Functional Replacement of Hamster Lysyl-tRNA Synthetase by the Yeast Enzyme Requires Cognate Amino Acid Sequences for Proper tRNA Recognition†

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ABSTRACT: We cloned the cDNA encoding a 597-aa hamster lysyl-tRNA synthetase. This enzyme is a close homologue of the 591-aa *Saccharomyces cerevisiae* enzyme, with the noticeable exception of their 60-aa N-terminal regions, which differ significantly. Several particular features of this polypeptide fragment from the hamster lysyl-tRNA synthetase suggest that it is implicated in the assembly of that enzyme within the multisynthetase complex. However, we show that this protein domain is dispensable *in vivo* to sustain growth of CHO cells. The cross-species complementation was investigated in the lysine system. The mammalian enzyme functionally replaces a null-allele of the yeast *KRS1* gene. Conversely, the yeast enzyme cannot rescue Lys-101 cells, a CHO cell line with a temperature-sensitive lysyl-tRNA synthetase. The yeast and mammalian enzymes, overexpressed in yeast, were purified to homogeneity. The hamster lysyl-tRNA synthetase efficiently aminoacylates both mammalian and yeast $tRNA^{Lys}$, whereas the yeast enzyme aminoacylates mammalian $tRNA^{Lys}$ with a catalytic efficiency 20-fold lower, as compared to its cognate tRNA. The 152-aa C-terminus extremity of the hamster enzyme provides the yeast enzyme with the capacity to complement Lys-101 cells. This hybrid protein is fairly stable and aminoacylates both yeast and mammalian $tRNA^{Lys}$ with similar catalytic efficiencies. Because this C-terminal polypeptide fragment is likely to make contacts with the acceptor stem of $tRNA^{Lys}$, we conclude that it should carry the protein determinants conferring specific recognition of the cognate tRNA acceptor stem and therefore contributes an essential role in the operational RNA code for amino acids.

Eukaryotic aminoacyl-tRNA synthetases are essentially made of three structurally distinct functional domains. One of these domains, the catalytic core of the enzyme, bearing the class-defining sequence motifs (Eriani *et al.*, 1990), might have contributed a primordial aminoacyl-tRNA synthetase with the capacity to selectively aminoacylate minimalist RNA molecules mimicking the present day acceptor stem of tRNA (Schimmel *et al.*, 1993). For a given synthetase, this unit is well conserved among species, from prokaryotes to eukaryotes. Later in evolution, a second protein module and L-shaped tRNA molecules would have conjointly emerged, providing more accurate discrimination properties. In many instances, this protein unit contributes an anticodon binding domain (Cavarelli & Moras, 1993). However, even when the anticodon moiety of tRNA plays a prominent role in tRNA·enzyme recognition, the loss of specificity in this attachment site is compensated by the discrimination properties provided by acceptor helix interactions (Auld & Schimmel, 1996). The univocal relationship that connects the nucleotide sequence of the acceptor stem of a tRNA to an amino acid, via specific RNA·protein interactions, is referred to as the operational RNA code for amino acids (Schimmel *et al.*, 1993). The catalytic and tRNA-binding modules of aminoacyl-tRNA synthetases are essentially preserved among species. In eukaryotes, synthetases have polypeptide extensions that contribute an additional domain, unrelated to catalysis (Mirande *et al.*, 1993). It confers on them the ability to bind to polyanionic structures or, in mammalian cells, to assemble into multienzyme complexes.

Lysyl-tRNA synthetase (LysRS)[1] is one of the 10 members of class II aminoacyl-tRNA synthetases. To date, the primary structure of LysRS has been determined from several sources, including various eubacteria, yeast, nematode, and plant, and the crystal structure of a bacterial species solved (Onesti *et al.*, 1995). In mammals, LysRS is one of the components of a multienzyme complex comprising the eight other synthetases specific for glutamic acid, proline, isoleucine, leucine, methionine, glutamine, arginine, and aspartic acid (Mirande *et al.*, 1993). Whereas the native enzyme, dissociated from the complex, displays hydrophobic and polyanion-binding properties, a proteolytically truncated yet fully active form did not (Cirakoglu & Waller, 1985). Because this truncated species behaved as a free enzyme, the domain lost upon proteolytic conversion is most likely involved in complex assembly. When expressed in Lys-101 cells, a Chinese hamster ovary (CHO) cell line with a temperature-sensitive LysRS (Adair *et al.*, 1979), yeast LysRS behaves as a free enzyme and cannot functionally replace the defective mammalian enzyme (Mirande *et al.*, 1992). These results raised two nonexclusive possibilities: (i) Association to the complex is a prerequisite, *in vivo*, to provide aminoacyl-tRNA to the protein synthesis machinery, and (ii) aminoacylation of mammalian $tRNA^{Lys}$ by the yeast enzyme is limiting.

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[1] Abbreviations: LysRS, lysyl-tRNA synthetase; AspRS, aspartyl-tRNA synthetase; CHO, Chinese hamster ovary.

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In the present study, the cDNA encoding hamster LysRS has been isolated and sequenced. Expression in yeast and CHO cells of variants of the yeast and hamster LysRS provided a suitable tool to identify molecular features responsible for cross-species specificities. We show here that the C-terminus of hamster LysRS confers on the yeast enzyme the ability to complement a Lys-101 strain. This segment of the catalytic core of the enzyme is believed to contain most if not all of the protein information involved in the operational RNA code for amino acids.

MATERIALS AND METHODS

DNA Manipulations. All recombinant manipulations were carried out using standard protocols (Sambrook *et al.*, 1989). Nucleotide sequences were determined by the dideoxy nucleotide chain termination method (Sanger *et al.*, 1977) using the T7 sequencing kit from Pharmacia. Restriction endonucleases and DNA modification enzymes were purchased from either Boehringer (Mannheim), New England Biolabs, BRL, or Perkin-Elmer Cetus and used as recommended by the suppliers. Radionucleotides were from Amersham.

Isolation and Sequencing of cDNA Encoding LysRS from CHO Cells. Mixed oligonucleotide primers (Table 1), in the sense orientation, were selected using the amino acid sequence of tryptic fragments of LysRS from rat liver (Jacobo-Molina *et al*., 1988). An *Eco*RI linker was introduced to facilitate the rescue of amplified cDNA. The antisense primer dT20(Bam), complementary to the poly(A) tail of mRNA and containing a *Bam*HI linker, was 5′-GGGATCC$(T)_{20}$-3′. Total cellular RNA from rat liver was prepared as described by Han *et al*. (1987), and poly$(A)^+$ RNA was selected by oligo(dT)-cellulose chromatography. The first-strand cDNA synthesis on poly$(A)^+$ RNA (1 μg) was primed by using a $(dT)_{15}$ (0.5 μg). Reverse transcription was initiated by the addition of 200 units of Mo-MLV reverse transcriptase (BRL) and allowed to proceed at 42 °C for 90 min. The single-stranded cDNA was subjected to PCR amplification by using 8 μM of one of the three mixed oligonucleotide primers (Table 1) deduced from amino acid sequences of rat LysRS, 2 μM oligonucleotide dT20(Bam), and 2.5 units of AmpliTaq DNA polymerase (Perkin-Elmer Cetus). The amplification was allowed to proceed for 40 cycles of 1 min at 94 °C, 2 min at 50 °C, and 3 min at 72 °C. A portion of each of the three PCR products was subjected to a second run (30 cycles) of PCR amplification with the specific oligonucleotides that were not used in the preceding step and dT20(Bam). PCR products were fractionated by electrophoresis on a 1.5% agarose gel in TBE buffer [50 mM Tris-borate (pH 8.3), 1 mM EDTA] and transferred on nylon membranes (NY 13 N from Schleicher & Schuell) by vacuum blotting (VacuGene, Pharmacia) using 20× SSPE (1× SSPE: 180 mM NaCl, 10 mM NaH_2PO_4, 1 mM EDTA, pH 7.4). DNA was fixed on the membranes by 2 min of UV exposure. After prehybridization in 5× SSPE, 2.5× Denhardt's solution, 2% Dextran sulfate, 1% SDS, 2% formamide, 150 μg/mL yeast tRNA, and 5 μg/mL poly(A), for 30 min at 28 °C, hybridization was conducted in the same solution containing radiolabeled mixed oligonucleotides (about 5 pmol/mL, 0.5 × 10^6 cpm/mL), for 16 h at 28 °C. Oligonucleotides were end-labeled with [γ-^{32}P]-ATP and T4 polynucleotide kinase. Filters were washed at 37 °C in 2× SSPE and 0.5% SDS and exposed to XAR film at −80 °C in the presence of intensifying screens. PCR products containing putative LysRS-related cDNAs were digested with *Eco*RI and *Bam*HI and ligated with the M13mp18 vector. Following transformation of *Escherichia coli* strain JM101Tr, positive transformants were identified by *in situ* hybridization (Benton & Davis, 1977) with the mixed oligonucleotide probes. Recombinant M13 phages containing DNA inserts of the expected size were selected and the nucleotide sequences determined.

The selected cDNA fragments were used to screen a λgt11 cDNA library constructed from total poly$(A)^+$ mRNA from CHO cells (Lazard & Mirande, 1993). Approximately 0.5 × 10^6 recombinant phages were plated on *E. coli* Y1090, and positive transformants were selected as described by Benton and Davis (1977). Prehybridization was carried out in 5× SSPE, 2.5× Denhardt's solution, 5% Dextran sulfate, 5% SDS, 50% formamide, 150 μg/mL yeast tRNA, and 5 μg/mL poly(A), for 30 min at 42 °C. Hybridization was in the same solution containing cDNA probes (about 1−2 ng/mL, 0.5−1.0 × 10^6 cpm/mL) radiolabeled by random oligonucleotide priming (Hodgson & Fisk, 1987). Following incubation for 16 h at 42 °C, filters were washed at 65 °C in 0.1× SSPE and 0.5% SDS and exposed to XAR film. The cDNA inserts were cloned into M13 vectors. The nucleotide sequence of the complete cDNA was determined on both strands.

Isolation of Stable Transformants of CHO Cells. For transformation of CHO cells, all constructs were placed under the control of SV40 promoter in the eukaryotic expression vector pSG5 (Green *et al.*, 1988). pSG/CKRS was constructed by inserting the full-length *Eco*RI-*Eco*RI cDNA from hamster LysRS into the *Eco*RI site of pSG5. To construct pSG/CKRS-ΔN, the *Sac*I-*Eco*RI fragment of hamster LysRS was ligated into the *Eco*RI site of pSG5 together with the *Eco*RI-*Sac*I linker made of the two complementary oligonucleotides 5′-AATTCAATAGCATAATGGCCGAGCT-3′ and 3′-GTTATCGTATTACCGGC-5′. The ensuing coding region of hamster LysRS has a 38-amino acid deletion, from Thr^3 to Lys^{40}. To construct pSG/CYKRS, the *Sac*I-*Hin*dIII fragment from the hamster cDNA in pSG/CKRS was replaced by addition of the *Bam*HI-*Hin*dIII DNA fragment from the yeast *KRS1* gene (Mirande & Waller, 1988) together with the *Sac*I-*Bam*HI linker made of the two complementary oligonucleotides 5′-CATTGAGAAACAACTAAGCCAAG-CTGCTGCTGCTGCTGCCACC-3′ and 3′-TCGAGTAA-CTCTTTGTTGATTCGGTTCGACGACGACGA-CGACGGTGGCTAG-5′. The encoded protein corresponds to a chimeric LysRS made of Met^1−Thr^{56} of hamster LysRS, Ser^{58}−Asp^{449} of yeast LysRS, and Lys^{446}−Val^{597} of the hamster enzyme. pSG/YKRS-N was constructed by replacing the 3′-terminal *Hin*dIII-*Bam*HI fragment from pSG/CYKRS by the *Hin*dIII-*Sna*BI yeast DNA fragment. The encoded protein consists of Met^1−Thr^{56} of hamster LysRS and Ser^{58}−Asn^{591} of yeast LysRS. The resulting plasmids were verified by DNA sequencing.

Wild-type CHO cells and Lys-101 cells, a mutant cell line with a temperature-sensitive LysRS (Adair *et al*., 1979), were grown and transformed as described earlier (Mirande *et al.*, 1992) following cotransfection with pSG derivatives and the pSV2neo plasmid carrying the aminoglycoside phosphotransferase gene as a selectable marker for neomycine resistance. Crude extracts were prepared as previously described (Mirande *et al.*, 1992). Expression of the various

LysRS derivatives from stable transformants grown from individual colonies was monitored by the tRNA aminoacylation assay and Western blotting using anti-yeast LysRS antibodies (Mirande *et al.*, 1986) or affinity-purified anti-sheep LysRS antibodies (Mirande *et al.*, 1982b), goat anti-rabbit IgG conjugated with peroxidase (Biosys), and the ECL detection reagents from Amersham.

Expression of Mammalian LysRS in Yeast. For transformation of yeast cells, all constructs were made in the yeast expression vector pYeDP10 (Urban *et al.*, 1990). The multicopy plasmid has the yeast 2μ origin of replication and the *URA3* selectable marker. Heterologous proteins are expressed under the control of the strong constitutive PGK promoter. pYeDP10/CYKRS was constructed in a two-step procedure: (i) The *Bgl*II-*Bam*HI fragment from pSG/CYKRS was cloned into the *Bgl*II site of pYeDP10. (ii) A *Bgl*II-*Bgl*II fragment was produced from pSG/CYKRS by PCR amplification between the oligonucleotides K01 (5′-GG-GAGCTCAGATCTTCATAATGGCCACGCTGCA-GGAGTGT-3′) and K02 (5′-CGTGAAACTTGTGTGGG-TAT-3′) and digestion with *Bgl*II. This fragment, bearing the 5′-coding region of CYKRS, was introduced at the *Bgl*II site. To construct pYeDP10/CKRS, the *Bgl*II-*Sac*I fragment, produced as above by PCR between K01 and K02 and digestion with *Bgl*II and *Sac*I, and the *Sac*I-*Eco*RI fragment from pSG/CKRS were inserted into pYeDP10 digested with *Bgl*II and *Eco*RI. The resulting plasmids were verified by DNA sequencing.

A null-allele of *KRS1*, the gene for *Saccharomyces cerevisiae* LysRS, was constructed. The plasmid pdYK01 was constructed by inserting the *Sph*I-*Bsm*I fragment from pYK04 into pYK06 digested with *Sph*I and *Sna*BI (Martinez & Mirande, 1992). The *Sph*I-*Sma*I fragment from pdYK01 contains the 5′- and 3′-flanking regions from *KRS1*, but the *KRS1* coding sequences are replaced by the *TRP1* gene. This fragment was used to transform to Trp^+ the diploid strain CC456 (*his3*/*his3*, *leu2*/*leu2*, *ura3*/*ura3*, *trp1*/*trp1*), to give CCdYK01. The replacement of one *KRS1* allele by a *Trp1* allele was verified by Southern blotting. After sporulation, only two of the four spores survived; they were Trp^- in phenotype. Plasmids pYeDP10/CKRS and pYeDP10/CYKRS were used to transform to Ura^+ the diploid strain CCdYK01. After sporulation, tetrads were dissected and the phenotype of viable spores was analyzed. The two haploid strains CdYK01:pYeDP10/CKRS and CdYK01:pYeDP10/CYKRS (*his3*, *leu2*, *trp1*) with a deleted *KRS1* allele and with functional, plasmid-encoded LysRS were selected.

Protein Purification. The yeast cells CdYK01:pYeDP10/CKRS and CdYK01:pYeDP10/CYKRS, which express the wild-type recombinant LysRS from CHO cells ($LysRS^C$) and the chimeric CHO:yeast LysRS ($LysRS^{CY}$), respectively, were grown at 28 °C in 4.5 L of YPG [0.5% yeast extract (Difco), 0.5% Bacto Peptone (Difco), 3% D-glucose] to an absorbance of $A_{600} \sim 8$. Cells were washed, resuspended in 60 mL of extraction buffer [100 mM Tris-HCl (pH 8.0), 100 mM KCl, 10 mM $MgCl_2$, 1 mM EDTA, 10 mM β-mercaptoethanol, 10% glycerol], and lysed in an Eaton Press at 6000 psi. The lysate was diluted by addition of 90 mL of extraction buffer containing protease inhibitors [1 mM phenylmethanesulfonyl fluoride, 1 mM diisopropyl fluorophosphate (Sigma), leupeptin, pepstatin, and chymostatin (Protein Research Foundation, Japan) at 0.5, 1, and 20 μg/mL, respectively] and centrifuged at 10000*g* for 30 min at 2 °C to remove cell debris.

All subsequent steps were performed at 4 °C. Following precipitation of nucleic acids by addition of Polymin P (BASF) at 0.15% and centrifugation, the clear supernatant was applied to a S Sepharose FF column (Pharmacia, 1.6 × 15.5 cm), equilibrated in 50 mM potassium phosphate (pH 7.5), 1 mM EDTA, 10 mM β-mercaptoethanol, 10% glycerol and eluted with a 400 mL linear gradient of potassium phosphate from 50 to 300 mM (pH 7.5). Fractions containing LysRS activity (eluted at ~150 mM for $LysRS^C$ or $LysRS^{CY}$) were pooled, 5-fold diluted with 10 mM β-mercaptoethanol and 10% glycerol, applied to a Q Sepharose FF column (Pharmacia, 1.6 × 5.0 cm) equilibrated in 30 mM potassium phosphate (pH 7.5), 1 mM EDTA, 10 mM β-mercaptoethanol, and 10% glycerol, and eluted with a 260 mL linear gradient of potassium phosphate from 30 to 250 mM (pH 7.5). Fractions containing LysRS activity (eluted at ~120 mM for $LysRS^C$ and ~90 mM for $LysRS^{CY}$) were combined, 1.5-fold diluted as above, applied to a phosphocellulose P11 column (Whatman, 1.6 × 8.0 cm) equilibrated in 10 mM potassium phosphate (pH 7.5), 1 mM EDTA, 10 mM β-mercaptoethanol, and 10% glycerol, and eluted with a 320 mL linear gradient of potassium phosphate from 10 to 400 mM (pH 7.5). Fractions containing LysRS activity (eluted at ~200 mM for $LysRS^C$ or $LysRS^{CY}$) were pooled, dialyzed against 200 mM potassium phosphate (pH 7.5), 10 mM β-mercaptoethanol, 0.01 mM $CaCl_2$, and 10% glycerol, and applied to a Macro-Prep ceramic hydroxyapatite column (type I from Bio-Rad Laboratories, 1.6 × 5.0 cm) equilibrated in the same buffer, at room temperature, and eluted with a 200 mL linear gradient of potassium phosphate from 200 to 600 mM (pH 7.5). Fractions containing LysRS activity (eluted at ~400 mM for $LysRS^C$ and ~350 mM for $LysRS^{CY}$) were pooled, concentrated by vacuum dialysis, dialyzed against 25 mM potassium phosphate (pH 7.5), 1.5 mM DTE (dithioerythritol, Fluka), and 50% glycerol, and stored at −20 °C.

Thermal Inactivation. Purified LysRS from yeast and hamster or the hybrid yeast/hamster protein, $LysRS^Y$, $LysRS^C$, or $LysRS^{CY}$, were incubated at a LysRS concentration of 0.1 μM (~13.6 μg/mL) in 10 mM Tris-HCl (pH 7.5), 10 mM β-mercaptoethanol, and 4 mg/mL bovine serum albumin, at varying temperatures. At different time intervals, samples were withdrawn from the incubation mixture and placed on ice. The residual LysRS activity in the samples was determined by the $tRNA^{Lys}$ aminoacylation assay.

Enzymatic Assays. Initial rates of tRNA aminoacylation were measured at 25 °C, unless otherwise stated, in 0.1 mL of 20 mM imidazole-HCl buffer (pH 7.5), 150 mM KCl, 0.5 mM DTE, 5 mM $MgCl_2$, 3 mM ATP, 60 μM ^{14}C-labeled lysine (DuPont NEN, 50 Ci/mol), and saturating amounts of beef liver or yeast tRNA. The incubation mixture contained catalytic amounts of enzymes (0.5−1.5 nM for $LysRS^C$ and $LysRS^{CY}$, 1−5 nM for $LysRS^Y$) appropriately diluted in 10 mM Tris-HCl (pH 7.5), 10 mM β-mercaptoethanol, and containing bovine serum albumin at 4 mg/mL. One unit of LysRS activity is the amount of enzyme producing 1 nmol of lysyl-tRNA/min, at 25 °C. Specific activities of purified enzymes were calculated with protein concentrations determined by using absorption coefficients of 0.547, 0.567, and 0.512 A_{280} units·mg^{-1}·cm^2 for $LysRS^C$, $LysRS^{CY}$, and $LysRS^Y$, respectively. For the determination

Table 1: Mixed Oligonucleotide Primers Deduced from the Amino Acid Sequence of the Tryptic Peptides of Lysyl-tRNA Synthetase

peptide sequence	sense primer synthesized
LPETSLFETEET	KRSRL1: 5′-ggaattcaTT(TC)GA(GA)AC(ATC)GA(GA)GA(GA)AC-3′
VLLFPAMKP	KRSRL2: 5′-ggaattcaTT(TC)CC(ATC)GC(ATC)ATGAA(GA)CC-3′
TYHPDGPEGQAYEIDF	KRSRL3: 5′-ggaattcaGC(ATC)TA(TC)GA(GA)AT(TC)GA(TC)TT-3′

[a] Amino acid sequences of tryptic fragments from rat liver lysyl-tRNA synthetase (Jacobo-Molina *et al*., 1988) used in this study are indicated. Three different mixed sense primers were synthesized from the underlined sequences. Codon degeneracy is presented in parentheses. Rare codons ACG (Thr), CCG (Pro), GCG (Ala), and ATA (Ile) were omitted. Nucleotides introduced to generate an *Eco*RI site are shown in lower script.

of K_M values for tRNA, $tRNA^{Lys}$ concentrations of 0.02−7.5 μM were used. Michaelian parameters were obtained by nonlinear regression of the theoretical Michaelis equation to the experimental curve using the KaleidaGraph 3.0.4 software (Abelbeck Software).

The isotopic [^{32}P]PP_i−ATP exchange reaction was assayed at 25 °C, unless otherwise stated, in 0.1 mL of 20 mM imidazole-HCl buffer (pH 7.5), 0.1 mM EDTA, 2.5 mM β-mercaptoethanol, 10 mM $MgCl_2$, 2 mM ATP, 2 mM lysine, and 2 mM [^{32}P]PP_i (DuPont NEN, 0.4 Ci/mol). Reaction was initiated by the addition of an appropriate enzyme dilution (in the 10 nM range). One unit of LysRS activity in the exchange reaction is the amount of enzyme producing 1 nmol of ATP/min, at 25 °C. For the determination of K_M values for ATP and amino acid, ATP and lysine concentrations of 0.01−2 mM and 0.4−400 μM were used, respectively.

RESULTS

Isolation of a Mammalian cDNA Encoding Lysyl-tRNA Synthetase. In mammalian cells, LysRS is a component of a multienzyme complex containing eight other synthetases as well. Among the 11 polypeptide components that characterize the purified enzyme preparation, that of an apparent molecular mass of 76 kDa was attributed to LysRS (Mirande *et al*., 1982a). The amino acid sequences of five peptides from the corresponding protein from rat liver were reported (Jacobo-Molina *et al*., 1988). Mixed oligonucleotide primers were derived from three of these peptide sequences to amplify cDNA sequences by PCR. To design degenerate primers, the amino acid sequences with minimal degeneracy were selected (Table 1). The extent of degeneracy was also optimized by taking into account codon bias for translation. The three sense primers (Table 1) and the antisense primer dT20(Bam) complementary to the mRNA poly(A)$^+$ tail were used to amplify cDNA prepared from poly(A)$^+$ mRNA from rat liver. The actual order of the primers along the cDNA sequence was unknown. When PCR was performed with primers KRSRL3 and dT20(Bam) followed by KRSRL1 or KRSRL2 and dT20(Bam), two fragments of ≈800 and ≈300 nucleotides were obtained. The cDNA sequences revealed that these two fragments encoded the C-terminal region of the same protein, which displayed strong sequence similarity with LysRS sequences from other organisms. These fragments were used to probe a λgt11 CHO cDNA library. Several inserts of various sizes were obtained and sequenced. The longest cDNA fragment was 2036 nucleotides long and comprised both the 5′- and 3′-untranslated regions. Accordingly, Northern blot analysis on total poly(A)$^+$ mRNA from CHO cells revealed a single transcript of ≈2100 nucleotides (data not shown). The cDNA sequence (accession number Z31711) displayed a single long open reading frame of 1791 nucleotides. The 5′- and 3′-untranslated regions are composed of 20 and 158 nucleotides, respectively. A canonical AATAAA polyadenylation signal precedes a 67-nucleotide long poly(A) tail.

Identification of the Deduced Protein as Lysyl-tRNA Synthetase. The cDNA sequence codes for a polypeptide of 597 amino acids, with a calculated molecular mass of 68 kDa. The five peptides recovered from rat LysRS (Jacobo-Molina *et al*., 1988) were identified in the deduced amino acid sequence of hamster LysRS (Figure 1). Alignment of hamster, yeast, and bacterial LysRS is shown in Figure 1. The homology between the hamster and yeast proteins extends over their entire length (55% of identical amino acid residues). Although both enzymes have a large N-terminal polypeptide extension and a small C-terminal extension as compared to the prokaryotic LysRS, the extent of sequence similarity in these regions (24% of identical residues) is much lower than for the core domain (60%). The common regions from the bacterial and mammalian enzymes share 43% of identical residues. As shown below, the cDNA-encoded protein, expressed in CHO cells or yeast, has an apparent molecular mass identical with that of the LysRS component of the multisynthetase complex and exhibits $tRNA^{Lys}$ aminoacylation activity.

In yeast, whereas the polypeptide extension of LysRS is not essential for growth, it could promote enzyme stability *in vivo* (Martinez & Mirande, 1992). According to several algorithms of secondary-structure prediction (Garnier *et al.*, 1978; Rost & Sander, 1993), a segment from the N-terminal extension of yeast and hamster LysRS, from Lys^{16} to Ala^{55} and from Asn^{16} to Lys^{55}, respectively, displays the propensity to fold into an α-helical structure (Figure 2). A particular feature of the helical segment of hamster LysRS is the segregation of lysine residues on one face of the helix and the stacking of hydrophobic and hydrophilic blocks on the opposite face (Figure 2). As in the case of mammalian aspartyl- and arginyl-tRNA synthetases (Mirande *et al*., 1992; Lazard & Mirande, 1993), two other components of the multisynthetase complex, the arrangement of hydrophobic and hydrophilic amino acids in the N-terminal extension of hamster LysRS could be involved in complex assembly.

Complementation of Lysyl-tRNA Synthetase from a Defective Lys-101 CHO Cell. To test for the involvement of the N-terminal polypeptide extension in the association of mammalian LysRS into the complex, the wild-type CHO LysRS, $LysRS^C$, and a derivative with a 38-amino acid deletion from Thr^3 to Lys^{40}, $\Delta N\text{-}LysRS^C$, were constructed in the eukaryotic expression vector pSG5 as described in Materials and Methods and expressed *in vivo*. In addition, two variants of the yeast LysRS were constructed. In the first of them, $N^C\text{-}LysRS^Y$, the N-terminal extension of the yeast enzyme was replaced by the N-terminal extension of the mammalian enzyme, from residues Met^1 to Lys^{56}. Because that derivative failed to complement a Lys-101 strain

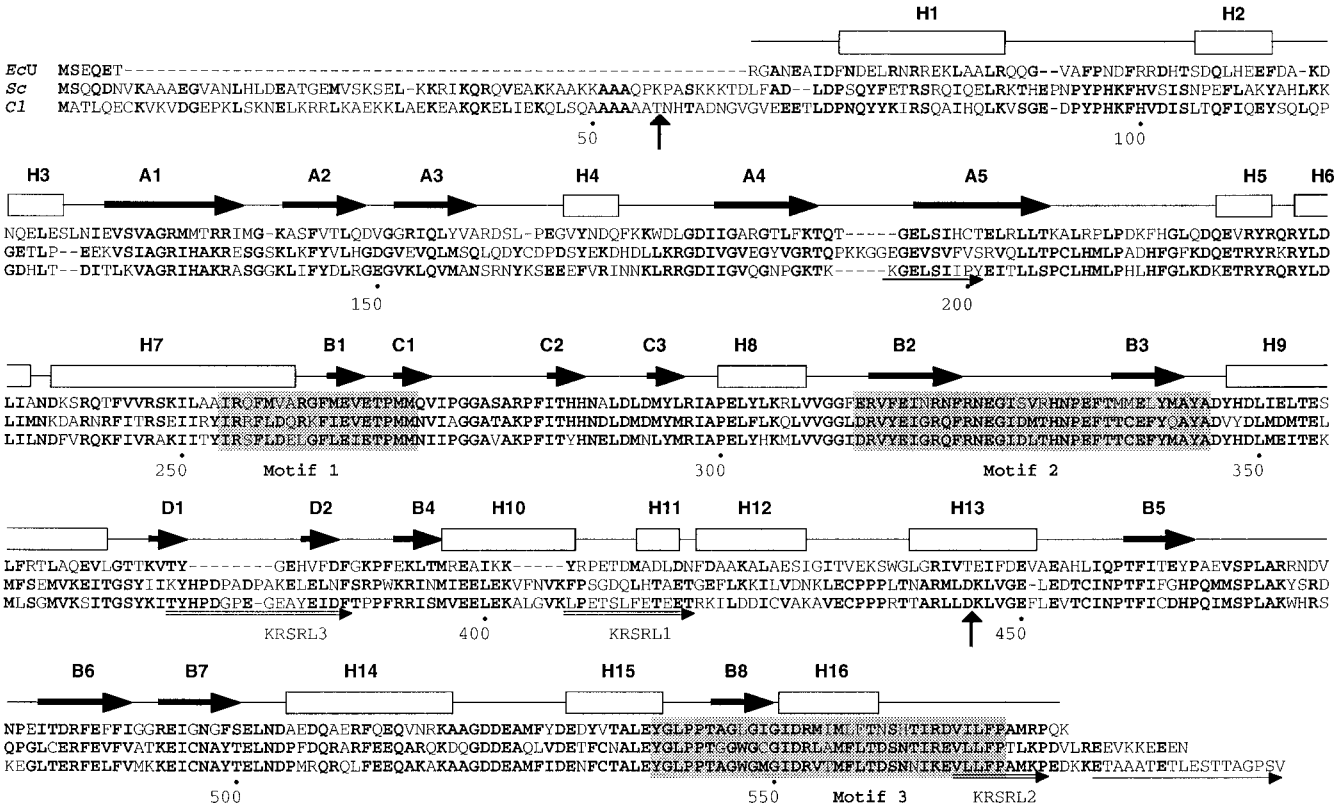

FIGURE 1: Alignment of the amino acid sequences of lysyl-tRNA synthetases. The complete amino acid sequences of LysRS from hamster (*Cricetulus longicaudatus*; *Cl*), yeast (*Saccharomyces cerevisiae*; *Sc*) (Mirande & Waller, 1988), and bacteria (*lysU* gene from *Escherichia coli*; *Ec*U) (Lévêque *et al*., 1990) were aligned using the Clustal W program by weighting with the mutation data matrix PAM 250 (Thompson *et al.*, 1994). Amino acid residues of the mammalian enzyme are numbered under the sequences. Residues which are identical or similar between two sequences are indicated in bold-faced type. The three sequence motifs characteristic of class II aminoacyl-tRNA synthetases are shaded. The secondary-structure elements of the bacterial enzyme, including α-helices (rectangles) and *β*-strands (arrows), are based on the crystal structure of the *lysU* gene product (Onesti *et al*., 1995). The vertical arrows show the fusion points in the mammal/yeast/mammal hybrid protein, LysRS^{CY}, that we constructed *in vitro*. The sequences of the five peptides reported for rat LysRS (Jacobo-Molina *et al.*, 1988) are underlined; those that were used to design oligonucleotide primers are double-underlined, and the name of the primer is indicated.

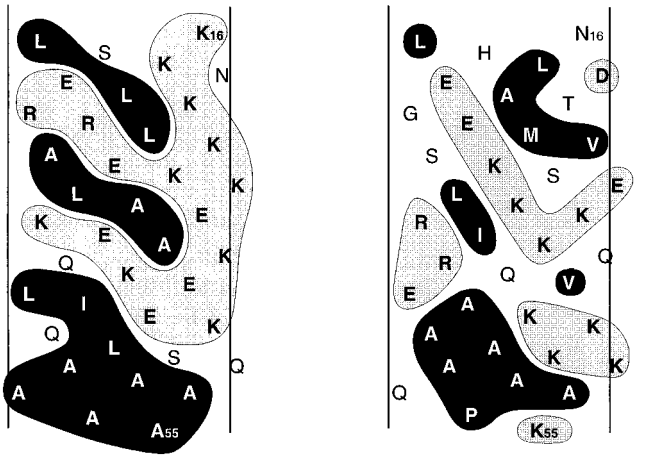

FIGURE 2: Structural organization of the polypeptide extensions of lysyl-tRNA synthetase from higher and lower eukaryotes. The segments from Lys^{16} to Ala^{55} and from Asn^{16} to Lys^{55} of LysRS from hamster (left) and yeast (right), respectively, predicted as α-helices structures according to the algorithm of Garnier *et al.* (1978), are shown as helical net diagrams. Hydrophobic and charged amino acid residues are indicated on black and shaded backgrounds, respectively.

(see below), a chimeric enzyme, LysRS^{CY}, was constructed. Since the purpose of that fusion was to test the possibility that accurate interactions with the acceptor stem of tRNA could be sufficient to confer on the yeast enzyme the capacity to aminoacylate mammalian tRNA, we chose to replace the C-terminus of N^{C}-LysRS^{Y} by the homologous region from the hamster enzyme, from residues Lys^{446} to Val^{597}. Indeed, according to the crystal structure of *E. coli* LysRS (Onesti *et al.*, 1995) and the tRNA·enzyme complex of yeast AspRS (Cavarelli *et al.*, 1993), a close homologue of LysRS, we assumed that this polypeptide fragment should exclusively interact with the acceptor stem region of tRNA. In addition, to favor a good structural complementarity between the yeast and hamster protein moieties, the point of fusion was chosen in a region where the two protein sequences are highly similar. A *Hin*dIII site shared by the two nucleotide sequences, located in a DNA segment coding for the very conserved helix H13 (Figure 1), fulfilled all of the above requirements. This fragment provides four of the six antiparallel *β*-strands that form the active site of the mammalian enzyme and the C-terminal extension.

These plasmids were used to transform a CHO cell line with a thermosensitive LysRS, the Lys-101 cell line (Adair *et al.*, 1979), in the presence of the pSV2neo plasmid which confers G418 resistance on the transfected cells. Stable transformants were selected. Since plasmid sequences are maintained following random integration into the genome, the gene copy number of the constructs and therefore the level of expression of the encoded proteins vary from one cell line to another. The expression of LysRS^{C}, ΔN-LysRS^{C}, N^{C}-LysRS^{Y}, and LysRS^{CY} in the cloned transformed cell lines

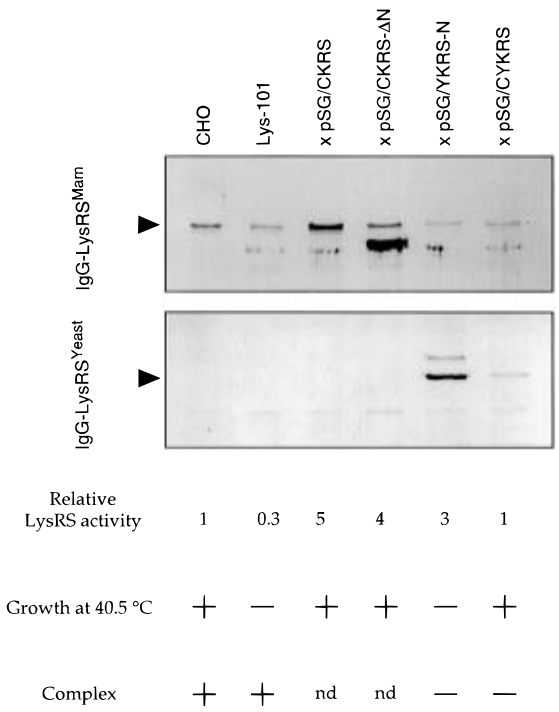

FIGURE 3: Yeast/hamster hybrid LysRS rescuing a Lys-101 strain. The expression of LysRS^{C} (68 kDa), ΔN-LysRS^{C} (63.5 kDa), N^{C}-LysRS^{Y} (68 kDa), or LysRS^{CY} (69 kDa) in Lys-101 cells transformed with pSG5 plasmids bearing the constructs CKRS, CKRS-ΔN, YKRS-N, or CYKRS, respectively, was detected by Western blotting. Wild-type CHO and Lys-101 cells were used as reference strains. Protein extracts were subjected to SDS−PAGE followed by immunoblot analysis with antibodies directed to sheep LysRS (IgG-LysRS^{Mam}) or yeast LysRS (IgG-LysRS^{Yeast}). The hybrid LysRS^{CY} protein reacted faintly with both antibodies. Arrow heads point to the 68 kDa polypeptides. LysRS activity in cell extracts was determined by the aminoacylation of tRNA and corrected for the protein concentration of the extracts. Relative specific activities are expressed by using wild-type CHO cells as the reference strain. At permissive temperature (34 °C) all strains grew approximately at the same rate (doubling time: 28 ± 3 h). At 40.5 °C, parental Lys-101 cells and Lys-101 cells transformed with pSG/YKRS-N did not grow, and cell lysis occurred. Other cell types developed with a doubling time of about 16 ± 2 h. Association of LysRS to the multisynthetase complex was monitored by molecular sieve chromatography analysis of protein extracts (nd: not determined). The two species N^{C}-LysRS^{Y} and LysRS^{CY} could not be detected in the high molecular weight fractions.

was determined by assaying tRNA aminoacylation activities in cell extracts. Relative LysRS activities are reported in Figure 3. Immunoblot analysis with anti-sheep and anti-yeast LysRS antibodies also detected expression of the various LysRS species in Lys-101 cells (Figure 3).

Stable transformants were tested for their ability to grow under nonpermissive conditions for the host Lys-101 cell. The two Lys-101 derivatives expressing the wild-type, LysRS^{C}, or the N-terminal-truncated, ΔN-LysRS^{C}, hamster LysRS were able to grow at 40.5 °C, in conditions were wild-type CHO cells grew but Lys-101 cells did not (Figure 3). This result indicates that the N-terminal polypeptide extension of mammalian LysRS is dispensable for growth. We have previously shown that the wild-type yeast LysRS cannot rescue a Lys-101 cell line (Mirande *et al.*, 1992). We show here that the yeast enzyme with a mammalian N-terminal polypeptide extension, N^{C}-LysRS^{Y}, is again not able to functionally complement a Lys-101 strain. Noteworthy is the fact that the hybrid LysRS construct, LysRS^{CY}, is stably expressed in Lys-101 cells and confers on them the ability to grow at nonpermissive temperature (Figure 3). Thus, the 152 amino acid residues from the C-terminus extremity of hamster LysRS, from Lys^{446} to Val^{597}, are likely to be readily accommodated in the structure of the yeast enzyme and give a fairly stable hybrid protein, providing the yeast enzyme with the capacity to replace the endogenous mammalian LysRS.

Because antibodies directed to the yeast enzyme do not cross-react with hamster LysRS (Figure 3), we could readily analyzed the structural behavior of N^{C}-LysRS^{Y} and LysRS^{CY} by submitting crude extracts of the corresponding transformed cell lines to size-exclusion chromatography on a TSK-G4000 SW column, as previously described (Mirande *et al.*, 1992). The column fractions corresponding to the elution volume of complex-associated and free enzymes were analyzed by immunoblotting (result not shown). The two yeast LysRS derivatives behaved exclusively as free entities. This result indicates that a free enzyme can functionally replace a complex-associated enzyme.

Complementation of a Null-Allele of Yeast KRS1 by the Mammalian Homologous Enzyme. In the yeast *S. cerevisiae* a single essential gene encodes the cytoplasmic form of LysRS (Martinez *et al.*, 1991). A null-allele of *KRS1* was constructed by the one-step disruption procedure (Rothstein, 1983). The *Sph*I-*Sma*I fragment from pdYK01, containing the 5′-upstream and 3′-downstream nucleotide regions from *KRS1* flanking the *TRP1* sequences, was used to transform to Trp^{+} the diploid strain CC456. The ensuing diploid strain CCdYK01 displays a wild-type and a *TRP1*-disrupted *KRS1* allele. The latter is devoid of any LysRS-coding sequence, therefore precluding subsequent recombination with *KRS1*-coding regions that could restore an active *KRS1* locus. We verified that after sporulation only two of the four spores survived; they were Trp^{-} in phenotype.

The diploid strain CCdYK01 was transformed to Ura^{+} with the 2μ plasmid pYeDP10/CKRS or pYeDP10/CYKRS that expressed the hamster enzyme LysRS^{C} and the hybrid LysRS^{CY}, respectively. Expression of the two plasmid-encoded enzymes was verified by tRNA aminoacylation activity and immunoblot analysis (not shown). Twenty tetrads were dissected from sporulated cultures of the two transformed diploid strains. All four spores survived. Two were Trp^{+} and Ura^{+} in phenotype, corresponding to haploid strains bearing a disrupted *KRS1* chromosomal allele rescued by the plasmid-encoded LysRS derivatives. The expression of the mammalian or the yeast/mammalian hybrid LysRS in yeast was detected by immunoblot analysis using antibodies directed to the sheep or yeast enzymes (Figure 4). We conclude that the wild-type hamster LysRS or the chimeric LysRS^{CY} can functionally replace the cytoplasmic yeast enzyme.

Functional Analysis of the Chimeric Enzyme LysRS^{CY}. The wild-type mammalian LysRS complements a yeast strain with a defective LysRS enzyme, whereas the wild-type yeast LysRS does not rescue the mammalian Lys-101 strain but the LysRS^{CY} does. To test for tRNA−enzyme cross-species specificities, we purified to homogeneity the wild-type yeast and hamster enzymes and the LysRS^{CY} hybrid. Since the 2μ plasmids from the haploid strains CdYK01:pYeDP10/CKRS and CdYK01:pYeDP10/CYKRS are the sole source of cytoplasmic LysRS, these two strains can be propagated in rich medium without loss of the multicopy plasmid.

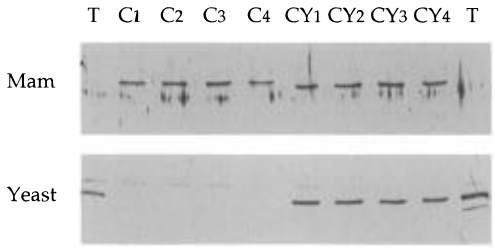

FIGURE 4: Hamster LysRS functionally complementing a *KRS1*-disrupted yeast strain. The expression of LysRS^{C} or LysRS^{CY} from four independent Trp^{+}Ura^{+} haploid strains recovered after sporulation of the diploid strain CCdYK01:pYeDP10/CKRS (C1−C4) or CCdYK01:pYeDP10/CYKRS (CY1−CY4) was detected by immunoblot analysis of protein extracts with antibodies directed to sheep LysRS (Mam) or yeast LysRS (Yeast). The diploid strain CCdYK01 was used as a control (T). In extracts from the haploid strains C1−C4, a polypeptide of 68 kDa that cross-reacted with anti-sheep LysRS antibodies is expressed and a polypeptide related to yeast LysRS could not be detected. The hybrid LysRS^{CY} protein expressed in the haploid strains CY1−CY4 reacted with anti-sheep LysRS (top) and anti-yeast LysRS (bottom) antibodies.

Because expression of the plasmid-encoded LysRS is driven by the strong PGK promoter, the recombinant enzymes were efficiently overproduced. The two enzymes LysRS^{C} and LysRS^{CY} were purified to homogeneity by the procedure described in Materials and Methods, including four chromatographic steps on S Sepharose FF, Q Sepharose FF, phosphocellulose P11, and ceramic hydroxyapatite. This purification procedure yields 10 mg of homogenous LysRS from a 4.5-L culture. Recently, the wild-type yeast LysRS has been overproduced in yeast and purified to homogeneity (unpublished results). The purified enzymes have similar apparent molecular masses of about 70 kDa and were judged to be at least 95% homogenous by SDS−PAGE analysis (not shown).

The three enzyme preparations LysRS^{Y}, LysRS^{C}, and LysRS^{CY} have very similar specific activities in the ATP−PP_i exchange reaction (Table 2). The Michaelien parameters K_M and k_{cat} with respect to ATP and Lys are indistinguishable for LysRS^{C} and LysRS^{CY}; LysRS^{Y} displays a slightly lower k_{cat}. These data already demonstrate that the C-terminus moiety of hamster LysRS is well fitted to the core of the yeast enzyme, generating a hybrid protein with a high catalytic efficiency. The steady-state kinetic parameters in the tRNA^{Lys} aminoacylation reaction further demonstrated the structural congruency of the yeast and hamster proteins.

When yeast tRNA^{Lys} was used as a substrate, the kinetics parameters k_{cat} and K_M of LysRS^{Y} and LysRS^{CY} were virtually identical; the k_{cat} and K_M values of LysRS^{C} were only 2-fold lower (Table 2). This result provides a rational explanation for the fact that both LysRS^{C} and LysRS^{CY} are able to sustain growth of a yeast strain with a disrupted *KRS1* gene. On the contrary, we found that beef tRNA^{Lys} is a poor substrate for the yeast enzyme, with a 10-fold higher K_M value and a 7-fold lower k_{cat} value as compared to the cognate mammalian enzyme (Table 2). The catalytic efficiency of LysRS^{Y} for beef tRNA^{Lys}, expressed by its specificity constant k_{cat}/K_M, is strongly decreased (60-fold), as compared with LysRS^{C}. The replacement of the C-terminus of the yeast enzyme by that of the mammalian enzyme confers on the yeast enzyme the capacity to efficiently aminoacylate tRNA^{Lys} from beef, as revealed by the 20-fold increase in catalytic efficiency. This result is in agreement with its ability to substitute *in vivo* for the mammalian protein.

Thermal Stability of the LysRS^{CY} Hybrid. To further evaluate the level of structural complementarity of the yeast and mammalian moieties in the hybrid, we compared the thermal stability of the hybrid with those of the parental proteins. The purified proteins were incubated at different temperatures, at a LysRS concentration of 0.1 μM. At various time intervals, the mixture was placed on ice and the residual activity of the samples measured in the tRNA aminoacylation reaction. After incubation at 41.5 °C, the enzymatic activity of the samples decreased with a half-life time of about 5, 40, and 295 min, respectively, for LysRS^{Y}, LysRS^{C}, and LysRS^{CY}. In Figure 5, residual activities from samples of the three LysRS species incubated for 60 min at different temperatures are shown as a function of temperature.

The profiles of thermal inactivation are strikingly different. Under these conditions, half-inactivation is achieved at 36 °C for the yeast protein and at 41 °C for the mammalian one, in good correlation with the relative optimal temperatures of growth of the two species (28 and 37 °C, respectively). The hybrid protein proved to be significantly more thermostable than the two parental proteins, its temperature of half-inactivation being shifted up to 43.5 °C, with a variation of +7.5 °C as compared to the yeast enzyme that accounts for two-thirds of the primary structure of the hybrid. This behavior is significantly different from the thermostability profiles obtained for hybrids made of the sequences of *E. coli* and *Bacillus stearothermophilus* tyrosyl-tRNA synthetases, where chimeric proteins proved to be invariably less stable than the parental *B. stearothermophilus* enzyme (Guez-Ivanier *et al.*, 1993). Our results also strengthen the view that it is not the most stable protein that fulfills the requirements of *in vivo* selection but the one that is the best adapted to a given biotope (Jaenicke, 1991).

DISCUSSION

The primary structure of hamster LysRS has been deduced from the nucleotide sequence of the cloned cDNA. It is highly similar to that of the homologous enzyme from the yeast *S. cerevisiae*, with the noticeable exception of their N-terminal 60-amino acid extensions which share only 14 identical residues. This difference could be related to the distinct structural behavior of the yeast and mammalian LysRS, the latter having in particular the propensity to associate within a multienzyme complex comprising eight other aminoacyl-tRNA synthetases (Mirande *et al.*, 1993). In this study, we show that the core catalytic unit of the mammalian enzyme, obtained after deletion of the polypeptide extension, is able to complement a Lys-101 strain, a CHO cell line with a thermosensitive LysRS (Adair *et al.*, 1979). This indicates that the truncated allele retains a high degree of specificity for its cognate tRNA and, therefore, that the extension does not play a major role *in vivo* to discriminate between tRNAs. This result strongly supports the assumption that these extensions are endowed with a functional role unrelated to catalysis. Since the N-terminal extensions of yeast and mammalian homologous synthetases have nonconserved amino acid sequences, the possibility that they are subjected to little selective pressure was suggested. However, for a given synthetase, the amino acid sequences of these extensions are well conserved among mammals. The cDNA sequence for human LysRS was recently deposited within the data libraries (accession number D31890). Only

Table 2: Kinetic Constants for LysRS from Yeast and Hamster or for the Yeast/Hamster Hybrid in the ATP−PP_i Exchange and tRNA Aminoacylation Reactions

kinetic constants	$LysRS^Y$	$LysRS^C$	$LysRS^{CY}$
	ATP−PP_i Exchange		
specific activity[a] (U/mg)	8300	13000	13200
K_M ATP (μM)	230 ± 25	200 ± 20	220 ± 20
K_M Lys (μM)	40 ± 6	38 ± 2	34 ± 2
k_{cat} (s^{-1})	15 ± 1	25 ± 2	24 ± 2
	tRNA aminoacylation		
yeast tRNA[c]			
specific activity[b] (U/mg)	493	432	399
K_M $tRNA^{Lys}$ (μM)	1.8 ± 0.2	0.8 ± 0.1	1.6 ± 0.3
k_{cat} (s^{-1})	2.5 ± 0.3	1.24 ± 0.04	2.1 ± 0.2
k_{cat}/K_M (s^{-1} μM^{-1})	1.4	1.6	1.3
beef tRNA[d]			
specific activity[b] (U/mg)	49	519	650
K_M $tRNA^{Lys}$ (μM)	3.2 ± 0.9	0.34 ± 0.06	1.3 ± 0.2
k_{cat} (s^{-1})	0.21 ± 0.03	1.52 ± 0.07	1.89 ± 0.04
k_{cat}/K_M (s^{-1} μM^{-1})	0.07	4.5	1.5

[a] One unit of LysRS activity in the exchange reaction is the amount of enzyme producing 1 nmol of ATP/min, at 25 °C. [b] One unit of LysRS activity in the aminoacylation reaction is the amount of enzyme producing 1 nmol of lysyl-tRNA/min, at 25 °C. [c] Crude yeast tRNA with a lysine acceptance of 42 pmol/A_{260} was used. [d] Crude beef liver tRNA with a lysine acceptance of 120 pmol/A_{260} was used.

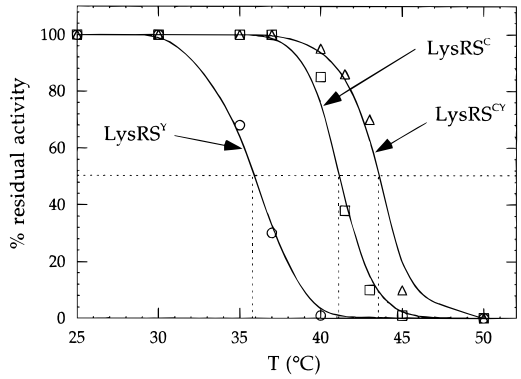

FIGURE 5: Thermal stability of the hybrid and parental LysRS species. The residual aminoacylation activity of LysRS samples incubated for 60 min at different temperatures, as described in Materials and Methods, is indicated; 100% corresponds to the initial LysRS activity, before heating.

37 amino acid residues differ from the sequence of the hamster protein. These changes are evenly distributed over the entire length of the polypeptide chain; only two changes occur in the segment shown in Figure 2. This supports the hypothesis in which the polypeptide extensions of synthetases from lower and higher eukaryotes fulfill different functions *in vivo*. Concerning those synthetases which form multienzyme complexes in mammals, the involvement of these extensions in the assembly of the complex was proposed (Mirande *et al.*, 1993).

It was shown that LysRS dissociated from the multisynthetase complex from sheep by hydrophobic interaction chromatography displays both hydrophobic and polyanion-binding properties, whereas a truncated fully active enzyme, obtained by controlled elastase treatment, did not (Cirakoglu & Waller, 1985). The site of cleavage by elastase has been localized within the stretch of alanine residues from Ala^{49} to Ala^{54} (J.-P. Waller, unpublished results). The particular amino acid composition revealed for the N-terminal polypeptide extension of hamster LysRS fully substantiates these earlier observations. Among the 40 amino acid residues of the predicted α-helical region from Lys^{16} to Ala^{55} (Figure 2) are 16 hydrophobic residues and 12 positively charged residues.

In the case of arginyl- and aspartyl-tRNA synthetases, two other components of the multisynthetase complex, the involvement of their N-terminal extensions in complex assembly could be established (Sivaram & Deutscher, 1990; Mirande *et al.*, 1992). However, several pieces of evidence suggest that the core domain of these enzymes forms protein interactions which are also essential for association into the complex [Mirande *et al.* (1992), Agou and Mirande, manuscript in preparation]. Accordingly, we observed that the N-terminal extension of hamster LysRS is not sufficient to promote association of yeast LysRS to the complex. Nevertheless, the hybrid protein $LysRS^{CY}$, which behaves as a free enzyme in extracts of transformed Lys-101 cells, functionally replaces the endogenous complex-associated species. Thus, association of LysRS to the complex does not appear to be a structural prerequisite for its *in vivo* activity. However, the possibility that the hybrid protein is weakly associated to the complex, and thus easily dissociates upon cell disruption, cannot be completely excluded.

Because the yeast and hamster proteins have very similar core catalytic units, a tRNA−enzyme cross-species recognition could be expected. The subset of nucleotides that contributes to the $tRNA^{Lys}$ acceptor identity in the *E. coli* system contains A73, the G3·C70 base pair in the acceptor stem, and U35 in the anticodon loop (Prather *et al.*, 1984; McClain *et al.*, 1990). Cytoplasmic $tRNA^{Lys}$ in eukaryotes have a conserved C3·G70 base pair, and the nucleotide at position 73 is G or U in yeast and G in mammal. Taking into account this identity set and the finding that yeast tRNA is a good substrate commonly used *in vitro* to assay the aminoacylation activity of mammalian LysRS (Cirakoglu & Waller, 1985), it was anticipated that they complement each other. Accordingly, $LysRS^C$ functionally replaces the yeast enzyme *in vivo* and efficiently aminoacylates yeast $tRNA^{Lys}$ *in vitro*. Conversely, the inability of $LysRS^Y$ to replace the mammalian enzyme is best explained by its weak capacity to aminoacylate beef $tRNA^{Lys}$, as revealed by *in vitro* kinetic measurements (Table 2). However, even if the catalytic efficiency of $LysRS^Y$ for beef $tRNA^{Lys}$ is too low to sustain growth of Lys-101 cells, it should be noted that at permissive temperature expression of yeast LysRS is not deleterious for growth, suggesting that little if any misaminoacylation

occurs. Three isoaccepting species of $tRNA^{Lys}$ have been described in mammals (Raba *et al.*, 1979). It is rather unlikely that one of these tRNA species cannot be aminoacylated by the yeast enzyme. Indeed, the plateau levels of aminoacylation of crude beef liver tRNA determined with nonlimiting amounts of $LysRS^{C}$, $LysRS^{Y}$, and $LysRS^{CY}$ were identical (120 pmol/A_{260} unit).

The C-terminal region of $LysRS^{C}$ confers on the yeast enzyme the ability to efficiently aminoacylate beef tRNA, without impairing its propensity to aminoacylate yeast tRNA. This effect can be *a priori* accounted for in two different ways: (i) The C-terminus of the mammalian enzyme promotes an essential productive interaction with its cognate tRNA, and (ii) the yeast homologous polypeptide fragment negatively discriminates a specific feature of mammalian tRNA. In the hybrid protein $LysRS^{CY}$, the core catalytic unit is made of 392 amino acid residues from the yeast enzyme and 152 from the mammalian LysRS. The crystal structure of *E. coli* LysRS has been solved in the absence of tRNA (Onesti *et al.*, 1995), but the mode of recognition of tRNA by the synthetase should be very similar to that reported for yeast aspartyl-tRNA synthetase (Cavarelli *et al.*, 1993), another class IIb enzyme sharing significant sequence similarity (Gatti & Tzagoloff, 1991). According to the aspartic model, only a few contacts are expected between the C-terminal 152-aa fragment of LysRS and the acceptor stem of $tRNA^{Lys}$. In the AspRS:$tRNA^{Asp}$ crystal structure, the residue Lys^{553}, close to the C-terminal extremity of the protein, binds to the phosphate group of C^{67} (Cavarelli *et al.*, 1993). The structure-based alignment of *E. coli* LysRS and yeast AspRS revealed that the C-termini of the two proteins coincide (Onesti *et al.*, 1995). Therefore, the short C-terminal polypeptide extensions of yeast and hamster LysRS, made of 11 and 20 amino acid residues (Figure 1), respectively, are likely to make contacts with the acceptor stem of $tRNA^{Lys}$.

The possibility to rescue a bacterial (Racher *et al.*, 1991; Weygand-Durasevic *et al.*, 1993) or yeast (Ripmaster *et al.*, 1995) aminoacyl-tRNA synthetase by a yeast or human enzyme, respectively, has already been demonstrated, but the *in vivo* replacement of a synthetase from a higher eukaryote by a distantly related enzyme from a prokaryote or lower eukaryote has never been reported. The strictly conserved elements between the acceptor stems of yeast and mammal $tRNA^{Lys}$ are limited to the C2·G71 and C3·G70 base pairs. The tRNA acceptor stem is thought to carry an operational RNA code for amino acids (Schimmel *et al.*, 1993). This implies that base changes occurring in the course of evolution are compensated at the protein level to ensure a proper tRNA·enzyme recognition.

Our finding that in the lysine system cross-species aminoacylation can be achieved *in vivo* but requires cognate amino acid sequences most certainly involved in triggering an accurate contact with the acceptor stem of tRNA also strongly supports the view that the fidelity of an early translation system could have been ensured by a limited set of specific contacts between a primordial tRNA, made of helical hairpin helices, and primordial synthetases, made of the core catalytic domains which discriminate between class I and class II enzymes (Schimmel *et al.*, 1993). The acceptor stem identity set differentiated independently from the anticodon set, anticodon binding domains and anticodons in the eukaryotic lysine system being mutually consistent. The coevolution of tRNA and synthetase is also strengthened by the observation that the C-terminus moiety of the hamster protein is readily accommodated by the core catalytic domain of the yeast enzyme. Noteworthy is the fact that the hybrid protein is fairly stable, even more thermostable than the parental proteins. Thus, small changes that trigger a positive recognition of the mammalian tRNA by the cognate synthetase have not been made at the expense of the overall structure of the synthetase and the catalytic center that remains essentially unchanged.

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