NADPH-Cytochrome P-450 Oxidoreductase Gene Organization Correlates with Structural Domains of the Protein†,‡

Todd D. Porter,§ Thomas W. Beck,‖ and Charles B. Kasper*
McArdle Laboratory for Cancer Research, University of Wisconsin—Madison, Madison, Wisconsin 53706
Received February 12, 1990; Revised Manuscript Received June 20, 1990

ABSTRACT: cDNA clones to rat liver NADPH-cytochrome P-450 oxidoreductase were used to isolate genomic clones from a Wistar-Furth inbred rat genomic DNA library. Fifteen exons containing the coding region and 3'-nontranslated segment of the P-450 reductase gene were identified, spanning 20 kilobases of DNA contained in 3 λ-Charon 35 clones. The organization of this single copy gene reveals a general correspondence between exons and structural domains of the protein, with the segment responsible for anchoring the reductase to the microsomal membrane and several segments involved in FMN, FAD, and NADPH binding encoded by discrete exons.

NADPH-cytochrome P-450 oxidoreductase is a 78 225-dalton flavoprotein associated with the endoplasmic reticulum of most eukaryotic cells (Williams & Kamin, 1962; Phillips & Langdon, 1962). The protein is responsible for electron transfer from NADPH to the cytochromes P-450 (Lu et al., 1969) and is unusual in that it contains 1 mol each of FAD and FMN (Iyanagi & Mason, 1973). We previously reported the amino acid sequence of rat liver P-450 reductase as determined from overlapping cloned cDNAs (Porter & Kasper, 1985) and have reported a structural characterization of the enzyme based on a computer-assisted comparison of the amino acid sequence to several other flavoproteins of defined structure (Porter & Kasper, 1986). Residues 77-228 are homologous with the bacterial flavodoxins, indicating that this portion of the reductase is likely to bind FMN; studies utilizing site-directed mutagenesis of several residues in this region have provided strong support for this hypothesis (Shen et al., 1989). Several segments in the carboxy-terminal region show considerable similarity to two FAD-containing proteins, ferredoxin-$NADP^+$ reductase and NADH-cytochrome b_5 reductase, suggesting that this portion of the reductase binds FAD. Comparison of these proteins to glutathione reductase, a flavoprotein whose three-dimensional structure is known, has allowed tentative identification of FAD- and cofactor-binding segments in these proteins. The similarity between P-450 reductase and these FAD-binding proteins, coupled with the homology of the amino-terminal domain with the bacterial flavodoxins, suggests that the reductase arose through a fusion of the ancestral genes for these two sets of flavoproteins. To further evaluate this hypothesis, we have cloned and characterized the rat P-450 reductase gene. Analysis of the exon organization reveals a general correspondence between exons and proposed functional or structural domains, and supports the postulate that P-450 reductase evolved from a fusion of two different flavoprotein genes.

Experimental Procedures

Construction and Screening of the Genomic DNA Library. Nuclei were isolated from male Wistar-Furth rat liver (Kasper, 1974), lysed with sodium dodecyl sulfate, and high molecular weight DNA was prepared according to the method of Blin and Stafford (1976). The DNA was partially digested with *Sau*3a restriction enzyme, and fragments between 18 and 22 kilobases (kb)[1] were isolated by sucrose density centrifugation (Maniatis et al., 1982). DNA was ligated into the *Bam*HI site of λ-bacteriophage Charon 35 (Loenen & Blattner, 1983), and phage were propagated in *Escherichia coli* strain K802. The library was screened with P-450 reductase cDNA clones pOR-7 and pOR-8 (Porter & Kasper, 1985) that had been radiolabeled by nick-translation, positive plaques were isolated, and phage DNA was prepared by large-scale liquid culture followed by density gradient centrifugation (Maniatis et al., 1982). Genomic DNA inserts were mapped by restriction digestion, and fragments obtained by *Bam*HI and *Hin*dIII digestion were subcloned into pSP64 or pSP65 plasmids (Promega, Madison, WI) for further characterization.

DNA Hybridization. DNA from selected λ clones, and genomic DNA prepared as described above, was digested with the indicated restriction enzymes and separated by electrophoresis on 0.8% agarose gels. The gels were stained with ethidium bromide and photographed, and the DNA was partially hydrolyzed and then denatured prior to transfer by capillary blotting to nitrocellulose membranes (Schleicher & Schuell) (Maniatis et al., 1982). Hybridization was carried out as described (Maniatis et al., 1982) at 65 °C with a near-full-length reductase cDNA labeled by nick-translation with ^{32}P (pORFL; Porter et al., 1987). The filters were washed 3 times in 3× SSC/0.5% SDS (1X SSC is 0.15 M NaCl/0.015 M sodium citrate) prior to autoradiographic exposure.

DNA Sequence Determination. Plasmid subclones containing exonic sequence, as determined by hybridization to the reductase cDNAs, were sequenced by using the dideoxynucleotide chain-terminating procedure (Sanger et al., 1977). Approximately 15 μg of each plasmid was linearized with *Bam*HI or *Hin*dIII and then digested with 1.8 units of *Bal*31 exonuclease (Bethesda Research Laboratories) at 20 °C, withdrawing 1-μg aliquots at various time points. The ends of the exonuclease-treated DNA were made flush with Klenow fragment of DNA polymerase, and the insert was then released by digestion with *Bam*HI or *Hin*dIII and ligated into M13. Transformants were checked for shortened insert on a 0.8%

† This work was supported by Grants CA-22484, CA-09020, and CA-09135 from the National Institutes of Health.
‡ The nucleic acid sequence in this paper has been submitted to GenBank under Accession Number J05291.
§ Present address: Department of Biological Chemistry, Medical School, The University of Michigan, Ann Arbor, MI 48109.
‖ Present address: National Cancer Institute, Laboratory for Viral Carcinogenesis, Frederick Cancer Research Facility, Frederick, MD 21701.

[1] Abbreviations: kb, kilobase(s); bp, base pair(s); SDS, sodium dodecyl sulfate.

0006-2960/90/0429-9814$02.50/0

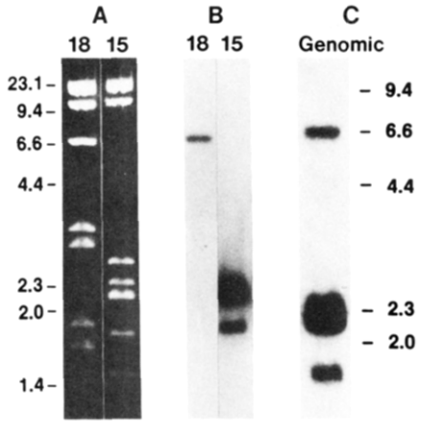

FIGURE 1: Hybridization analysis of reductase clones λOR18 and λOR15, and comparison to genomic DNA. λOR clones 18 and 15 were digested with *Bam*HI and *Hin*dIII and fractionated by gel electrophoresis, and the gel was stained with ethidium bromide (A). The gel was transferred to nitrocellulose and hybridized with pORFL, and the resulting autoradiographic image is shown (B). Male Wistar–Furth genomic DNA was digested with *Bam*HI and *Hin*dIII, fractionated by electrophoresis, transferred to nitrocellulose, and hybridized with a near-full-length reductase cDNA, pORFL (Porter et al., 1987). The resulting autoradiographic image is shown (C). The positions of kilobase size markers (*Hin*dIII-digested λ DNA) are shown on the left for (A) and (B), and on the right for (C).

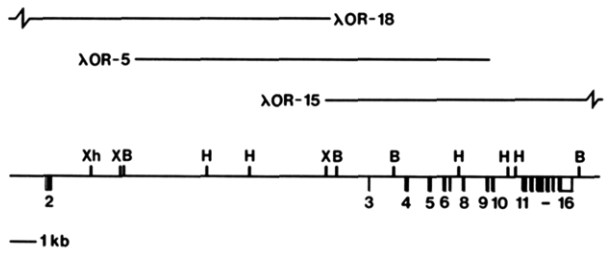

FIGURE 2: Restriction map and location of exons in the cytochrome P-450 reductase gene. The λ clone inserts are positioned above the exon map. Closed boxes represent coding sequence; open portions are nontranslated. The indicated restriction sites are as follows: X, *Xba*I; B, *Bam*HI; H, *Hin*dIII; Xh, *Xho*I.

agarose gel, and single-stranded template was prepared from selected clones. DNA was polymerized by using the Klenow fragment (Lyphozyme, Bethesda Research Laboratories) in the presence of [^{35}S]dATP (New England Nuclear) and fractionated on 6% linear and buffer gradient gels (Biggin et al., 1983). The sequence was determined 2–3 times from both strands.

Results

Three λ-Charon 35 recombinants that hybridized to the P-450 reductase cDNAs were isolated from the inbred Wistar–Furth library and characterized by restriction mapping and hybridization analysis.[2] As shown in Figure 1, digestion of clones 15 and 18 with *Bam*HI and *Hin*dIII yielded 10 prominent (nonvector) fragments, with sizes ranging from 6.6 to 1.6 kb, which lead to the restriction map presented in Figure 2. Hybridization with a near-full-length reductase cDNA identified a 6.6-kb fragment containing exon 2, a broad region between 2.2 and 2.4 kb containing exons 4–7 and 11–16, and an intensely labeled group of fragments at approximately 1.8 kb, containing exons 3 and 8–10. Exon 1 was not detected, since this nontranslated segment is not contained in the cDNA probe. These bands correspond perfectly with the pattern obtained upon hybridization of identically digested Wistar–Furth rat genomic DNA (Figure 1C), demonstrating that the reductase clones accurately reflect the native organization of the gene and that it is present in a single copy. Intron–exon junctions were determined by DNA sequence analysis of selected subclones, with comparison to the reductase cDNA sequence. The 3 reductase clones encompass 32 kb of DNA, with the coding portion of the gene divided into 15 exons (exons 2–16) spanning 20 kb of DNA (Figure 2). The majority of the coding sequence is clustered at the 3′ end of the gene within 8 kb of DNA (exons 3–16). Exon 2 contains the start codon and 13 nucleotides of untranslated sequence; it is preceded by a 3′-splice recognition site and at least 5 kb of intronic sequence.[3]

Table I: Exon and Intron Positions and Lengths

exon	position of first bp	position of first aa[b]	length bp	length aa	intron	length[a] (bp)
2	1	1	192	60	2	11900
3	193	61	49	16	3	1300
4	242	77	129	43	4	710
5	371	120	150	50	5	412
6	521	170	125	42	6	91
7	646	212	90	30	7	398
8	736	242	99	33	8	777
9	835	275	117	39	9	88
10	952	314	119	39	10	1000
11	1071	353	182	61	11	103
12	1253	414	150	50	12	102
13	1403	464	271	90	13	79
14	1674	554	146	49	14	89
15	1820	603	86	29	15	126
16	1906	632	502	47		

[a] The lengths of introns 2, 3, and 10 were estimated by restriction analysis. [b] aa, amino acid; codons split by introns are assigned to the exon containing two of the three nucleotides.

The positions of the introns with regard to the mature mRNA sequence are shown in Figure 3. Exon lengths, shown in Table I, range from 49 bp (exon 3) to 502 bp (exon 16), the latter of which includes the complete 3′-nontranslated segment. There are five nucleotide differences between the Wistar–Furth gene and the Sprague Dawley cDNA (Porter & Kasper, 1985). Of the two differences that occur in the coding sequence, at positions 584 and 777, only the latter results in an amino acid replacement (Val to Ala). A three base pair deletion is present in the 3′-nontranslated region at position 2158. Resequencing of the cDNAs across these positions demonstrated that these are bona fide differences and not the result of sequencing errors.

Intron lengths are also shown in Table I, and range from 79 bp (intron 13) to 11.9 kb (intron 2). Interestingly, introns 6, 9, 13, and 14 approach the minimal length (78 bp) thought to be consistent with efficient and accurate splicing (Wieringa et al., 1984; Upholt & Sandell, 1986). All junctional sequences conform to the GT-AG rule (Breathnach & Chambon, 1981).

Discussion

As an integral component of the microsomal (but not mitochondrial) cytochrome P-450 system, NADPH–cytochrome

[2] An earlier report from this laboratory (Gonzalez & Kasper, 1983) had identified the 5′ end of the reductase gene by differential hybridization to cDNA synthesized from reductase-immunoenriched RNA and to the reductase clone pOR-7, by S_1 nuclease mapping, and by translational arrest of P-450 reductase synthesis in vitro by a putative reductase gene 5′ DNA segment. Present studies have revealed that the cap site identified in this segment of DNA probably represents the 5′ end of a second, unidentified gene lying approximately 4 kb 3′ to the reductase gene. The translational arrest of reductase synthesis by this DNA was artifactual (P. McQuiddy and C. B. Kasper, unpublished results).

[3] Characterization of the promoter region and first exon of the reductase gene will be reported separately.

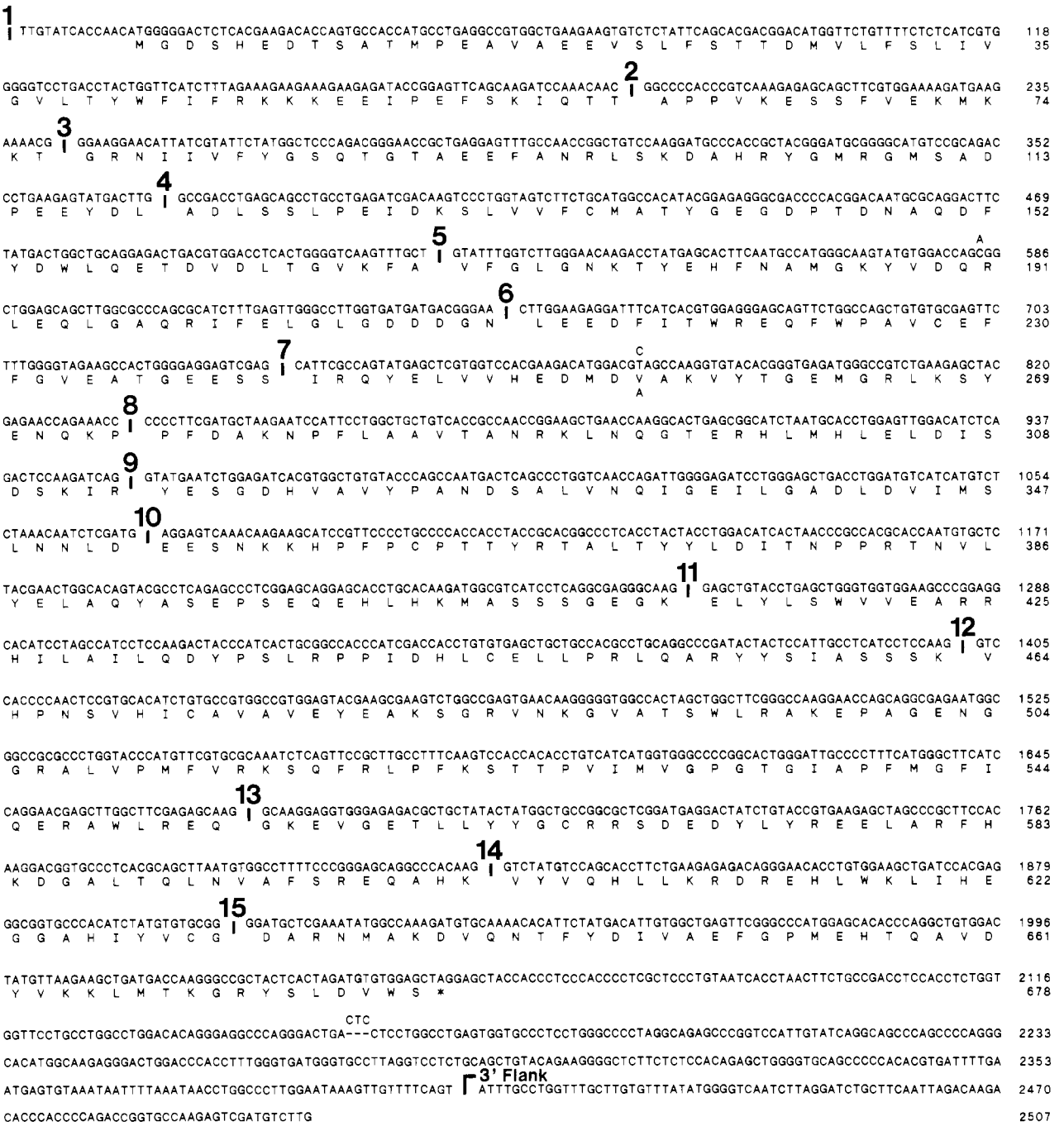

FIGURE 3: Exonic sequence and amino acid translation of the P-450 reductase gene. Intron positions are indicated (numbered 1–15) with 100 nucleotides of the 3′-flanking region of the gene also shown. The two nucleotide differences in the Sprague Dawley cDNA (positions 584 and 777) and the three-nucleotide insertion (position 2158) in the 3′-nontranslated segment are shown above the gene sequence.

P-450 reductase is present in many, if not all, tissues. Reductase expression differs, however, from that of the cytochromes P-450 in several important aspects. It is expressed at a lower level than P-450, with a ratio of reductase to P-450 of approximately 1:10 or less in most tissues (Masters & Okita, 1980). Reductase is largely refractory to induction by agents which elevate P-450 levels; a maximal increase of 2–3-fold was found after treatment of rats with either phenobarbital or dexamethasone, both potent inducers of cytochrome P-450 (Hardwick et al., 1983; Simmons et al., 1987). In marked contrast to the cytochromes P-450, most studies have indicated that only a single form of the reductase existed. The present studies confirm that supposition. A single set of overlapping genomic clones was isolated from a Wistar–Furth inbred rat genomic library by hybridization to reductase cDNAs, and these clones were shown to correspond perfectly with the restriction–hybridization pattern of rat genomic DNA. This result, combined with an earlier study from this laboratory revealing the presence of a single hybridizing band in genomic DNA from a wide variety of species (Simmons et al., 1985), demonstrates that P-450 reductase is encoded by a single-copy gene and this singular enzyme must therefore be capable of interacting with the multiple cytochromes P-450 found in each organism. Hence, those structural features of cytochrome P-450 responsible for its highly specific recognition by the reductase must be preserved in the many diverse P-450 isoforms. The mechanism of this interaction is currently an active area of investigation.

The hypothesis that genes have been assembled from discrete exons coding for small functional domains (Gilbert, 1978) has gained considerable support in recent years. One implication of this hypothesis, that the positions of introns within a pro-

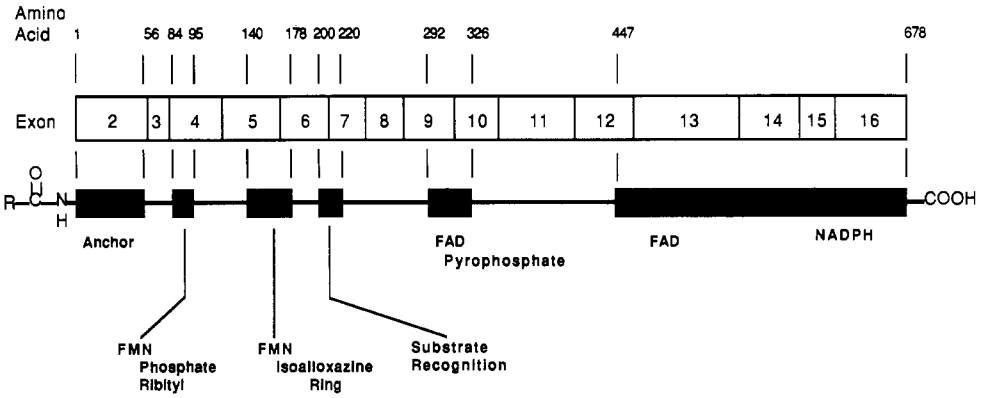

FIGURE 4: Correlation of the structural domains of cytochrome P-450 reductase with the exonic organization of the gene. The open boxes indicate the exons, with the closed boxes on the lower line indicating the proposed functional or structural segments. The amino acid positions of these segments are indicated at the top of the figure.

tein-coding sequence should correspond to structural and functional divisions of the protein, has been borne out in many instances (Craik et al., 1980; Tonegawa, 1983; Stone et al., 1985; Sudhof et al., 1985; Marchionni & Gilbert, 1986), although examples of genes with intron positions that do not correlate with domain divisions have also been documented (Benyajati et al., 1981; Venta et al., 1985). Although the three-dimensional structure of cytochrome P-450 reductase is not yet known, we recently reported a structural characterization of the protein based on computer-assisted comparison of the amino acid sequence to other flavoproteins, including flavodoxin and glutathione reductase, for which three-dimensional structures have been determined. This study revealed that the FMN domain of cytochrome P-450 reductase appears to be structurally similar to flavodoxin, while the FAD and NADPH domains share structural elements with glutathione reductase, and more so with ferredoxin–$NADP^+$ reductase and NADH–cytochrome b_5 reductase (Porter & Kasper, 1986). Recently reported studies utilizing site-directed mutagenesis and bacterial expression of the reductase have provided strong support for the assignment of FMN binding to the flavodoxin-like domain (Shen et al., 1989). Here we compare the exon organization of the reductase gene with the proposed structural features of the reductase protein; this relationship is represented schematically in Figure 4. We also note several significant similarities between the cytochrome P-450 reductase gene organization and that of the recently reported cytochrome b_5 reductase gene (Tomatsu et al., 1989).

The first translated exon (exon 2) encodes the first 60 amino acids of the hydrophobic amino-terminal region and contains the sequence responsible for anchoring P-450 reductase to the endoplasmic reticulum and nuclear envelope. The uniquely sensitive trypsin site, between Lys-56 and Ile-57, is the traditional site of demarcation between the membrane-binding segment and the catalytic portion of the reductase; thus, the intron–exon junction interrupting codon 60 corresponds closely to the physical boundary between these two structural domains.

The third exon of the reductase gene is the smallest (49 bp), encoding amino acids 61–76, which appear to serve as a bridge between the membrane-binding segment and the FMN domain of the enzyme. The FMN domain is divided into three exons, the first of which (exon 4) encodes the FMN phosphate-binding residues, amino acids 84–95, while exons 5 and 6 each encode a tyrosine (Tyr-140 and Tyr-178) involved in shielding and stabilizing the FMN isoalloxazine ring. Mutagenesis studies involving these tyrosyl residues have established their essential role in FMN binding (Shen et al., 1989). Recent studies also indicate that acidic residues between positions 200 and 220 (exons 6 and 7) may be involved in cytochrome P-450 and cytochrome *c* interactions (Nisimoto, 1986; Shen and Kasper, unpublished results).

Exons 7 and 8 together encode a 37 amino acid segment that joins the FMN and FAD domains of the reductase. This segment, which we have postulated arose when the ancestral genes coding for the precursor FMN- and FAD-containing proteins fused (Porter & Kasper, 1986), extends approximately from residue 229 to residue 266; exon 7 begins just before this, at position 212, and exon 8 ends just after it, at position 274.

The subsequent FAD domain of cytochrome P-450 reductase extends for 60 residues before being interrupted by a 120 amino acid segment which appears unrelated to other flavoproteins (Porter & Kasper, 1986). The initial 60 amino acid segment (residues 267–326) bears strong similarity to the amino-terminal regions of ferredoxin–$NADP^+$ reductase and cytochrome b_5 reductase, and contains those residues likely to interact with the pyrophosphoryl portion of the FAD molecule. This segment is largely encoded by exon 9, which includes residues 293–296, the amino acids proposed to directly interact with the pyrophosphate group. This segment is also encoded by a discrete exon in the cytochrome b_5 reductase gene (Tomatsu et al., 1989), with the position of the third intron of this gene corresponding closely to the position of intron 9 of P-450 reductase.

Exons 10–12 encode the 120 amino acid "insertion" in the cytochrome P-450 reductase sequence, relative to the ferredoxin–$NADP^+$ reductase and cytochrome b_5 reductase sequences; we have proposed that this segment, from residues 327 to 446, may be involved in orienting the FMN and FAD domains so as to facilitate electron transfer between the two flavins. A second segment of the FAD domain (residues 447–479) follows this insertion, but is split between exons 12 and 13. A similar observation is also made for the cytochrome b_5 reductase gene, in which an intron divides this segment. Exon 13 also encodes the beginning of the NADPH domain of the reductase, including the predicted NADPH pyrophosphate binding residues, positioned by sequence alignment near position 488. The remaining 125 amino acids of the NADPH domain are encoded by exons 14–16, with the position of intron 14 differing by only one amino acid from the position of intron 8 of b_5 reductase (Tomatsu et al., 1989). Introns 14 and 15 are also associated with gaps in the sequence alignment of this region (Porter & Kasper, 1986).

Traut (1988), in examining the correlation of exons with protein structural or functional units for a variety of genes, concluded that this relationship reflects an ancestral correspondence between exons and structure–function modules in proteins. It is evident from the present analysis that there is also a general correspondence between exons and structural

domains of P-450 reductase; in particular, the membrane-binding segment and the subsequent connecting segment are encoded by discrete exons (2 and 3), as is the proposed phosphate-binding segment of the FMN domain (exon 4), the segment joining the FMN and FAD domains (exons 7 and 8), and the pyrophosphate-binding residues of the FAD domain (exon 9). Similarly, Chang et al. (1988) found that the first exon of the human adrenodoxin gene encoded the signal peptide for this protein, and Shih et al. (1988) demonstrated a correspondence between exons and structural units of the NAD^+-binding domain of glyceraldehyde-3-phosphate dehydrogenases. In contrast, the second FAD-binding segment of P-450 reductase is split by the junction of exons 12 and 13. This correlation is similar to that seen with other genes, where some, but not all, intron–exon junctions correspond to functional boundaries within the protein (Traut, 1988).

It has been proposed that loss of introns may be a common occurrence in evolution and that ancestral genes may have been assembled from multiple exons encoding small structural units (Naora & Deacon, 1982; Gilbert, 1985). An analysis of fibrinogen genes indicated that a selective loss of introns had occurred for these genes and that the ancestral gene must have been composed of numerous small exons encoding perhaps 20–30 amino acids each (Crabtree et al., 1985). Intron loss was also strongly indicated in the evolution of glyceraldehyde-3-phosphate dehydrogenase genes (Shih et al., 1988), and also appears likely with cytochrome P-450 genes (Nebert et al., 1988). Thus, a loss of introns at some positions in the reductase gene may explain the lack of correspondence between some domain boundaries and intron positions. It is worth noting that the reductase is evidently an ancient protein, as a highly similar reductase has been found in a species of *Bacillus* as part of a cytochrome P-450 (Ruettinger et al., 1989), and in *Escherichia coli* and *Salmonella typhimurium* as a component of sulfite reductase (Ostrowski et al., 1989). An examination of the reductase gene in other organisms, as well as an examination of other flavoprotein genes, and in particular the ferredoxin–$NADP^+$ reductase gene, should reveal additional features of the ancestral flavoprotein genes from which this group of proteins has evolved.

ACKNOWLEDGMENTS

We thank Lori Manney and John Sheehan for excellent technical assistance.

REFERENCES

Benyajati, C., Place, A. R., Powers, D. A., & Sofer, W. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 2717–2721.

Biggin, M. D., Gibson, T. J., & Hong, G. F. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 3963–3965.

Blin, N., & Stafford, D. W. (1976) *Nucleic Acids Res. 3*, 2303–2308.

Breathnach, R., & Chambon, P. (1981) *Annu. Rev. Biochem. 50*, 349–383.

Chang, C.-Y., Wu, D.-U., Lai, C.-C., Miller, W. L., & Chung, B.-C. (1988) *DNA 7*, 609–615.

Crabtree, G. R., Comeau, C. M., Fowlkes, D. M., Fornace, A. J., Jr., Malley, J. D., & Kant, J. A. (1985) *J. Mol. Biol. 185*, 1–19.

Craik, C. S., Buchman, S. R., & Beychok, S. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 1384–1388.

Gilbert, W. (1978) *Nature 271*, 501.

Gilbert, W. (1985) *Science 228*, 823–824.

Gonzalez, F. J., & Kasper, C. B. (1983) *J. Biol. Chem. 258*, 1363–1368.

Hardwick, J. P., Gonzalez, F. J., & Kasper, C. B. (1983) *J. Biol. Chem. 258*, 8081–8085

Iyanagi, T., & Mason, H. S. (1973) *Biochemistry 12*, 2297–2308.

Kasper, C. B. (1974) *Methods Enzymol. 31*, 279–292.

Loenen, W. A. M., & Blattner, F. R. (1983) *Gene 26*, 171–179.

Lu, A. Y. H., Junk, K. W., & Coon, M. J. (1969) *J. Biol. Chem. 244*, 3714–3721.

Maniatis, T., Fritsch, E. F., & Sambrook, J. (1982) in *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Marchionni, M., & Gilbert, W. (1986) *Cell 46*, 133–141.

Masters, B. S. S., & Okita, R. T. (1980) *Pharmacol. Ther. 9*, 227–244.

Naora, H., & Deacon, N. J. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 6196–6200.

Nebert, D. W., Jones, J. E., Owens, J., & Puga, A. (1988) in *Oxidases and Related Redox Systems* (King, T. E., Mason, H. S., & Morrison, M., Eds.) pp 557–576, A. R. Liss, New York.

Nisimoto, Y. (1986) *J. Biol. Chem. 261*, 14232–14239.

Ostrowski, J., Barber, M. J., Rueger, D. C., Miller, B. E., Siegel, L. M., & Kredich, N. M. (1989) *J. Biol. Chem. 264*, 15796–15808.

Phillips, A. H., & Langdon, R. G. (1962) *J. Biol. Chem. 237*, 2652–2660.

Porter, T. D., & Kasper, C. B. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 973–977.

Porter, T. D., & Kasper, C. B. (1986) *Biochemistry 25*, 1682–1687.

Porter, T. D., Wilson, T. E., & Kasper, C. B. (1987) *Arch. Biochem. Biophys. 254*, 353–367.

Ruettinger, R. T., Wen, L.-P., & Fulco, A. J. (1989) *J. Biol. Chem. 264*, 10987–10995.

Sanger, F., Nicklen, S., & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5463–5467.

Shen, A. L., Porter, T. D., Wilson, T. E., & Kasper, C. B. (1989) *J. Biol. Chem. 264*, 7584–7589.

Shih, M.-C., Heinrich, P., & Goodman, H. M. (1988) *Science 242*, 1164–1166.

Simmons, D. L., Lalley, P. A., & Kasper, C. B. (1985) *J. Biol. Chem. 260*, 515–521.

Simmons, D. L., McQuiddy, P., & Kasper, C. B. (1987) *J. Biol. Chem. 262*, 326–332.

Stone, E. M., Rothblum, K. N., & Schwartz, R. J. (1985) *Nature 313*, 498–500.

Sudhof, T. C., Goldstein, J. L., Brown, M. S., & Russell, D. W. (1985) *Science 228*, 815–822.

Tomatsu, S., Kobayshi, Y., Fukumaki, Y., Yubisui, T., Orii, T., & Sakaki, Y. (1989) *Gene 80*, 353–361.

Tonegawa, S. (1983) *Nature 302*, 575–581.

Traut, T. W. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 2944–2948.

Upholt, W. B., & Sandell, L. J. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 2325–2329.

Venta, P. J., Montgomery, J. C., Hewett-Emmett, D., Wiebauer, K., & Tashian, R. E. (1985) *J. Biol. Chem. 260*, 12130–12135.

Wieringa, B., Hofer, E., & Weissman, C. (1984) *Cell 37*, 915–925.

Williams, C. H., Jr., & Kamin, H. (1962) *J. Biol. Chem. 237*, 587–595.