# Nucleotide Sequence of a Gene from the *Pseudomonas* Transposon Tn501 Encoding Mercuric Reductase†

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ABSTRACT: We have determined the nucleotide sequence of the *mer*A gene from the mercury-resistance transposon Tn501 and have predicted the structure of the gene product, mercuric reductase. The DNA sequence predicts a polypeptide of $M_r$ 58 660, the primary structure of which shows strong homologies to glutathione reductase and lipoamide dehydrogenase, but mercuric reductase contains as additional N-terminal region that may form a separate domain. The implications of these comparisons for the tertiary structure and mechanism of mercuric reductase are discussed. The DNA sequence presented here has an overall G+C content of 65.1 mol%, typical of the bulk DNA of *Pseudomonas aeruginosa* from which Tn501 was originally isolated. Analysis of the codon usage in the *mer*A gene shows that codons with C or G at the third position are preferentially utilized.

The mercury-resistance transposon Tn501 was originally isolated from *Pseudomonas aeruginosa* PAT on the nonconjugative plasmid pVS1 (Stanisich et al., 1977) and was shown to confer resistance to mercuric ions. The transposon was transferred to *Escherichia coli*, in which both the transposition and the mercury-resistance functions are expressed. Mercuric ion resistance is the most widely distributed of the prokaryotic resistance determinants, and that on Tn501 is regarded as the prototype of mercury-resistance determinants found on IncFII plasmids [J. Miller and R. H. Rownd, unpublished observations reported in Jackson & Summers (1982b)]. In all cases examined, mercury resistance has been shown to be inducible and to involve the reduction of Hg(II) ions to elemental mercury Hg(0) (Summers & Silver, 1978). The mercury resistance specified by Tn501 is narrow spectrum and, in addition to resistance to mercuric ions, also confers resistance to the organomercurials merbromin, *p*-(hydroxymercuri)-benzoate, and fluorescein mercuric acetate, by an unknown mechanism that does not involve volatilization of mercury (Clark et al., 1977). No resistance was found with other organomercurials. The enzyme catalyzing the reduction of Hg(II) to Hg(0), mercuric reductase, has been shown to be a dimeric flavoprotein, containing bound FAD,[1] utilizing NADPH as an electron donor, and requiring an excess of exogenous thiols for activity in vitro. Mechanistic studies have shown that the enzyme has similarities to glutathione reductase and lipoamide dehydrogenase (Fox & Walsh, 1982).

In the plasmid R-100, the mercuric reductase protein is the product of the *mer*A gene (Foster et al., 1979). We have located the equivalent gene in Tn501 by molecular cloning of Tn501 DNA and by examining the mercury-resistance phenotype of the recombinants and the polypeptides produced by them. Together with peptide sequence data described in the accompanying paper (Fox & Walsh, 1983), this has allowed us to unambiguously identify the coding sequence for the mercuric reductase in Tn501 and to predict the structure of the polypeptide. The region of Tn501 DNA sequenced in this work is shown on the outline physical map in Figure 1.

The DNA sequence of the Tn501 *mer*A gene and the predicted amino acid sequence of the mercuric reductase gene product are presented in this paper. The predicted primary structure of mercuric reductase shows extensive homologies with human erythrocyte glutathione reductase, suggesting that the tertiary structures of these dimeric enzymes may be similar. The major differences are in the N- and C-terminal regions. We discuss the mechanistic implications of the homologies and differences to other thiol oxidoreductases. The frequency of use of degenerate codons (codon usage) shows a high proportion of codons with C or G at the third position. The base composition of the *mer*A gene is similar to that of bulk *Pseudomonas* DNA, and we suggest that this codon usage is typical of *Pseudomonas* genes. this is the first gene from this genus whose sequence has been reported.

## Experimental Procedures

*Materials.* *E. coli* strains C600(pUB781), DS714-(pJOE114), and CSR603 were generous gifts of P. M. Bennett (University of Bristol, U.K.), R. Schmitt (University of Regensberg, FRG), and A. G. Hepburn (John Innes Institute, U.K.), respectively. *E. coli* strains 71–18 and JM101 and bacteriophage M13mp7 were generous gifts of J. Messing (University of Minnesota). Plasmid DNA and bacteriophage M13mp7 replicative-form DNA were prepared by CsCl–ethidium bromide isopycnic centrifugation of cleared lysates. Plasmids pUB781 and pICI arose from transposition of Tn501 into ColE1 and pMB8, respectively (Bennett et al., 1978; S. J. Ford, unpublished data); plasmid pJOE114 is an in vitro recombinant made by replacement of the *Eco*RI fragments of Tn1721 with those of Tn501 in the pBR322 (Δ*Sal*GI-*Eco*RI)::Tn1721 recombinant pJOE105 (Schöffl et al., 1981).

Restriction endonucleases, DNA polymerase I, DNA ligase, and exonuclease III were obtained from Boehringher Corporation London, Ltd., U.K., Bethesda Research Laboratories, U.K., or P-L Biochemicals Inc., U.K., or were prepared in the laboratory. All chemical reagents were from British Drug Houses Ltd., U.K., and were AnalaR grade whenever possible. Antibiotics were obtained from Sigma Chemical Co. Ltd., London, U.K. Deoxyadenosine 5′-[$\alpha$-$^{32}$P]triphosphate (>400

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[1] Abbreviations: FAD, flavin adenine dinucleotide; NADPH, reduced nicotinamide adenine dinucleotide.

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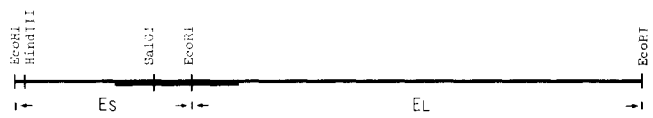


FIGURE 1: Outline physical map of Tn501 showing restriction sites for *Eco*RI, *Hin*dIII, and *Sal*GI and *Eco*RI fragments ES and EL. The sequence presented in this paper is represented by the broad band.

Ci/mmol) and [$^{35}$S]methionine (>800 Ci/mmol) were purchased from Amersham International plc, U.K.

*Molecular Cloning of Tn501 DNA Fragments.* A partial *Eco*RI digest of pICI DNA was incubated with excess stoichiometric amounts of RNA polymerase and filtered through nitrocellulose filters as described by Hinkle & Chamberlin (1972). All partial products containing pMB8 DNA were retained on the filter as they contain a strong RNA polymerase binding site. The Tn501 DNA in the filtrate was then precipitated and ligated with *Eco*RI-digested pAC184 DNA. Competent *E. coli* C600 was transformed with the products of ligation, and transformed cells were selected on tetracycline nutrient agar medium. Recombinant DNAs in the transformants were identified by screening for chloramphenicol sensitivity and by restriction analysis of plasmid DNA prepared from single-colony lysates. The procedures used were essentially those of Hepburn (Hepburn & Hindley, 1979). Five different recombinant types were identified, and representative plasmids were designated pSJF301–pSJF305, respectively.

*Phenotypic Analysis of Recombinants.* Recombinants were plated as a lawn on nutrient agar plates, a $HgCl_2$-saturated paper disk was placed on the agar, and the plates were incubated at 37 °C overnight (Foster et al., 1979). The size of the zone of inhibition around the disk was measured and compared with those of known *mer* phenotypes. Resistant, sensitive, and supersensitive phenotypes could be discriminated.

*"Maxicell" Labeling of Plasmid-Coded Polypeptides.* The five recombinant plasmids pSJF301–pSJF305 were isolated and used to transform *E. coli* CSR603. The protocol of Sancar et al. (1979) was used to specifically label the plasmid-coded polypeptides and prepare them for gel electrophoresis, with two modifications: cycloserine (200 $\mu$g mL$^{-1}$) was added to the culture 1 h after irradiation to kill viable cells; the method of Gill et al. (1979) was used to prepare the proteins for electrophoresis after labeling. Gel electrophoresis was performed by a modification of the method of Laemmli (1970), and sodium salicylate fluorography was used to detect the $^{35}$S-labeled polypeptide (Chamberlain, 1979).

*DNA Sequence Analysis.* The chain-termination procedure of Sanger et al. (1977) was used, employing templates generated by the random cloning in M13mp7 (Messing et al., 1981) of restriction endonuclease fragments of pJOE114 DNA or of DNA purified from pUB781. In the later stages of the DNA sequence analysis, specific DNA fragments, identified from the predicted restriction map of Tn501, were purified by gel electrophoresis prior to cloning in M13mp7. The cloning, transformation, template preparation, and sequence analysis were essentially as described by Sanger et al. (1980). Templates of *Taq*I fragments cloned into the *Acc*I site of M13mp7 grew better in *E. coli* JM101 than in 71-18. Exonuclease-treated 96-bp primer (Heidecker et al., 1980) or a small synthetic primer was used. The DNA sequence was determined on both strands of DNA throughout the region described in this paper.

*Computer Methods.* Sequence data were compiled and analyzed by using minor modifications of the programs DBUTIL (and related programs), TRAONE, SEARCH, CODSUM, and BASSUM described by Staden (1977, 1980; R. Staden, unpublished data), on a PDP 11/45 with the RSX11M V3.1 operating system. Some comparisons of predicted mercuric reductase amino acid sequence with that of glutathione reductase were kindly done by G. E. Schulz (Max-Planck-Institut für Medizinische Forschung, Heidelberg, FRG) using the computer program described by Williams et al. (1982).

Table I: Structures and Phenotypes of Plasmids Containing Tn501 DNA

| plasmid[a] | structure[b] | phenotype[c] |
|---|---|---|
| pUB781 | ColE1::Tn501 ($\vec{ES}$:$\vec{EL}$) | Col$^{IMM}$ Hg$^{R}$ |
| pSJF301 | pAC184:$\overleftarrow{ES}$ | Tc$^{R}$ Hg$^{SS}$ |
| pSJF302 | pAC184:$\vec{ES}$ | Tc$^{R}$ Hg$^{SS}$ |
| pSJF303 | pAC184:$\overleftarrow{EL}$ | Tc$^{R}$ Hg$^{S}$ |
| pSJF304 | pAC184:$\vec{EL}$ | Tc$^{R}$ Hg$^{S}$ |
| pSJF305 | pAC184:$\vec{ES}$:$\vec{EL}$ | Tc$^{R}$ Hg$^{R}$ |
| pJS120 | pBR322:$\vec{ES}$:$\overleftarrow{EL}$ | Tc$^{R}$ Ap$^{R}$ Hg$^{SS}$ |

[a] pUB781 is the product of in vivo transposition. [b] Arrows show the relative orientations of the Tn501 DNA fragments. The orientation of the vector is constant in all pSJF clones. [c] IMM, immune; R, resistant; S, sensitive; SS, supersensitive.

## Results

*Molecular and Phenotypic Analysis of pAC184::Tn501 Recombinants.* Restriction analysis of the five plasmids pSJF301–pSJF305 showed that they contained one or both of the *Eco*RI fragments of Tn501 and showed the relative orientation of the fragments in the vector (Table I). Only recombinants that could be generated by a single insertion of a fragment of Tn501 DNA with pAC184 were observed. The insert in pSJF305 is probably a single fragment. A pBR322::Tn501 recombinant (pJS120) in which the two *Eco*RI fragments of Tn501 are cloned such that the larger fragment (EL) is inverted relative to the smaller (ES) was provided by J. Stephenson, and its phenotype was also tested.

The data show that only when the *Eco*RI fragments ES and EL are cloned together in the same relative orientation as in Tn501 (i.e., pSJF305) is the mercury-resistance phenotype conferred on the host strain. Clones containing only the larger *Eco*RI fragment (EL; pSJF303 and pSJF304) are mercury sensitive, whereas those containing fragment ES alone (pSJF301 and pSJF302) or ES with EL in the wrong relative orientation (pJS120) are mercury supersensitive. These data show that the mercury transport functions are transcribed and translated in ES (presumably from the *mer* promoter) but that the expression of mercury resistance requires both ES and EL in their relative orientations.

*Maxicell Labeling of Polypeptides Produced from Tn501.* An autoradiograph of a sodium dodecyl sulfate–polyacrylamide gel of the radioactively labeled polypeptides produced from plasmids pSJF301–pSJF305 in the maxicell system (Sancar et al., 1979) is shown in Figure 2. With plasmid pSJF305, which contains both ES and EL fragments of Tn501 in their correct orientation and which confers the mercury-resistance phenotype, three radioactive polypeptide bands are seen on the gel. The same three bands are seen with plasmid pUB781 and are increased in intensity relative to the colicin E1 band following induction of the *mer* operon with $HgCl_2$ (data not shown). The largest band (band A, Figure 2) is only seen with plasmid pSJF305 and is probably the mercury reductase subunit. It has an estimated molecular weight of 68 000 on this gel system. The other two bands (bands B and C, Figure 2) seen with all plasmids containing fragment ES are probably the mercury transport protein and at least one

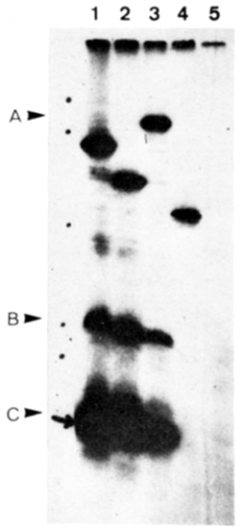


FIGURE 2: Autoradiograph of polypeptides encoded by pAC184::Tn501 recombinant plasmids, analyzed on a sodium dodecyl sulfate–polyacrylamide (10–25% gradient) gel. The samples were prepared as described under Experimental Procedures from *E. coli* CSR 603 strains transformed with plasmids (1) pSJF302, (2) pSJF301, (3) pSJF305, (4) pSJF304, and (5) pSJF303. The bands marked A–C are discussed in the text.

other unidentified gene product. Band C is broad and intense and may contain more than one molecular species. The estimated molecular weights of bands B and C are 17 000 and <12 000, respectively. The remaining radioactive bands apparently arise as fusion products of Tn501 and pAC184 sequences. Only in one orientation (pSJF304) is a band seen with large *Eco*RI fragment clones. The most likely explanation is that transcription originates in pAC184 and reads through into EL but that no highly expressed transcription/translation unit initiates in EL.

*Location of merA Gene in Tn501.* The simplest interpretation of the data from phenotypic studies and maxicell analysis of the plasmid-coded polypeptides on plasmids pSJF301–pSJF305 is that the small *Eco*RI fragment ES contains the *mer* promoter and the gene(s) for mercury transport and confers the mercury-supersensitive phenotype due to uptake of Hg(II) in the absence of the detoxifying enzyme, mercuric reductase, and that the structural gene for the mercuric reductase (*mer*A) lies across the internal *Eco*RI site of Tn501 (Figure 1). The *mer*A gene cannot be contained solely within ES as plasmids pSJF301, pSJF302, and pJS120 confer the supersensitive phenotype; nor is it likely to be contained solely within EL as plasmids pSJF304 and pSJF305 confer the mercury-sensitive phenotype, yet in one orientation transcription into EL from the constitutive chloramphenicol acetyltransferase promoter on pAC184 should occur. The band seen with plasmid pSJF304 is probably due to translation of this transcript. Plasmid pAC184 in the maxicell system produces one band, that corresponding to chloramphenicol acetyltransferase (CAT): the coding sequence for which contains the *Eco*RI site used for cloning. Only with plasmid pSJF305 is the mercury-resistance phenotype conferred. If the coding sequence for the mercuric reductase spans the central *Eco*RI site, then all bands seen in the maxicell experiment can be explained. Band A is the mercuric reductase subunit; bands B and C are other *mer* gene products. The "fusion bands" in pSFJ301 and pSFJ302 would both be N-terminal mercuric reductase fused to either the C-terminal CAT or the product of another open-reading frame in the other strand. The fusion product in pSJF304 is likely to be the N terminal of CAT fused to the C terminal of mercuric reductase. Data on the insertional inactivation of *mer*A by Tn802 mutagenesis (Choi, 1982) agree with the localization of the *mer*A gene across the internal *Eco*RI site.

*DNA Sequence Analysis.* The sequence of 1747 nucleotides across the internal *Eco*RI site of Tn501 is shown in Figure 3, and the predicted amino acid sequence of the mercuric reductase subunit is given. The nucleotide sequence lies between coordinates 0.16 and 0.36 on the Tn501 map (unpublished data). The predicted gene product has an unprocessed length of 561 amino acids and a molecular weight of 58 661. The amino-terminal sequence of the predicted gene product agrees with that determined for Tn501 mercuric reductase by Fox & Walsh (1983) and indicates that only the N-terminal formylmethionyl residue is removed during posttranslational processing.

Inspection of the predicted amino acid sequence of the mercuric reductase revealed a sequence homologous to the active site peptides of other dithiol reductases (Williams et al., 1982). All but the last two residues of this sequence, Thr-Ile-Gly-Gly-Thr-Cys-Val-Asn-Val-Gly-Cys-Val-Pro-Ser-Lys, have been confirmed by amino acid sequence analysis of the alkylated peptide (Fox & Walsh, 1983). Other peptide homologies are seen between mercuric reductase and glutathione reductase (Figure 4; Krauth-Siegel et al., 1982) and lipoamide dehydrogenase (Williams et al., 1982; J. Guest, personal communication).

The G+C content of the *mer*A gene DNA is 65.1 mol %, which is similar to that found elsewhere in Tn501 (unpublished data) and is within the range observed in total *P. aeruginosa* DNA (Normore, 1976). The distribution of the 562 codons used in the *mer*A gene is given in Figure 5 and shows a marked preference for codons ending in C or G over those ending in U or A.

## Discussion

The mercury-resistance determinants of Tn501 and plasmid R-100 are closely related [J. Miller and R. H. Rownd, unpublished observations reported in Jackson & Summers (1982b)]. Mercuric reductase is the product of the *mer*A gene of plasmid R-100 (Foster et al., 1979). The *mer* operon of R-100 was shown to contain at least three genes and the *mer* promoter in the order *mer*R, promoter, *mer*T, *mer*A. The *mer*R gene product is the regulatory protein, and the *mer*T gene product is the transport protein (Foster et al., 1979). Recent work by N. Ni'Bhriain, S. Silver, and T. J. Foster (personal communication) has suggested that two additional genes are present in the R-100 *mer* operon, one each side of the *mer*A gene. The location of the *mer*A gene in Tn501, the phenotypic properties of plasmids containing *Eco*RI subfragments of Tn501, and the number of polypeptides produced from these plasmids are all consistent with the existence of an analogous *mer* operon with the same gene order in Tn501. Preliminary DNA sequence data on regions of Tn501 outside the *mer*A gene suggest that the additional genes predicted by Ni'Bhriain, Silver, and Foster in R-100 also exist in Tn501. The product of the promoter-proximal additional gene and the *mer*T gene product are probably the two bands B and C seen in maxicell labeling experiments.

A detailed study of the polypeptides encoded by the *mer* operon of R-100 and some comparison with the polypeptides encoded by Tn501 has been done by using the minicell system (Jackson & Summers, 1982a,b). The minicell studies are approximately in agreement with our data, except that we did not detect two inducible proteins in the 14 000–17 000 molecular weight range. There is considerable variation in the estimated molecular weights of the mercuric reductase mon-

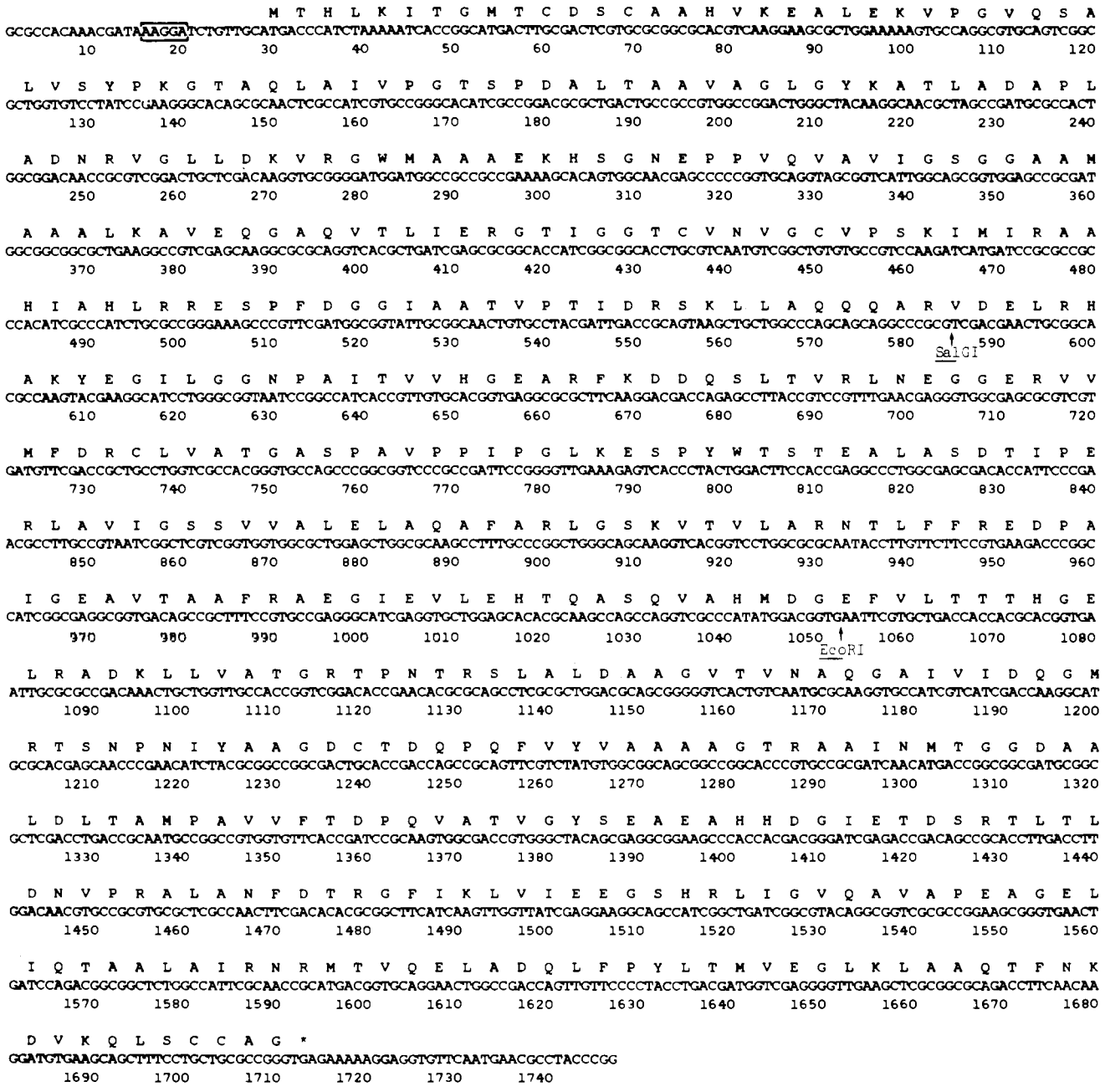


FIGURE 3: DNA sequence of 1747 base pairs of Tn501 DNA and predicted amino acid sequence of the mercuric reductase polypeptide. The Shine–Dalgano sequence is boxed, and the *Eco*RI and *Sal*GI sites are marked.

omer, from 69 000 (Jackson & Summers, 1982a) to 62 000 (Fox & Walsh, 1982). The gene sequence presented in this paper gives a polypeptide molecular weight of 58 660 (excluding processing and FAD cofactor). This apparently large discrepancy is within the range of error for molecular weight calculations from sodium dodecyl sulfate–polyacrylamide gels for proteins of this size range.

The N-terminal and active site peptide sequence data presented in the accompanying paper (Fox & Walsh, 1983) confirm our assignment of the coding sequence for the *mer*A gene product. Earlier studies of the Tn501 mercuric reductase by Fox & Walsh (1982) have shown that the enzyme contains redox-active cysteine residues and that it is mechanistically similar to the disulfide oxidoreductases glutathione reductase and lipoamide dehydrogenase. Alkylation studies (Fox & Walsh, 1983) confirm this similarity.

The predicted amino acid sequence of Tn501 mercuric reductase is aligned with the sequence of human erythrocyte glutathione reductase (Krauth-Siegel et al., 1982) in Figure 4. The amino acid sequences of mercuric reductase and glutathione reductase show striking homologies over about 65% of the mercuric reductase sequence. Residues 101–463 of mercuric reductase align well with residues 24–396 of glutathione reductase, and the two polypeptides would be expected to have similar secondary structures through this region. The closest homologies are seen in the adenine binding site (residues 102–109) of the FAD-binding domain and in the redox-active cysteine region (residues 131–142). There is also homology between mercuric reductase and *E. coli* lipoamide dehydrogenase (J. Guest, personal communication). Spectroscopic and kinetic data and alkylation studies (Fox & Walsh, 1982, 1983) suggest that the active site organization of mercuric reductase is similar to that of glutathione reductase. The major differences between the primary sequence of these two enzymes are in the N-terminal region (residues 1–100) and the C-terminal region (residues 464–561).

The tertiary structure of human erythrocyte glutathione reductase is known at 2-Å resolution (Thieme et al., 1981),

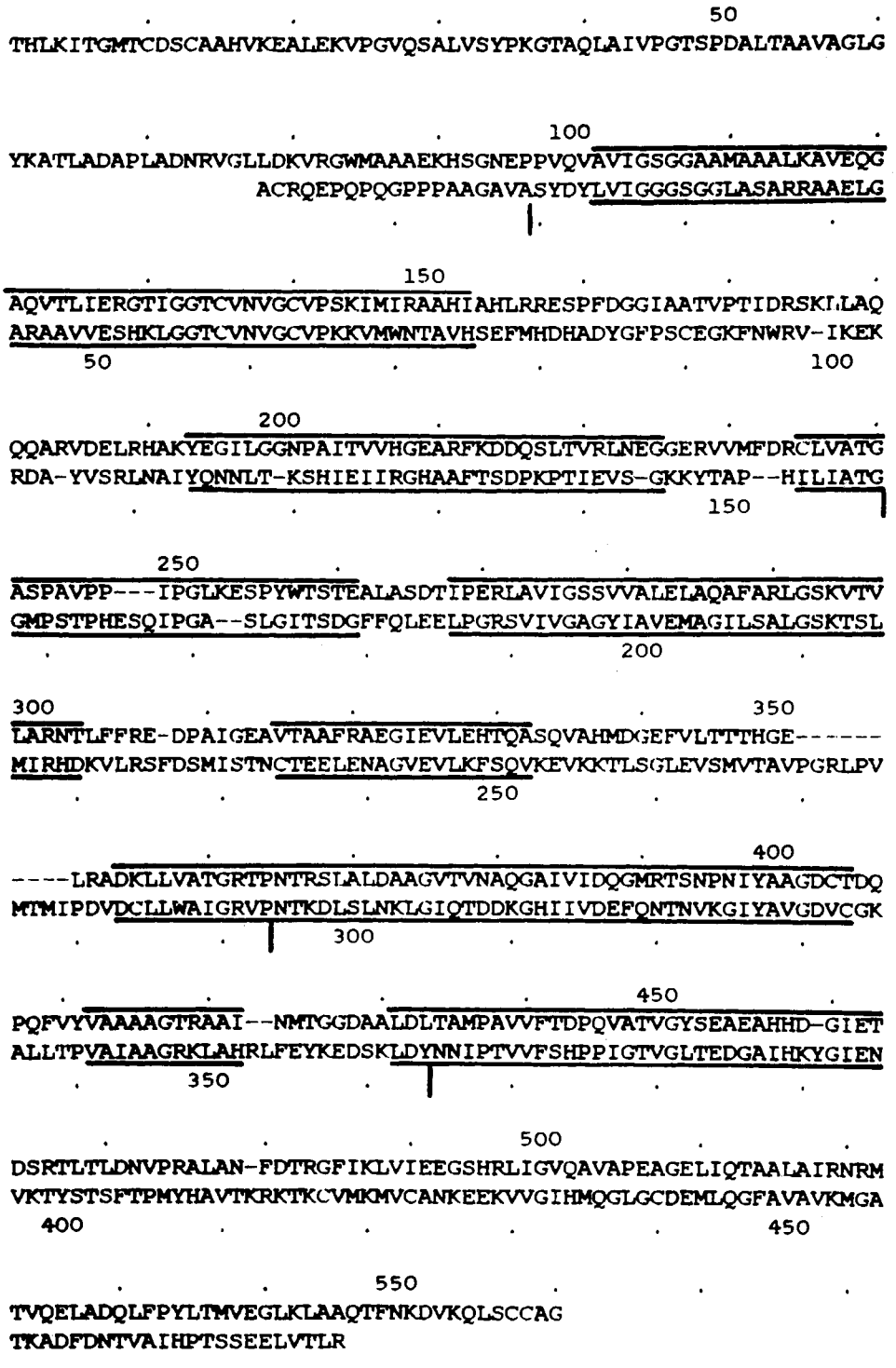


FIGURE 4: Alignment of amino acid sequences of Tn501 mercuric reductase (top line) and human erythrocyte glutathione reductase (bottom line). Homologous sequences were determined by the computer program described in Williams et al. (1982) on the basis of the natural substitution frequencies of homologous proteins, allowing for deletions and insertions. Homologies scoring significantly higher than the mean score of all comparisons between the two polypeptides are shown by horizontal lines. Vertical lines represent the domain boundaries in glutathione reductase. Hyphens are spacing to align the sequences and are not included in the numbering.

and mercuric reductase may have a similar tertiary structure in the homologous core region (residues 101–463). The N- and C-terminal nonhomologous regions may form separate domains, which are important in the specific differences between the two enzymes, as discussed below.

Mercuric reductase contains eight cysteine residues. Two of these are the redox-active cysteines in the active site (Cys-135 and Cys-140). The only other cysteine that may have a counterpart in glutathione reductase is Cys-403, which lies at one end of a homologous region in the two enzymes. No function has been proposed for this residue. The remaining five cysteines have no obvious counterpart in glutathione reductase, and we can speculate on the roles of four of these in the reduction of Hg(II). There are two cysteine residues (Cys-557 and Cys-558) at the C terminus of mercuric reductase. These residues are probably those found in the minor alkylated peptide in the experiments of Fox & Walsh (1983) and are therefore accessible to electrophiles. We propose that these cysteine residues are responsible for the binding of Hg(II) at the active site of mercuric reductase. By analogy with

| | | | |
|---|---|---|---|
| UUU PHE 1 | UCU SER 0 | UAU TYR 2 | UGU CYS 1 |
| UUC PHE 13 | UCC SER 4 | UAC TYR 6 | UGC CYS 7 |
| UUA LEU 0 | UCA SER 1 | UAA TER 0 | UGA TER 1 |
| UUG LEU 9 | UCG SER 5 | UAG TER 0 | UGG TRP 2 |
| CUU LEU 3 | CCU PRO 1 | CAU HIS 4 | CGU ARG 5 |
| CUC LEU 6 | CCC PRO 4 | CAC HIS 9 | CGC ARG 18 |
| CUA LEU 2 | CCA PRO 2 | CAA GLN 7 | CGA ARG 0 |
| CUG LEU 30 | CCG PRO 19 | CAG GLN 16 | CGG ARG 6 |
| AUU ILE 6 | ACU THR 5 | AAU ASN 4 | AGU SER 2 |
| AUC ILE 21 | ACC THR 22 | AAC ASN 11 | AGC SER 12 |
| AUA ILE 0 | ACA THR 5 | AAA LYS 4 | AGA ARG 0 |
| AUG MET 12 | ACG THR 12 | AAG LYS 15 | AGG ARG 0 |
| GUU VAL 3 | GCU ALA 2 | GAU ASP 5 | GGU GLY 11 |
| GUC VAL 21 | GCC ALA 40 | GAC ASP 23 | GGC GLY 29 |
| GUA VAL 3 | GCA ALA 5 | GAA GLU 15 | GGA GLY 4 |
| GUG VAL 24 | GCG ALA 40 | GAG GLU 17 | GGG GLY 5 |

FIGURE 5: Synonymous codon usage in Tn501 *mer*A gene (including initiation and termination codons).

glutathione reductase, these cysteines are in a region of the molecule that may be expected to interact with the other subunit.

.The major structural difference between mercuric reductase and glutathione reductase is the additional N-terminal 77 amino acids in the former. This region is also absent in *E. coli* lipoamide dehydrogenase (J. R. Guest, personal communication). The first 19 residues of glutathione reductase have no defined X-ray structure (Thieme et al., 1981), nor do they show homology with mercuric reductase. The first 96 amino acids of mercuric reductase may form an additional domain that includes two cysteine residues in the sequence -$Cys^{10}$-$Asp^{11}$-$Ser^{12}$-$Cys^{13}$-. We propose that these cysteine residues may be involved in the direct transfer of Hg(II) from the transport protein to the active site of mercuric reductase. Such a mechanism would be biologically advantageous in protecting the cellular contents from the toxic effects of Hg(II). There is some circumstantial evidence for the N-terminal domain having a role in membrane binding (Jackson & Summers, 1982b), and proteolytic removal of these cysteine residues has no significant effect on the specific activity of the enzyme (Fox & Walsh, 1983).

In human glutathione reductase, His-467 is thought to promote formation of the nucleophilic thiolate anion at Cys-63 and to catalyze cleavage of the enzyme–glutathione mixed disulfide (Untucht-Grau et al., 1979). There is no equivalent base in the corresponding amino acid sequence of mercuric reductase, and, if a similar catalytic mechanism occurs in mercuric reductase, the base must be contributed by a different part of the primary structure. In this regard, Boggaram & Mannervik (1978) observed that mammalian glutathione reductase was almost completely inhibited by ethoxyformic anhydride, which modifies His-467, whereas yeast glutathione reductase was only 10–15% inhibited. This suggest that the reactivity and possibly the identity of the basic group are different in these two classes of glutatione reductase.

The overall G+C content of the *mer*A gene (65.1 mol %) is close to that of bulk *P. aeruginosa* DNA (64–69 mol %; Normore, 1976). This suggests that codon usage in the *mer*A gene may be typical of that in *P. aeruginosa*. An analysis of codon usage in the *mer*A gene is given in Figure 5. This shows that in all cases of synonymous codons there is a strong preference (about 81%) for codons with C or G at the third position. This observation is not surprising in view of the fact that, in the absence of large noncoding regions, the G+C content of a DNA must reflect the codon usage. The significance of this codon usage with respect to relative concentrations of isoaccepting tRNAs in *Pseudomonas* is not known as these concentrations have not been measured, but those codons that are recognized by rare tRNA species in *E. coli* (AUA, AGA, AGG, etc.) are absent or used infrequently in the *mer*A gene. The tRNA concentrations are not thought to affect the choice between synonymous codons ending with U or C, as these are probably recognized by the same tRNA species. A similar codon usage is found in other Tn501 structural genes (N. L. Brown, R. D. Pridmore, and D. C. Fritzinger, unpublished data).

Grosjean & Fiers (1982) have summarized evidence for the proposal that efficient translation is facilitated by the choice of codons that give codon–anticodon interactions of intermediate strength, avoiding very strong or weak interaction energies. A recent analysis of codon usage in *E. coli* (Gouy & Gautier, 1982) showed that in highly expressed genes, U or A residues were found more frequently than C or G residues in the third position of codons that have C or G bases at the first two positions. In genes that were weakly expressed, the converse was true. In the *mer*A gene, the codons CCN, CGN, and GGN have C or G at the third position in 69–92% of the codons. The *mer*A gene is under transcriptional control, being transcribed in response to Hg(II) in the cell, and codes for the Hg(II)-detoxifying enzyme. The gene product is therefore likely to be efficiently translated. If this is the case, then the argument that efficient translation is facilitated by codon–anticodon interactions of intermediate strength, proposed on the basis of data from *E. coli*, does not apply without modification to other genera. In genera with very high or low G+C contents, the relative availability of isoaccepting tRNA species, and the use or avoidance of particular codons from the larger families of synonymous codons, may be more important than codon–anticodon interaction energy in translational efficiency. This interaction energy may allow an additional sophistication of control in the Enterobacteriacae. A preference for codons ending in C or G similar to that observed in *mer*A has been reported in the bacteriorhodopsin gene from the archaebacterium *Halobacterium halobium* (Dunn et al., 1981), which has an overall G+C content of about 67 mol %.

## Added in Proof

The nucleotide sequence of the metapyrocatechase gene from the TOL plasmid of *Pseudomonas putida* mt-2 has recently been reported (Nakai et al., 1983). These workers report a similar codon usage to that described in this paper.

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# Histones of Terminally Differentiated Cells Undergo Continuous Turnover†

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ABSTRACT: In contrast to the widely accepted idea of the nearly absolute metabolic stability of histones, our experiments support the view that the histones of nonproliferating, terminally differentiated cells undergo continuous replacement. This conclusion is based on the incorporation of labeled amino acids into the histones of mouse kidney and liver cells after their intraperitoneal introduction. We have found that the intranuclear uptake of the histones made in the absence of replicative synthesis and their integration into chromatin proceed with striking delay. The metabolic rates of individual histones measured by calculating their half-lives suggest that each histone turns over at a specific rate. With regard to the basic chromatin structure, the nucleosome, such unequal turnover should mean that the histone core does not participate in this process as a single unit but rather as a protein mosaic in which each partner follows its own rate of removal. Additional experiments suggested that intact nucleosomes take part in the replacement, but the relative proportion of the nucleosomes involved should be limited. The nonnucleosomal H1A and H1° histones have been found to undergo faster replacement than the core histones. Moreover, in comparison to each other, these two histone subfractions are also replaced at a different rate. The results of autoradiography of isolated kidney and liver nuclei after continuous labeling with [$^3$H]-thymidine suggest that the histone replacement is not associated with the repair of DNA.

A large body of evidence has indicated that histone synthesis is tightly coupled to DNA synthesis [for reviews, see Elgin & Weintraub (1975) and Tsanev (1980)]. This suggestion has received additional support by the finding that G1 cells possess a reduced amount of histone mRNA (Melli et al., 1977). One of the implications that has been raised from these results considers the metabolic stability of the histones as being almost absolute. A number of reports have suggested, however, that the coupling may not hold true for all systems. Gurley et al. (1972) have demonstrated that histone synthesis occurs in G1-arrested cells at a detectable rate and that this synthesis has been accompanied by a slow turnover of the histones. Adamson & Woodland (1974, 1977) have found that during

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