA synthetase from various organisms by PILEUP (Genetics Computer Group). Gaps are represented by dots. Hs-Ib represents the human cytoplasmic enzyme. Numbers represent amino acid residues of the human cytoplasmic enzyme. Regions from which degenerate primers were designed are shown by arrows above the alignment. Chemically and/or evolutionarily related residues were calculated by the PRETTY program with Dayhoff's relatedness odds matrix (24) and are shaded. Residues whose identities are conserved among all five sequences are darkly shaded. After residue 788, the *E. coli* enzyme was not considered further (separated by space in the figure) and the remaining four sequences including the type B enzyme were aligned separately. In this second half of the C-terminal domain, only identical residues are boxed and shaded. The conserved HIGH, KMSKS, and PXXP motifs and a proposed anticodon-binding determinant

MATERIALS AND METHODS

Sequence Analysis. Programs provided in the Genetics Computer Group (Madison, WI) package were used for sequence analysis. Multiple sequence alignments were performed with the PILEUP program, which aligns multiple sequences by the method of Needleman and Wunsch (23). From the aligned sequences, the "distances" between any two selected sequences, the evolutionarily conserved residues, and the average similarity among all members at each position were calculated by use of the DISTANCE, PRETTY, and PLOTSIMILARITY programs, respectively. These programs use the modified Dayhoff comparison table (24) for calculation. The DISTANCE values appearing in the text were obtained by dividing the number of matches between each sequence pair by the length of the shorter sequence. For these comparisons, the C-terminal domain after the conserved PXXP motif was omitted from each sequence.

Nucleic Acid Manipulations. Human fetal fibroblast cell strain TIG-2 (25) was cultured in Dulbecco's modified Eagle's medium (GIBCO/BRL) supplemented with 5% fetal bovine serum (Cell Culture Laboratories, Cleveland) Poly(A)$^+$ mRNA was isolated as described (26). Details of the PCR amplification are available on request. The products were cloned into the *Eco*RI site or *Sma* I site of phagemid pTZ19R (27).

A cDNA library was constructed from poly(A)$^+$ mRNA derived from human T-cell line KUT-2 as described (28) by using plasmid vector pSI4001 (29). cDNA inserts were recloned into phagemid vector pTZ19R or pBluescript II (Stratagene) and a unidirectional deletion was made by using the Kilo-Sequence deletion kit (Takara Shuzo, Ohtsu). Single-stranded DNA was obtained by superinfection with helper M13 K07 phage (27), and sequences were determined with an automated DNA sequencer (Pharmacia). The 5′ regions of type A and type B cDNA (see below) were extended by the modified rapid amplification of cDNA ends (RACE)–PCR method (30) using 5′-RACE-Ready cDNA from human brain (Clontech).

For Northern blot analysis (26) 5 μg of poly(A)$^+$ RNA isolated from the human HeLa–fibroblast hybrid cell line CGL-1 (ref. 31, provided by T. Yudate, Cancer Institute) was fractionated through a 2.2 M formaldehyde/1% agarose gel, transferred onto a Duralon-UV nylon membrane (Stratagene), and probed with a 1.7-kb *Nco* I–*Nco* I fragment from type B cDNA.

Expression of Type B Core Enzyme in *E. coli* and Assay for Charging Activity. Plasmid pKS402, which encodes the core region of the type B enzyme (aa 6–1086), was constructed by using a T7 promoter vector, pET-3a (31) (details of the construction will be published elsewhere). Strain HMS174 (*recA*, *hsdR*, *rpoB*) containing plasmid pKS402 was grown in 50 ml of LB broth (26) containing carbenicillin (50 μg/ml, Sigma) and maltose (0.04%) at 37°C. At OD_{600} = 0.3, glucose (0.4%), $MgSO_4$ (10 mM), and λ phage CE6 (3 × 10^9 plaque-forming units/ml) were added. Cells were grown at 37°C for an additional 4 hr, harvested, suspended in 10 ml of 50 mM 2-mercaptoethanol/0.1 M NaCl/50 mM sodium phosphate, pH 7.5/1 mM phenylmethylsulfonyl fluoride, disrupted by sonication (two cycles of 15 sec, Bioruptor, Cosmo), and clarified by centrifugation (100,000 × *g* for 30 min). Charging activity was assayed as described (7) with ≈25 μg of protein (Bio-Rad protein assay kit), and calf liver tRNA (Boehringer Mannheim) at 0.4 mg/ml.

RESULTS AND DISCUSSION

Design of Primers for Cloning Human Isoleucyl-tRNA Synthetase by PCR. The sequences of one prokaryotic [*E. coli* (9)], one archaebacterial (*Methanobacterium thermoautotrophicum* (32)], and two lower eukaryotic [*Saccharomyces cerevisiae* (33, 34) and *Tetrahymena thermophila* (35)] isoleucyl-tRNA synthetases have been reported. Alignment of these sequences with the unpublished sequence of *Thermus thermophilus* isoleucyl-tRNA synthetase (S. Yokoyama, personal communication) was used to identify regions of the sequence that were well conserved among five enzymes but not shared by other class I enzymes. Well-conserved regions in the class-defining catalytic core in the N-terminal domain, such as the 11-aa signature sequence which ends in the tetrapeptide HIGH (9) and the KMSKS pentapeptide (36), were excluded from consideration because they are shared by all class I enzymes. Instead, we located sequences on the C-terminal side of the signature sequence (region 1), the beginning of CP1 (region 2), and the beginning and end of CP2 (regions 3 and 4) which were well conserved amongst the five isoleucine enzymes but not amongst the other class I enzymes. In regions 1 and 3 we made two different primers for each, in order to take account of the high similarity of certain subgroupings of the five sequences that appeared more related. The selected primers were 23–32 nt long and their sequences are available on request.

With these primers, PCR was performed with cDNA prepared from a human fibroblast strain. Two fragments were amplified which had distinct sequences, each of which showed strong similarity to the five aforementioned sequences of isoleucyl-tRNA synthetase. We designate these two products as type A and type B. These fragments were used as probes with the cDNA plasmid library prepared from a human T-cell line, and the cDNA inserts [3.2 kb (A) and 4.3 kb (B)] cloned thereby were extended at the 5′ end by RACE–PCR and subsequently sequenced. The sequences obtained were translated and, from comparison of the sequences with the five available sequences of isoleucyl-tRNA synthetase, we noted that the type A enzyme had a much higher similarity (DISTANCE value, 0.59, see *Materials and Methods*) to the *E. coli* enzyme compared to the similarity seen with the sequence of the type B enzyme (0.43), while the converse was true when the two sequences were compared with that of the *S. cerevisiae* cytoplasmic enzyme [0.45 (A) and 0.73 (B)]. The type A enzyme has an N-terminal extension not found in the type B protein or in any of the other available synthetase sequences, and we suggest that this N-terminal addition is a mitochondrial targeting sequence. These considerations led us to believe that the type B sequence was that of human cytoplasmic isoleucyl-tRNA synthetase, and we investigated further that sequence and enzyme.

Primary Structure of Human Cytoplasmic Enzyme Shows a C-Terminal Repeated Sequence. The type B cDNA sequence codes for a 1266-aa-polypeptide that aligns with the four aforementioned sequences for isoleucyl-tRNA synthetase (Fig. 1) and with the unpublished sequence of the enzyme from *Thermus thermophilus*. Northern blot analysis of poly(A)$^+$ RNA from the human HeLa–fibroblast hybrid cell line CGL-1 (38) with a type B cDNA-specific probe identified a transcript of 4500 nt, large enough to encode the 1266-aa protein (data not shown). The alignment of the six sequences is sufficiently precise that the predicted locations of second-

(a.b.d.) are shown. The secondary structure elements of *E. coli* methionyl-tRNA synthetase are shown across the top (see ref. 6) and the nomenclatures for β-strand (arrows), α-helix (tubes), and loop are based on ref. 37. CP1, CP2, and the second C-terminal domain that are predicted by modeling (6) are shown by arrows. The amino acid sequence of the appended C-terminal peptide of human cytoplasmic enzyme (shown in Fig. 2) is shown as CX. Hs, *Homo sapiens*; Sc, *Saccharomyces cerevisiae* (33, 34); Tt, *Tetrahymena thermophila* (35); Mt, *Methanobacterium thermoautotrophicum* (32); Ec, *Escherichia coli* (9).

FIG. 2. Repeated sequence motif observed in the C-terminal appended domain of type B human isoleucyl-tRNA synthetase (shown as CX in Fig. 1). Identical residues are shaded.

ary structure elements in the human enzyme, based on the model of the related *E. coli* methionyl-tRNA synthetase, whose three-dimensional structure is known (37), are the same as that of the other enzymes (Fig. 1).

The human enzyme is 328 aa longer than its *E. coli* counterpart and virtually all of this additional length is found at the C-terminal end. Approximately 60% of this additional sequence is due to a repeated element of about 90 aa (Fig. 2) which is not found in any of the other sequences. The sequence of this 180-aa extra domain is not similar to any sequence in other tRNA synthetases or in proteins compiled in the Protein Identification Resource database (National Biochemical Research Foundation, Release 39.0). The N-terminal 34-aa extension of human cytoplasmic aspartyl-tRNA synthetase (39), the N-terminal 73-aa extension of rat cytoplasmic argininyl-tRNA synthetase (40), and the three 75-aa internal repeats of the human cytoplasmic glutamyl-prolyl fusion tRNA synthetase (41) have all been proposed as motifs needed for assembly of the multisynthetase tRNA complex (42). If the repeated sequence in human cytoplasmic isoleucyl-tRNA synthetase is required for assembly into this complex, and if the unique additional sequences in the other mammalian sequences have the same function, then it would appear that the sequence for complex assembly and its location are idiosyncratic to the human enzyme.

Expression and Species-Specific Aminoacylation by the Human Cytoplasmic Enzyme. The sequences of the bacterial $tRNA^{Ile}$ and mammalian $tRNA^{Ile}$ (43) are diverged, and not all of the identity-determinant residues determined for *E. coli* $tRNA^{Ile\text{-}1}$ (44) are conserved in mammalian cytoplasmic $tRNA^{Ile}$ [for example, the C29·G41 base pair is important for aminoacylation by *E. coli* isoleucyl-tRNA synthetase, and this base pair is transversed to a G29·C41 base pair in the mammalian $tRNA^{Ile}$ (44)]. For these reasons, we anticipated that the *E. coli* enzyme would not charge the mammalian cytoplasmic isoleucine tRNAs. With that consideration in mind, we thought that expression of the human enzyme in *E. coli* might confer an activity for aminoacylation of mammalian tRNA with isoleucine on extracts of *E. coli*. For this purpose, we cloned the nearly full-length human enzyme (codons 6–1266) behind a T7 promoter in plasmid pET-3a and the recombinant plasmid was introduced into strain HMS174 (*recA*, *hsdR*, *rpoB*). These cells were then infected with λ phage CE6 (*cI857*, *Sam7*), which carries the T7 phage polymerase gene under control of the P_L and P_I promoters (31), and soluble fractions were prepared. However, expression of the entire human protein seemed to result in protein precipitation, and charging activity was not observed. Alternatively, the core enzyme, missing the repeated sequence at the C-terminal end and comprising codons 6–1085, was cloned into the same vector. Expression of this protein resulted in detection of a new protein of 125 kDa, when cell extracts were subjected to SDS/PAGE (Fig. 3). Extracts of cells containing the pET-3a vector alone showed no aminoacylation activity toward the mammalian tRNA. In contrast, aminoacylation with isoleucine was measured in extracts containing the truncated human cytoplasmic enzyme (Fig. 3).

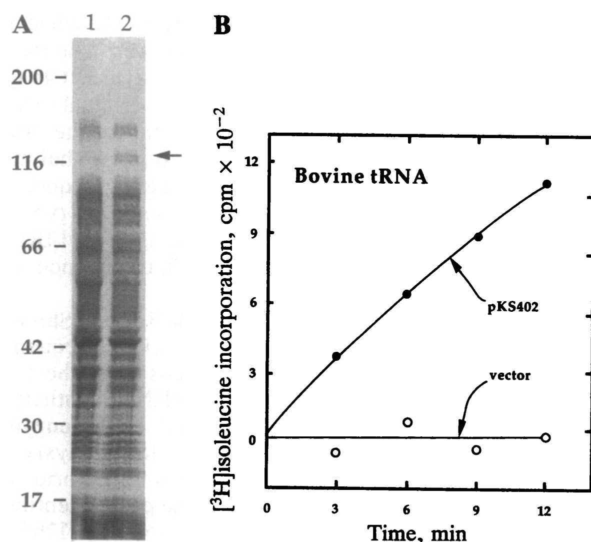

FIG. 3. Expression of type B enzyme in *E. coli* and its aminoacylation activity. (*A*) Total cell extract from HMS174/pKS402 (lane 2) and HMS174/pET3a (lane 1) infected with λ phage CE6 was analyzed by SDS/PAGE with a 4–20% gradient gel (Dai-ichi Pure Chemicals, Tokyo). Locations of size markers (Dai-ichi Pure Chemicals) are indicated at left ($M_r \times 10^{-3}$). Protein of the expected size is indicated by an arrow. (*B*) Cell extracts were assayed for isoleucine charging activity as described in *Materials and Methods*.

Selective Conservation of N-Terminal Domain, Including its Two Major Insertions. Within the class-defining nucleotide-binding fold, the conservation of sequence across the five widely separated species is relatively high. In contrast, the C-terminal domain, excluding the extra sequences associated with the human enzyme, diverges sharply (Fig. 4). Remarkably, the CP1 insertion, which varies about 5-fold in length (see above) among the five *E. coli* tRNA synthetases in the class I subgroup, is virtually fixed in size (262–277 aa) among the five sequences for isoleucyl-tRNA synthetase shown in Fig. 1. Likewise, the length of the CP2 insertion is relatively constant (105–112 aa) for the isoleucine enzymes. The profile of the similarity score for the five sequences shows that CP1 and CP2 are almost as well conserved (identity residue scores of 16–20%) as the core secondary structure elements (19–20%) that are common to class I enzymes (Fig. 4). But the first half of the C-terminal domain (aa 632–787 of the human enzyme), which extends to the helix–loop motif involved in anticodon recognition, has only a 7% identity residue score, and the second half has a score near zero.

We conclude that the N-terminal catalytic domain, including the two major insertions, acts as an integral unit. Because it is likely that at least CP1 contributes to the part of the enzyme needed for the operational RNA code for isoleucine—for sequence-specific aminoacylation of RNA substrates based on the acceptor stem of $tRNA^{Ile}$ (45)—we infer that these interactions coevolved with those associated with amino acid activation. In contrast, the anticodon-binding domain diverges strongly.

After the completion of this work, we learned that R. Nichols and P. H. Plotz had independently cloned and determined the sequence of the type B enzyme. Their sequence and ours are identical. We thank Professor S. Yokoyama for providing unpublished sequence data for *Thermus thermophilus* isoleucyl-tRNA synthetase, Dr. T. Yudate for providing mRNA of human HeLa–fibroblast cell line CGL-1, and Mr. J. Kuno for preparing TIG-2 mRNA. This work was supported in part by a Grant-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Science, and Culture of Japan, by a grant from the Human Frontiers Science Organization, and by Grant GM15539 from the National Institutes of Health.

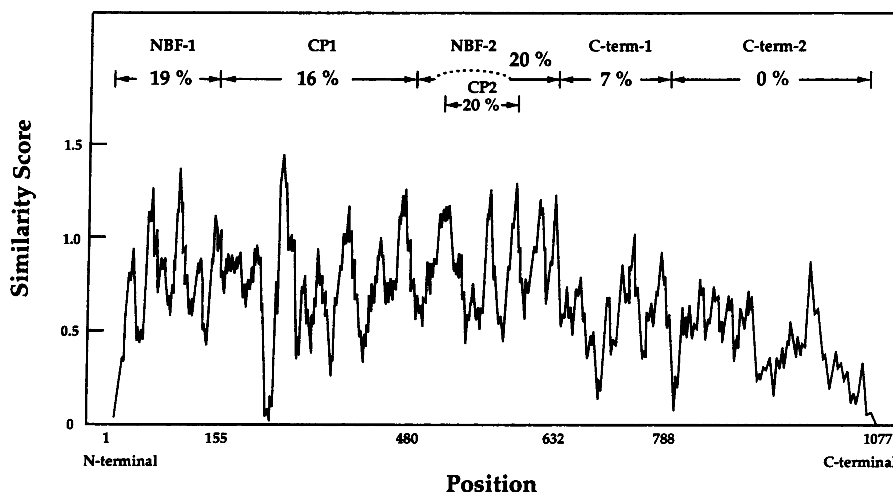

FIG. 4. Similarity score and identity residue score for the aligned sequences (shown in Fig. 1) of five isoleucyl-tRNA synthetases. The similarity score was obtained with the PLOTSIMILARITY program by calculating the average of the Dayhoff scores of all possible pairwise comparisons between sequences at each position with a window range of 10 residues. Similarity score values are shown on the ordinate. With five sequences, these scores range from about −0.3 to 1.5 (identical). Fractions of conserved residues in each domain are shown across the top as identity residue scores by percentages, which were calculated by dividing the number of identical residues among all five sequences by the total length of the shortest sequence of the five. Numbers on the abscissa represent residues of the human type B enzyme. NDF-1 and -2 correspond to the first and the second halves of the N-terminal nucleotide-binding fold, respectively; C-term-1 and C-term-2 are the first and the second halves of the C-terminal domain, respectively, without the appended sequence shown in Fig. 2.

1. Schimmel, P. (1987) *Annu. Rev. Biochem.* **56**, 125–158.
2. Moras, D. (1992) *Trends Biochem. Sci.* **17**, 159–164.
3. Giegé, R., Puglisi, J. D. & Florentz, C. (1993) *Prog. Nucleic Acid Res. Mol. Biol.* **45**, 129–206.
4. Saks, M. E., Sampson, J. R. & Abelson, J. N. (1994) *Science* **263**, 191–197.
5. de Duve, C. (1991) *Blueprint for a Cell. The Nature and Origin of Life* (Patterson Publishers, Carolina Biological Supply Company, NC), Chapt. 8, pp. 173–198.
6. Shiba, K. & Schimmel, P. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 1880–1884.
7. Shiba, K. & Schimmel, P. (1992) *J. Biol. Chem.* **267**, 22703–22706.
8. Landro, J. A. & Schimmel, P. (1993) *Curr. Opin. Struct. Biol.* **3**, 549–554.
9. Webster, T. A., Tsai, H., Kula, M., Mackie, G. A. & Schimmel, P. (1984) *Science* **226**, 1315–1317.
10. Eriani, G., Delarue, M., Poch, O., Gangloff, J. & Moras, D. (1990) *Nature (London)* **347**, 203–206.
11. Cusack, S., Berthet-Colominas, C., Härtlein, M., Nassar, N. & Leberman, R. (1990) *Nature (London)* **347**, 249–255.
12. Schimmel, P., Giege, R., Moras, D. & Yokoyama, S. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 8763–8768.
13. Rould, M. A., Perona, J. J., Söll, D. & Steitz, T. A. (1989) *Science* **246**, 1135–1142.
14. Cavarelli, J., Rees, B., Ruff, M., Thierry, J. C. & Moras, D. (1993) *Nature (London)* **362**, 181–184.
15. Park, S. J. & Schimmel, P. (1988) *J. Biol. Chem.* **263**, 16527–16530.
16. Biou, V., Yaremchuk, A., Tukalo, M. & Cusack, S. (1994) *Science* **263**, 1404–1410.
17. Buechter, D. D. & Schimmel, P. (1993) *Biochemistry* **32**, 5267–5272.
18. Shepard, A., Shiba, K. & Schimmel, P. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 9964–9968.
19. Hou, Y.-M., Shiba, K., Mottes, C. & Schimmel, P. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 976–980.
20. Eriani, G., Dirheimer, G. & Gangloff, J. (1991) *Nucleic Acids Res.* **19**, 265–269.
21. Starzyk, R. M., Webster, T. A. & Schimmel, P. (1987) *Science* **237**, 1614–1618.
22. Schimmel, P., Shepard, A. & Shiba, K. (1992) *Protein Sci.* **1**, 1387–1391.
23. Needleman, S. B. & Wunsch, C. D. (1970) *J. Mol. Biol.* **48**, 443–453.
24. Gribskov, M. & Burgess, R. R. (1986) *Nucleic Acids Res.* **14**, 6745–6763.
25. Ohashi, M., Aizawa, S., Ooka, H., Ohsawa, T., Kaji, K., Kondo, H., Kobayashi, T., Nomura, T., Matsudo, M., Mitsui, Y., Murota, S., Yamamoto, K., Ito, H., Shimada, H. & Utakoji, T. (1980) *Exp. Gerontol.* **15**, 121–133.
26. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY), 2nd Ed.
27. Mead, D. A., Szczesna-Skorupa, E. & Kemper, B. (1986) *Protein Eng.* **1**, 67–74.
28. Zu, Y., Shigesada, K., Nishida, E., Kubota, I., Kohno, M., Hanaoka, M. & Namba, Y. (1990) *Biochemistry* **29**, 8319–8324.
29. Shigesada, K., Itamura, S., Kato, M., Hatanaka, M., Imai, M., Tanaka, M., Masuda, N., Nagai, J. & Nakashima, K. (1987) *Gene* **53**, 163–172.
30. Dumas, J. B., Edwards, M., Delort, J. & Mallet, J. (1991) *Nucleic Acids Res.* **19**, 5227–5232.
31. Studier, F. W., Rosenberg, A. H. & Dunn, J. J. (1990) *Methods Enzymol.* **185**, 60–89.
32. Jenal, U., Rechsteiner, T., Tan, P.-Y., Bühlmann, E., Meile, L. & Leisinger, T. (1991) *J. Biol. Chem.* **266**, 10570–10577.
33. Englisch, U., Englisch, S., Markmeyer, P., Schischkoff, J., Sternbach, H., Kratzin, H. & Cramer, F. (1987) *Biol. Chem. Hoppe-Seyler* **368**, 971–979.
34. Martindale, D. W., Gu, Z.-M. & Csank, C. (1989) *Curr. Genet.* **15**, 99–106.
35. Csank, C. & Martindale, D. W. (1992) *J. Biol. Chem.* **267**, 4592–4599.
36. Hountondji, C., Dessen, P. & Blanquet, S. (1986) *Biochimie* **68**, 1071–1078.
37. Brunie, S., Zelwer, C. & Risler, J.-L. (1990) *J. Mol. Biol.* **216**, 411–424.
38. Stanbridge, E. J., Flandermeyer, R. R., Daniels, D. W. & Nelson-Rees, W. A. (1981) *Somatic Cell Genet.* **7**, 699–712.
39. Mirande, M., Lazard, M., Martinez, R. & Latreille, M. T. (1992) *Eur. J. Biochem.* **203**, 459–466.
40. Lazard, M. & Mirande, M. (1993) *Gene* **132**, 237–245.
41. Cerini, C., Kerjan, P., Astier, M., Gratecos, D., Mirande, M. & Semeriva, M. (1991) *EMBO J.* **10**, 4267–4277.
42. Mirande, M. (1991) *Prog. Nucleic Acid Res. Mol. Biol.* **40**, 95–142.
43. Sprinzl, M., Hartmann, T., Weber, J., Blank, J. & Zeidles, R. (1989) *Nucleic Acids Res.* **17**, r1–r172.
44. Nureki, O., Niimi, T., Muramatsu, T., Kanno, H., Kohno, T., Florentz, C., Giege, R. & Yokoyama, S. (1994) *J. Mol. Biol.* **236**, 710–724.
45. Nureki, O., Niimi, T., Muto, Y., Kanno, H., Kohno, T., Muramatus, T., Kawai, G., Miyazawa, T., Giege, R., Florentz, C. & Yokoyama, S. (1993) in *The Translaton Apparatus*, eds. Nierhaus, K. H., Franceschi, F., Subramanian, A. R., Erdmann, V. A. & Wittmann-Liebold, B. (Springer, Berlin), pp. 59–66.