Defining the Active Site of Yeast Seryl-tRNA Synthetase

MUTATIONS IN MOTIF 2 LOOP RESIDUES AFFECT tRNA-DEPENDENT AMINO ACID RECOGNITION*

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The active site of class II aminoacyl-tRNA synthetases contains the motif 2 loop, which is involved in binding of ATP, amino acid, and the acceptor end of tRNA. In order to characterize the active site of Saccharomyces cerevisiae seryl-tRNA synthetase (SerRS), we performed in vitro mutagenesis of the portion of the SES1 gene encoding the motif 2 loop. Substitutions of amino acids conserved in the motif 2 loop of seryl-tRNA synthetases from other sources led to loss of complementation of a yeast SES1 null allele strain by the mutant yeast SES1 genes. Steady-state kinetic analyses of the purified mutant SerRS proteins revealed elevated $K_m$ values for serine and ATP, accompanied by decreases in $k_{cat}$ (as expected for replacement of residues involved in aminoacyl-adenylate formation). The differences in the affinities for serine and ATP, in the absence and presence of tRNA are consistent with the proposed conformational changes induced by positioning the 3′-end of tRNA into the active site, as observed recently in structural studies of Thermus thermophilus SerRS (Cusack, S., Yaremchuk, A., and Tukalo, M. (1996) EMBO J. 15, 2834–2842). The crystal structure of this moderately homologous prokaryotic counterpart of the yeast enzyme allowed us to produce a model of the yeast SerRS structure and to place the mutations in a structural context. In conjunction with structural data for Thermus thermophilus SerRS, the kinetic data presented here suggest that yeast seryl-tRNA synthetase displays tRNA-dependent amino acid recognition.

The formation of aminoacyl-tRNA, catalyzed by aminoacyl-tRNA synthetases, is a crucial step in maintaining the fidelity of protein biosynthesis. This family of enzymes can be partitioned into two classes of 10 enzymes each, based on conserved sequences (1) and structural motifs (2). All members of class I contain a common loop with the signature sequence KMSKS (3) and a region of homology with the HIGH peptide (4) as part of a Rossmann dinucleotide binding fold of parallel β-sheets (5). Class II synthetases have a different topology of dinucleotide binding based on antiparallel β-sheets (2, 6, 7). The three common signature motifs of class II synthetases are found in this domain. Motif 1 forms part of the conserved inter-subunit interface of homodimeric (6, 8) and heteromeric (9) synthetases. Motifs 2 and 3 contain many of the active-site residues important for ATP, amino acid, and tRNA acceptor stem recognition (10–15). The elucidation of the crucial role of sequence motifs in substrate binding have resulted from the solution of several crystal structures of enzymes and enzyme-substrate complexes from both class I and class II (16) and numerous biochemical studies involving mutant synthetases (17–22).

The evolution of tRNA recognition systems has recently gained much attention (23–27). The primary structure of several prokaryotic and eukaryotic seryl-tRNA synthetases (Ref. 23; see also the legends to Fig. 1 and 3), including the enzyme that probably functions in yeast mitochondria (28), have been determined. While two prokaryotic enzymes, from Escherichia coli and Thermus thermophilus, have been crystallized in different contexts with substrates and subjected to biochemical analysis in order to identify the domains important for tRNA and amino acid binding (23), information on structure/function relationships in eukaryotic seryl-tRNA synthetases is still scarce by comparison. In contrast to their prokaryotic counterparts, these enzymes contain C-terminal extensions abundant in basic amino acids, which may be important for both stability and optimal substrate recognition in eukaryotic SerRS,1 as recently shown for yeast SerRS (29). To gain further insight into the mechanisms of substrate recognition employed by yeast SerRS and to identify catalytically important residues in the active site, we have replaced a number of amino acids in the motif 2 loop and analyzed the resulting mutants in vivo and in vitro. The altered kinetic parameters of the mutant SerRS proteins correlate with predictions based upon structural studies of the prokaryotic system.

MATERIALS AND METHODS

General Materials—[3H]Serine (30.0 Ci/mmol) was from Amersham Corp. [14C]Serine (166.1 mCi/mmol) and tetrasodium [32P]pyrophosphate (3.09 Ci/mmol) were purchased from DuPont NEN.

Plasmids and Strains—In vitro mutagenesis of the SES1 gene was carried out (see below) in pBluescript SK−, where the BamHI cassette containing SES1 (30) was inserted, giving pSKSES1. The resulting mutant SES1 genes, named SES1mut, were recloned as 1.4-kilobase pair BamHI fragments into pVTL-100 behind the ADH (alcohol dehydrogenase) promoter and pG11 behind the GAL promoter for complementation of the null-allele strains, or in pET3 behind the T7 RNA polymerase promoter for overproduction of mutated proteins in E. coli (29, 30). Construction of the Saccharomyces cerevisiae SES1 disruption strain BR2727ΔSES1 (MATa ade2–1 arg4–9 his4 leu2–3, 112 lys2 trp1 ura3–1 SES1::LYS2) has been described (29). The deletion could be rescued by supplying a functional SES1 gene on a centromeric plasmid pUN70 (29). Selected haploids with a disrupted SES1 gene (Lys+ phenotype), where SerRS function was provided in trans (from

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1 The abbreviation used is: SerRS, seryl-tRNA synthetase.
pUN70SES1 (plasmid), were used for complementation experiments via plasmid shuffling involving pVT1-100SES1\textsubscript{mut}. Since the parental strain BR2727 was not fully galactose-inducible, as shown by its inability to ferment galactose after streaking the colonies on bromthymol blue indicator plates (1% yeast extract, 2% peptone, 2% agar, 2% galactose, and 50 μg/ml bromthymol blue; Ref. 31), pUN70SES1 transformants of BR2727ΔSES1 were crossed with S. cerevisiae S2088 (MATα ura3–52 trp 1 lys2–801 leu2A1 his3–Δ200 pep4–HIS3 pro–Δ16R can 1 GAL). Diploids were sporulated, tetrad dissected, and haploid segregants of the pUN70SES1 transformant with a disrupted SES1 gene (Ly*) were selected, and the ability to ferment galactose (produce a yellow halo on induction plates) were selected. The strain was named SD816 and used for complementation experiments via plasmid exchange with pCJ11\textsubscript{S}ES1\textsubscript{mut}.

In Vitro Mutagenesis—Saturation mutagenesis was performed with a 5:1 molar ratio of synthetic phosphorylated oligonucleotides to U-containing single-stranded pSKSES1 DNA. A mutagenic oligonucleotide, a 50-mer complementary to the codons corresponding to amino acids 278–294, was synthesized doped to 4% with an equal mixture of A/C/G/T at all positions. A new NcoI restriction site was introduced internally by silent mutations to allow screening by restriction analysis. Annealing, extension, and ligation of the mutagenic mixture was performed with T. thermophilus DH5α, plasmid DNA was isolated from a large pool of individual transformants. The pertinent regions of all NcoI-containing pSKSES1 plasmids were subjected to sequencing in order to determine the mutational changes. Multiple mutations in the SES1 gene were separated by exchanging either 880-base pair PstI/NcoI or 560-base pair NcoI/EigAl fragments with the corresponding fragments of pSKSES1\textsubscript{mut}.

Purification of Mutant SerRS from an E. coli Overproducing Strain—The overproduction of mutated SerRS enzymes was achieved by transformation of E. coli strain BL21(DE3) with pETISES1\textsubscript{mut} constructs, followed by induction of the cultures with 2 mM isopropyl-1-thio-

**results and discussion**

**Motif 2 Loop Mutants of Yeast Seryl-tRNA Synthetase**

There is significant similarity among the primary structures of all known SerRS proteins. Pairwise similarities range from 26% between S. cerevisiae cytoplasmic and a recently identified S. cerevisiae putative second SerRS (28) to 78% between E. coli and Haemophilus influenzae SerRS. The second yeast SerRS is most likely a mitochondrial enzyme. This is based on its position in the phylogenetic tree, the presence of a putative N-terminal mitochondrial targeting sequence (as diagnosed by multiple sequence alignment, and with high confidence by the method of Nakai and Kanehisa; Ref. 38) and the lack of a C-terminal extension (29). The phylogenetic tree showing evolutionary relationships among seryl-tRNA synthetases from different organisms is presented in Fig. 1. The cytoplasmic enzymes of eukaryotes form a separate group, and the divergence pattern of eubacterial enzymes is in accord with the contemporary phylogenetic trees (39). Surprisingly, an archaean SerRS (Halocella marismortui) clusters with the enzymes of B. subtilis and the three Gram-negative bacteria regardless of the method used to construct the tree. This suggests that, at some point in evolution, a eubacterial gene for SerRS replaced the ancestral H. marismortui gene by horizontal transfer.

In spite of primary sequence similarity, immunological cross-reactivity has been observed neither between eukaryotic and prokaryotic seryl-tRNA synthetases nor between the cytoplasmic and organellar proteins (40, 41). As revealed by the crystal structures of two prokaryotic seryl-tRNA synthetases from E. coli (2) and T. thermophilus (42), each subunit of the homodimeric enzymes is composed of two domains: an N-terminal "helical arm" comprising a 60-Å solvent-exposed, antiparallel coiled-coil, which binds the variable arm of cognate tRNA\textsuperscript{Ser} and the catalytic domain based on a seven-stranded β-sheet.

**sequence analysis and protein modeling**

Multiple alignment of protein sequences was performed using CLUSTALW (34). The PHYLIP package (35) was used to calculate protein distance matrices and to construct phylogenetic trees. Percent similarities between proteins were calculated by the method of Myers and Miller (36). Theoretical models of the yeast SerRS three-dimensional structure were produced and optimized using the PROMOD program suite (37). Images were produced using RasMol Molecular Renderer version 2.6.

**fig. 1. Unrooted tree of seryl-tRNA synthetases based on whole sequences alignment.** BS, Bacillus subtilis (accession no. P37464); CB, Caxiella burnetii (P39919); EC, E. coli (P09156); HI, H. influenzae (P43833); HS, Homo sapiens (P49591); MG, Mycoplasma genitalium (P47251); TT, T. thermophilus (P34845); SC, S. cerevisiae (P07284); SCM, S. cerevisiae (putative mitochondrial, P38705); HM, H. marismortui (EMBL X91007); CE, Caenorhabditis elegans (EMBL 268852); AT, Arabidopsis thaliana (EMBL 270313). The accession numbers are from SwissProt, except where indicated. The tree was constructed using CLUSTALW (34) and PROTDIST, NEIGHBOR, and DRAWTREE of the PHYLIP package (35).
As shown by primary sequence alignment (Fig. 3), this is the most conserved region among all SerRS proteins.

In Vivo Analysis of SerRS Mutants—From the many SerRS mutants isolated, which carry one to five amino acid substitutions in region 278–294 of the motif 2 loop, we wanted to select those with the most severely affected substrate binding or catalytic properties. This was first assessed in vivo by the ability of the mutants to complement S. cerevisiae SES1 null-allele strains via plasmid shuffling (Table I). The haploid strain BR2727ΔSES1 (where viability of the cell is ensured by a wild-type SES1 gene on the URA3-containing plasmid pUN70SES1), was transformed with pVT1-100SES1mut constructs (which carry the LEU2-selectable marker and SES1mut behind the ADH promoter). Four of the motif 2 loop mutants were found to have lost the ability to complement the ΔSES1 null allele. They were named SES1mut2, SES1mut3, SES1mut4, and SES1mut5. The type and the position of mutational changes in the resulting SerRS proteins are presented in Fig. 3B. In order to check whether the function of wild-type SerRS can be restored by the overproduction of mutated SerRS proteins, the complementation of S. cerevisiae SD816 (which also carries an inactive SES1::LYS2 allele but has a normal galactose uptake), was performed via plasmid shuffling with pCJ11SES1mut constructs. After induction of the GAL promoter by switching the cultures of SD816/pCJ11SES1mut from glucose to galactose medium, significant amounts of mutant proteins accumulated in the cell, as detected by SDS-polyacrylamide gel analysis of protein extracts (not shown). pUN70SES1 was cured by growing induced double transformants in the presence of uracil, followed by plating the cells on selective galactose plates. Lys+Leu+ colonies were replicated to 5-fluoro-orotic acid-containing medium (31), which counterselects against colonies containing URA3 plasmids carrying the wild-type S. cerevisiae SES1 gene. Some very slow growing transformants of pCJ11SES1mut4 and pCJ11SES1mut5 appeared on 5-fluoro-orotic acid plates after several days of incubation at 30°C, while all the cells containing pCJ11SES1mut2 or pCJ11SES1mut3 as the only source of seryl-tRNA synthetase activity were nonviable (Table I). It is thus apparent that several amino acid substitutions between residues 278 and 294 of yeast SerRS are responsible for enzyme inactivation and consequently for the phenotypic alteration.

Purification and in Vitro Characterization of SerRS Mutants—To determine the steps of the aminoacylation reaction at which the altered enzymes are defective, noncomplementing (SES1mut2 and SES1mut3) or weakly complementing mutants (SES1mut4 and SES1mut5) which normally accumulate in yeast cells (Table I) were overexpressed in E. coli, purified to apparent homogeneity as described under “Materials and Methods,” and characterized in vitro. As discussed previously (29), there are some indications that S. cerevisiae SerRS purified from bacterial overproducing strains may differ in modification and/or conformation compared to the native protein. However, since the kinetic parameters for overexpressed yeast SerRS are very much alike regardless of the overproducing system used (29, 30), the comparison of the substrate binding and catalytic constants for the wild-type and mutant yeast SerRS was performed with proteins isolated from E. coli.

Since the aminoacylation assay did not reveal significant changes in the affinity for tRNA (Table II), but instead showed impaired ATP and serine binding capacities resulting in elevated $K_m$ values for both substrates accompanied by decreased turnover rates, the impact of the mutations on the steady-state kinetic parameters were independently determined in the amino acid activation reaction. Mutants SerRS2 (R279C/E821Q/G283A/D288V/W290L) and SerRS3 (R279P/A282R) have no detectable in vitro enzyme activity and could not be used for kinetic studies. This is in agreement with the inability...
of SES1mut2 and SES1mut3 to substitute for the wild-type SES1 function in vivo. In both mutants Arg279, which aligns with an invariant arginine in the class II synthetases, was replaced with other amino acids. This change probably causes the major inactivating effect in SerRS2 and SerRS3, since this position corresponds to Arg256 in T. thermophilus SerRS, which is in direct contact with the phosphate of the seryl-adenylate. Supersposition of the models of the wild-type and SerRS2 motif 2 loops, with the seryl-adenylate analog positioned as in the crystal structure of T. thermophilus SerRS (10), is shown in Fig. 2. In order to determine the contribution of particular amino acid alterations, to enzyme function, the five mutations found in SerRS2 were separated. This was achieved by reconstruction of pSKSES1mut2, carrying the unique NcoI site introduced in SES1mut as silent mutations at the position of the Gly256 and Ser261 codons. The PsiI-NcoI and NcoI-EcoI fragments of pSKSES1mut2 were individually replaced with corresponding fragments excised from pSKSES1wt. The new constructs, named SES1mut2 and SES1mut3, recloned as BamHI cassettes behind the T7 promoter of pET3, were used for the overproduction of SerRS6 (R270C/E281Q/G283A) and SerRS7 (D288V/W290L), respectively. As expected, the mutation of three absolutely conserved residues in motif 2 (see below), render the mutant SerRS6 totally inactive in both the amino acid activation and aminoacylation reactions. The kinetic analysis of SerRS7 showed slightly reduced specificity constants for both ATP and serine, relative to the wild-type enzyme. The affinity for tRNA of this mutant was slightly increased. This is of considerable interest, since the occurrence of tryptophan at position 290 is unique to yeast SerRS, and thus could be expected to be involved in tRNA binding. All other seryl-tRNA synthetases contain an arginine or lysine at this position. The exception is SerRS from A. thaliana, which contains a leucine. This indicates that the replacement of one bulky and hydrophobic amino acid (Trp) for another (Leu) is tolerated.

Kinetic analysis of the aminoacylation and amino acid activation reactions revealed that substitution of the class II invariant glycine in SerRS4 (G291V) and SerRS5 (E281D/E281Q/G283A) has the most dramatic effect on ATP and serine binding. The enzymes exhibit more than an order of magnitude elevated $K_a$ for ATP and 35-fold decrease in $k_{cat}$. The parameters for serine were changed to a similar extent, while the $K_a$ for the tRNA (for mutant SerRS5) is almost identical to the wild-type value. Gly291 of yeast SerRS occupies a position that is strictly conserved in the primary structures of all prokaryotic and eukaryotic seryl-tRNA synthetases. Its analogue is not exposed in the active site of the T. thermophilus enzyme, thus it is probably involved in maintaining an overall motif 2 loop conformation (10). As recently discussed by Cusack et al. (13), the occurrence of several glycines in the loop, surrounded by other small residues, may provide the necessary flexibility and reduced steric hindrance to facilitate the conformational switch imposed by tRNA binding. In agreement with this structural data, alteration of the nonpolar Ala282 in yeast SerRS (mutant SerRS10) to serine, another small but polar amino acid, causes the least dramatic change in kinetic parameters.

To single out the effect of the E281Q replacement, two mutations in SES1mut1 were separated by the same experimental procedure as described above for SES1mut2. Two new mutant enzymes were obtained by expression of the reconstructed SES1mut1 and SES1mut2 genes: SerRS8 (E281D) and SerRS9 (G291A), respectively. Glu291 in yeast SerRS is of special interest since it aligns with Glu291 in T. thermophilus SerRS, which is a part of the Arg256-X-Glu peptide. The side chain conformation of this residue is fixed upon binding of ATP or adenylate, which is believed to be the first step toward the stabilization of the motif 2 loop, which is also involved in tRNA acceptor stem interactions (10, 13). Glu291 is severely reoriented upon tRNA
**Motif 2 Loop Mutants of Yeast Seryl-tRNA Synthetase**

**Table II**

| Enzyme | Aminoacylation | PP exchange |
|--------|----------------|-------------|
|        | $k_{cat}$     | $K_m$       | Relative $k_{cat}/K_m$ | $k_{cat}$     | $K_m$       | Relative $k_{cat}/K_m$ |
| Ser    |               |             |                         | SerRS        | 0.99       | 2.5 x 10$^{-5}$       | 1              | 3.5                | 4.0 x 10$^{-5}$       | 1              |
| SerRS4 | 0.09           | 4.5 x 10$^{-5}$ | 0.005                   | SerRS4      | 0.09       | 1.0 x 10$^{-5}$       | 1              | 1.0                | 3.0 x 10$^{-5}$       | 0.33            |
| SerRS5 | 0.04           | 2.0 x 10$^{-5}$ | 0.03                    | SerRS5      | 0.04       | 1.0 x 10$^{-5}$       | 1              | 5.0                | 1.0 x 10$^{-5}$       | 5.0             |
| SerRS7 | 0.15           | 2.0 x 10$^{-5}$ | 0.06                    | SerRS7      | 0.15       | 1.0 x 10$^{-5}$       | 1              | 9.0                | 3.0 x 10$^{-5}$       | 3.0             |
| SerRS8 | 0.09           | 3.0 x 10$^{-5}$ | 0.05                    | SerRS8      | 0.09       | 1.0 x 10$^{-5}$       | 1              | 10.0               | 3.0 x 10$^{-5}$       | 3.0             |
| SerRS9 | 0.13           | 3.0 x 10$^{-5}$ | 0.06                    | SerRS9      | 0.13       | 1.0 x 10$^{-5}$       | 1              | 15.0               | 3.0 x 10$^{-5}$       | 5.0             |
| SerRS10| 0.07           | 3.0 x 10$^{-5}$ | 0.05                   | SerRS10     | 0.07       | 1.0 x 10$^{-5}$       | 1              | 20.0               | 3.0 x 10$^{-5}$       | 6.67            |
| ATP    | 0.99           | 6.3 x 10$^{-5}$ | 0.02                    | ATP         | 0.99       | 2.5 x 10$^{-5}$       | 1              | 3.5                | 4.0 x 10$^{-5}$       | 1              |
| tRNA$^{Ser}$ | 0.13       | 3.0 x 10$^{-5}$ | 0.06                   | tRNA$^{Ser}$| 0.13       | 1.0 x 10$^{-5}$       | 1              | 14.0               | 3.0 x 10$^{-5}$       | 4.67            |

The $K_m$ values for wild-type yeast SerRS, with respect to both ATP and serine, are considerably different when determined by the PP$_i$ exchange or aminoacylation reactions. The affinity for serine is more than an order of magnitude higher in the reaction performed in the presence of tRNA rather than in the activation step. Furthermore, the apparent affinity of SerRS for serine, as measured during seryl-adenylate formation, is not significantly changed by amino acid replacements. In contrast, an order of magnitude difference in $K_m$ for wild-type and mutant SerRS, with respect to serine, was observed during aminoacylation. These results are consistent with the possibility that new binding sites for serine are created upon tRNA binding. When the kinetic data presented here are considered in conjunction with structural data for *T. thermophilus* SerRS, it is clear that SerRS displays tRNA-dependent amino acid recognition, as described previously for *E. coli* glutaminyl- and tryptophanyl-tRNA synthetases (48).

In agreement with the *T. thermophilus* SerRS structural studies, the apparent affinity for ATP to the yeast enzyme decreases 6.3-fold in the aminoacylation reaction compared to PP$_i$ exchange. This may support the idea that conformational changes in the active site are induced by substrate binding, as recently observed for eukaryotic aspartyl- (14) and prokaryotic seryl-tRNA synthetases (13). Mutational changes in the 278–294 region of yeast SerRS may interfere with the proposed structural flexibility essential to the function of the motif 2 loop. It is also likely that the impaired interaction of mutant SerRS enzymes with ATP and serine makes the amino acid activation reaction, instead of the transfer reaction, rate-limiting. This conclusion is based on the comparison of the $k_{cat}$ values for wild-type and mutant enzymes in PP$_i$ exchange and aminoacylation. In contrast to wild-type SerRS, which more rapidly turns over ATP and serine in the absence of tRNA, the catalytic rate constants characterizing the most affected mutant proteins (SerRS4 and SerRS5) are comparable in the activation and aminoacylation reactions. In summary, the results of *in vivo* and *in vitro* functional analyses of yeast SerRS mutants, combined with structural data for the *T. thermophilus* and *E. coli* enzymes which allowed the prediction of yeast SerRS structure, strongly suggest that seryl-tRNA synthetase from *S. cerevisiae* structurally and functionally resembles its prokaryotic counterparts in the active site.
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REFERENCES

1. Eriani, G., Delarue, M., Poch, O., Gangloff, J., and Moras, D. (1990) Nature 347, 203–206
2. Cusack, S., Berthet-Colominas, C., Hartlein, M., Nassar, N., and Leberman, R. (1990) Nature 347, 249–255
3. Houtondji, C., Dessen, P., and Blanquet, S. (1986) Biochimie 68, 1071–1078
4. Webster, T., Tsai, H., Kula, M., Mackie, G. A., and Schimmel, P. (1984) Science 226, 1315–1317
5. Rossmann, M. G., Moras, D., and Olsen, K. W. (1974) Science 185, 1091–1098
6. Cusack, S., Hartlein, M., and Leberman, R. (1991) Nucleic Acids Res. 13, 3489–3496
7. Ruff, M., Krishnaswamy, S., Boeglin, M., Petterszmann, A., Mitschler, A., Podjarny, A., Rees, B., Thierry, J. C., and Moras, D. (1991) Science 252, 1682–1689
8. Cavarelli, J., Rees, B., Ruff, M., Thierry, J.-C., and Moras, D. (1993) Nature 362, 181–184
9. Mosyak, L., and Safro, M. (1993) Biochimie 75, 1091–1098
10. Belrhali, H., Yaremchuk, A., Tukalo, M., Berthet-Colominas, C., Leberman, R., Reijer, B., Sproat, B., Als-Nielsen, J., Grubel, G., Legrand, J.-F., Lehmann, M., and Cusack, S. (1994) Structure 3, 341–352
11. Belrhali, H., Yaremchuk, A., Tukalo, M., Berthet-Colominas, C., Rasmussen, B., Bosccke, P., Diat, O., and Cusack, S. (1995) Structure 3, 341–352
12. Bizu, Y., Yaremchuk, A., Tukalo, M., and Cusack, S. (1994) Science 263, 1404–1410
13. Cusack, S., Yaremchuk, A., and Tukalo, M. (1996) EMBO J. 15, 2834–2842
14. Cavarelli, J., Eriani, G., Rees, B., Ruff, M., Boeglin, M., Mitschler, A., Martin, F., Gangloff, J., Thierry, J.-C., and Moras, D. (1994) EMBO J. 13, 327–337
15. Petterszmann, A., Delarue, M., Thierry, J.-C., and Moras, D. (1994) J. Mol. Biol. 244, 158–167
16. Cusack, S. (1995) Nat. Struct. Biol. 2, 824–831
17. Fersht, A. R. (1987) Biochemistry 26, 8031–8037
18. Vincent, C., Borel, F., Wilson, J. C., Leberman, R., and Hartlein, M. (1995) Nucleic Acids Res. 23, 1113–1118
19. Lu, Y., and Hill, K. A. W. (1994) J. Biol. Chem. 269, 12137–12141
20. Sever, S., Rogers, K., Rogers, M. J., Carter, C., and Soll, D. (1996) Biochemistry 30, 632–640
21. Borel, F., Vincent, C., Leberman, R., and Hartlein, M. (1994) Nucleic Acids Res. 22, 2963–2969
22. Ibba, M., Thomann, H.-U., Hong, K.-W., Sherman, J. M., Weygand-Durašević, I., Sever, S., Stange-Thomann, N., Praetorius, M., and Soll, D. (1995) Nucleic Acids Symp. Ser. 33, 40–42
23. Hartlein, M., and Cusack, S. (1985) J. Mol. Biol. 40, 519–530
24. Saks, M. E., and Sampsen, J. R. (1995) J. Mol. Biol. 240, 509–518
25. Eriani, G., Cavarelli, J., Martin, F., Ador, L., Rees, B., Thierry, J.-C., Gangloff, J., and Moras, D. (1995) J. Mol. Biol. 240, 487–498
26. Nagel, G. M., and Doolittle, R. F. (1995) J. Mol. Biol. 240, 487–498
27. de Pouplana, L. R., Frugier, M., Quinn, C. L., and Schimmel, P. (1996) Proc. Natl. Acad. Sci. U.S.A. 93, 166–170
28. Johnston, M., Andrews, S., Brinkman, R., Cooper, J., Ding, H., Dover, J., Du, Z., Favello, A., Fulton, L., Gattung, S., Geisel, C., Kirsten, J., Kucaba, T., Hillier, L., Jier, M., Johnston, L., Langston, Y., Lattrell, P., Louis, E. J., Macri, C., Mardis, E., Menezes, S., Mouser, L., Nhan, M., Rikli, L., Riles, L., St. Peter, H., Trevasakis, E., Vaughn, K., Vignati, D., Wilecox, L., Wohldman, P., Waterston, R., Wilson, R., and Vaudin, M. (1994) Science 265, 2077–2082
29. Weygand-Durašević, I., Lenhard, B., Filipič, S., and Soll, D. (1996) J. Biol. Chem. 271, 2455–2461
30. Weygand-Durašević, I., Ban, N., Jahn, D., and Soll, D. (1993) Eur. J. Biochem. 214, 869–877
31. Guthrie, C., and Fink, G. R. (1991) Guide to Yeast Genetics and Molecular Biology, Academic Press, Inc., New York
32. Weygand-Durašević, I., Schwab, E., and Soll, D. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 210–214
33. Calendar, R., and Berg, P. (1966) Biochemistry 5, 1681–1690
34. Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994) Nucleic Acids Res. 22, 4673–4680
35. Felsenstein, J. (1989) Cladistics 5, 164–166
36. Myers, E. W., and Miller, W. (1988) Comp. Appl. Biochem. 4, 11–17
37. Peitsch, M. C. (1996)
38. Nakai, K., and Kanehisa, M. (1992) Genomics 14, 897–911
39. Brown, J. R., and Doolittle, W. F. (1995) Proc. Natl. Acad. Sci. U.S.A. 92, 2441–2445
40. Weygand-Durašević, I., and Jerala, R. (1990) Period. Biol. 92, 295–304
41. Sidorik, L. L., Guderza, O. I., Dragovez, D. A., Tukalo, M. A., and Beresten, S. F. (1991) FEBS Lett. 292, 76–78
42. Fujinaga, M., Berthet-Colominas, C., Yaremchuk, A. D., Tukalo, M. A., and Cusack, S. (1995) J. Mol. Biol. 234, 222–238
43. Price, S., Cusack, S., Borel, F., Berthet-Colominas, C., and Leberman, R. (1993) FEBS Lett. 324, 167–170
44. Himeno, H., Hasegawa, T., Ueda, T., Watanabe, K., and Shimizu, M. (1990) Nucleic Acids Res. 18, 6815–6819
45. Normany, J., Ollick, T., and Abelson, J., (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 5680–5684
46. Asohara, H., Himeno, H., Tamura, K., Nameki, N., Hasegawa, T., and Shimizu, M. (1994) J. Mol. Biol. 236, 738–748
47. Wu, X. Q., and Gross, H. J. (1993) Nucleic Acids Res. 21, 5589–5594
48. Ibba, M., Hong, K.-W., Sherman, J. M., Sever, S., and Soll, D. (1996) Proc. Natl. Acad. Sci. U.S.A. 93, 6953–6958