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Cloning of two novel P450 cDNAs from German cockroaches, *Blattella germanica* (L.): CYP6K1 and CYP6J1

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Abstract

Two novel P450 cDNAs, CYP6K1 and CYP6J1, were isolated from German cockroaches, *Blattella germanica* (L). Both CYP6K1 and CYP6J1 are typical microsomal P450s and their deduced amino acid sequences share a number of common characteristics with other members of the P450 superfamily. Both CYP6K1 and CYP6J1 showed the highest per cent identity (based on the deduced amino acid sequence) to CYP6L1 from *B. germanica* and CYP6H1, a putative ecdysone 20-hydroxylase from *Locusta migratoria*. Using a CYP6K1 probe, two mRNA signals (~2.5 and ~2.1 kb) were detected in all life stages. Both signals were just detectable in the eggs and became stronger in later instars. The strongest signals were detected in the fifth and sixth instars as well as in adults. These two bands were also detected in the abdomens and in the remainder of bodies of both male and female adults. Southern blots suggest the two mRNA bands detected in the Northern blot might be a result of alternative splicing. No signal could be detected at any life stage using the CYP6J1 probe, suggesting that CYP6J1 was expressed at a low level.

Keywords: cytochrome P450 monooxygenase, cDNA cloning, *Blattella germanica*, Insecta.

Introduction

The cytochrome P450 monooxygenases are important because they metabolize structurally diverse endogenous and exogenous compounds (Hodgson, 1985; Porter & Coon, 1991). However, there are many P450s in each metazoan species. Genomic sequencing has revealed eighty putative P450s in *Caenorhabditis elegans* (Consortium, 1998) and eighty-six in *Drosophila melanogaster* (Adams *et al.*, 2000). In insects, P450s are involved in the metabolism of plant allelochemicals and insecticides, as well as physiologically important endogenous compounds including juvenile hormones (JHs), ecdysteroids and pheromones (Hodgson, 1985). The isolation and characterization of insect P450s is a critical first step towards understanding the P450s involved in these important metabolic processes. Such studies have provided great insight into herbivore–plant interactions, insecticide resistance, insect development and physiology (Berenbaum, 1999; Feyereisen, 1999; Scott, 1999).

Cockroaches are one of the most primitive group of winged insects. Their body morphology has remained virtually unchanged for approximately 350 million years (Appel, 1995). Among the approximately 4000 known species of cockroaches, only about thirty are considered pests, with German cockroaches (*Blattella germanica*) being arguably the most important (Herrick, 1914; Ross & Cochran, 1975). German cockroaches are human pathogen-carriers and allergen-producers. Cockroaches carry a number of microorganisms potentially pathogenic to humans, including polio viruses, mycobacteria and helminthes (Brenner, 1995). The development of allergic respiratory diseases, especially bronchial asthma, is consistently correlated with cockroach infestation, and occurs world-wide wherever living conditions are suitable for cockroaches (Brenner, 1995; Chapman *et al.*, 1997).

Studies of insecticide metabolism and resistance triggered the discovery of P450s in many insects, including German cockroaches. The earliest indication of P450s in German cockroaches was the discovery of a microsomal enzyme system capable of converting DDT to dicofol (Agosin *et al.*, 1961; Agosin, 1985). Involvement of monooxygenases in the metabolism of various insecticides was later supported by *in vitro* metabolism studies or bioassays using various insecticides coupled with P450 inhibitors or inducers (Matsumura *et al.*, 1967; Khan & Matsumura, 1972; Siegfried *et al.*, 1990; Siegfried & Scott, 1991; Hemingway *et al.*, 1993a; Hemingway *et al.*, 1993b; Mahmood *et al.*, 1993; Valles *et al.*, 1994; Lee *et al.*, 1996; Scharf *et al.*, 1997; Scott & Wen, 1997;

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Wen & Scott, 1997; Valles, 1998; Wu *et al*., 1998). In these studies on German cockroaches, P450s have often been implicated in resistance to various insecticides.

The first report on individual P450 isoforms in German cockroaches was the successful purification of P450 MA from Munsyana (MA) strain, which had multiple resistance to several insecticides (Scharf *et al*., 1998). Western blot analysis showed that an anti-P450 MA recognized a single protein band that was detectable in the MA individuals, but not in individuals susceptible to insecticides. P450 MA could be induced by pentamethylbenzene in both insecticide susceptible and MA strains. Currently, only one P450 gene has been cloned from German cockroaches (*CYP6L1* (Wen & Scott, 2001)) although one other P450 (CYP4C1) has been cloned from another cockroach species, *Blaberus discoidalis* (Bradfield *et al*., 1991). In this paper, we report the cloning and expression of two new P450 cDNAs, CYP6K1 and CYP6J1, from German cockroaches.

Results and discussion

Characterization of CYP6K1 and CYP6J1 cDNAs

The full length cDNA sequence (based on clones FB2, FB6 as well as 3′ and 5′ RACEs) for MCHB (accession number: AF281328) is shown in Fig. 1. MCHB was named CYP6K1 by the P450 Nomenclature Committee. CYP6K1 has an open reading frame of 1572 nucleotides with a deduced protein of 524 amino acids (AAs) and a molecular mass of 59.6 kDa. CYP6K1 is most similar to other members of the CYP6 family (Table 1), having the highest per cent AA identities with CYP6H1 (43.3%) from *Locusta migratoria* (Winter *et al*., 1999), CYP6L1 (38.2%) from *B. germanica* (Wen & Scott, 2001), CYP6A2 (37.7%) and CYP6A4 (37.1%) from *D. melanogaster* (Adams *et al*., 2000).

The consensus sequence of MCHC (accession number: AF281325) (based on clones FC6, FC12, FC13 and FC15 as well as 3′ and 5′ RACE results) is shown in Fig. 2. MCHC was named CYP6J1. CYP6J1 has an open reading frame of 1503 nucleotides with a deduced protein of 501 AAs and a molecular mass of 57.9 kDa. CYP6J1 is most similar to other members of the CYP6 family (Table 1), having the highest per cent AA identities with CYP6K1 (37.1%), from *B. germanica*, CYP6A17 (36.1%) from *D. melanogaster* (Adams *et al*., 2000) and CYP6H1 (35.9%) from *L. migratoria* (Winter *et al*., 1999).

Both CYP6K1 and CYP6J1 are typical microsomal P450s. Their deduced AA sequences share a number of common characteristics with other members of the P450 superfamily (the underlined AAs, Figs 1 and 2): the hydrophobic N-terminal region, the conserved signature motif FxxGxxxCxG in the heme binding region, the consensus sequence (A/G)GxxT) within the I-helix region, the charge pair consensus (ExxR) within the K-helix, the consensus (WxxxR) in the C-helix and the consensus (aromatic)xx(P/D) followed four residues later by PERF (Nelson, 1998). Instead of a conserved P residue in the position 16 residues from T in the I-helix is a Q residue in CYP6J1 (Fig. 2). The conserved family 6 specific AAs immediately following the heme binding region (consensus: (M/L/E/K)RF(A/G)xxQ (Fogleman *et al*., 1997) appeared as LRFGYMA in CYP6K1 (Fig. 1 and Table 2) and MRFGSMQ in CYP6J1 (Fig. 2 and Table 2). Alignment of the available insect P450 protein sequences indicates that this region is less conserved than previously thought. The relatively conserved R (underlined in Table 2) residue in this region can also be found in P450s in families 9 and 28.

The sequence analyses indicate that CYP6K1 and CYP6J1 are both more similar to some members of the CYP9 family than to many members of the CYP6 family. For example, comparison of CYP6K1 and CYP6J1 with CYP6T3 from *D. melanogaster* (Adams *et al*., 2000) indicates they are 26.7 and 23.2% identical, respectively. However, they are 35.7% and 36.5% identical to CYP9E2 (Wen & Scott, unpublished), respectively. These results point out some of the contradictory aspects of the current P450 nomenclature system.

Northern and Southern analyses

When the CYP6K1 probe was used, two bands (~2.5 kb and ~2.1 kb) were detected in all life stages of German cockroaches by Northern blot analyses (Fig. 3). Both signals were hardly detectable in the eggs, but became stronger in later instars. The strongest signals were detected in the fifth and sixth instars as well as adults. These two bands were also detected in the abdomens and in the remainder of bodies of both male and female adults (Wen, 2000).

Genomic DNA (2.5 μg) was digested with four restriction enzymes (*BamH* I, *Not*I, *Sma*I and *Xba*I) which have no cutting site within the full length CYP6K1 cDNA. Southern blots probed with CYP6K1 consistently gave a single band for each enzyme (Fig. 4). These results suggest that the CYP6K1 probe is specific. Therefore, the two mRNA bands detected by Northern blots might be the result of alternative splicing.

Expression of CYP6J1 in German cockroaches was examined in different life stages as well as different body parts of male and female adults by Northern blots. When probed with CYP6J1, no mRNA signal was detected in any of these samples (10 μg of total RNA was used). Given that CYP6L1 (Wen & Scott, 2001) and CYP6K1 mRNA signals were readily detectable with the same amount of total RNA, we conclude that CYP6J1 is expressed at much lower levels than the above mentioned P450s.

Although gene expression patterns can provide valuable clues about the biological role of the gene, our results with CYP6K1 and CYP6J1 offer very limited information. CYP6K1 is expressed in all life stages and in both sexes of adult German cockroaches. This is similar to the expression of several other insect P450s (refs in (Scott *et al*., 1998)).

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AA                                                                                        NT

                           ttatttagttcttgtgaaacactggcttactgctgccaactgagaaatatcacaggtgaacc      62
     ATGGTGACCATCACAGGCTGTGCACTCTGTGATGCATTGGTCATCCTTGCGACACTGATAGTTGCAGCATACCTTTACTATGCTGTTAGA   152
  1   M  V  T  I  T  G  C  A  L  C  D  A  L  V  I  L  A  T  L  I  V  A  A  Y  L  Y  Y  A  V  R

     TTTACATACTGGAAGCGCAAGGGTGTGGTAAATCCAAAACCATTGCCAGTTTTTGGAAACTTTCTTCCATCTGTACTACAGAAACGATCT   242
 31   F  T  Y  W  K  R  K  G  V  V  N  P  K  P  L  P  V  F  G  N  F  L  P  S  V  L  Q  K  R  S

     CCTGGTCAAATATTGTGGGATATCTACAAAGCAGCAGAGGCACCCTTTGTTGGATTCTATATCTTTGCAAGACCTGCAATTCTAATAAAA   332
 61   P  G  Q  I  L  W  D  I  Y  K  A  A  E  A  P  F  V  G  F  Y  I  F  A  R  P  A  I  L  I  K

     GATCCAAATATAATAAAACATGTATTAGTAAAGGATTTCAATGCATTTTCTGATCGTCATGCATCAGCAGCAGAGAGTGACACTTTGGGA   422
 91   D  P  N  I  I  K  H  V  L  V  K  D  F  N  A  F  S  D  R  H  A  S  A  A  E  S  D  T  L  G

     TCTCAGAATTTATTCACATTGAATGGAGCACCATGGAAATACCTTCGTGTGAAGCTTTCTCCCACATTCACATCTGGACGTATGAAGAAA   512
121   S  Q  N  L  F  T  L  N  G  A  P  W  K  Y  L  R  V  K  L  S  P  T  F  T  S  G  R  M  K  K

     ATGTACCCTCTTGTAGAAAGCTGTGCAAAACAGCTACAGGATTATCTGAAGGAAAATTGTAATACTAAAGCCATTGAGGTGAAAGAGACG   602
151   M  Y  P  L  V  E  S  C  A  K  Q  L  Q  D  Y  L  K  E  N  C  N  T  K  A  I  E  V  K  E  T

     ACAGCAAAATATGCCACAGATGTGATATCTACATGTGCCTTTGGAATTGAGAGCAACTCTCTTAAAGACCCTAATGCTGAATTTCGAGAA   692
181   T  A  K  Y  A  T  D  V  I  S  T  C  A  F  G  I  E  S  N  S  L  K  D  P  N  A  E  F  R  E

     TTTGGAAGGAAGATATTTGAGTTCACAAGGTACCGTACATTTGAAGTGATGGCATTGTTCTTTAGTCCAGGACTTGTCAAGTTTCTGAAT   782
211   F  G  R  K  I  F  E  F  T  R  Y  R  T  F  E  V  M  A  L  F  F  S  P  G  L  V  K  F  L  N

     GGTAATTTCTTCACCAAGGAAACTACTGAATTCCTCAGGAAGGTATTTTGGGACACCATCAATTTCAGAGAGTCGAACAAAATTTCAAGA   872
241   G  N  F  F  T  K  E  T  T  E  F  L  R  K  V  F  W  D  T  I  N  F  R  E  S  N  K  I  S  R

     GATGACTTTATGGACTTACTCATACAGCTGAAGAATAAAGGCACTATTGATAATGAAGATGGAGAGGTCACTGAAAAAGTTGATAAAATT   962
271   D  D  F  M  D  L  L  I  Q  L  K  N  K  G  T  I  D  N  E  D  G  E  V  T  E  K  V  D  K  I

     GATAAAGATTCCCATCTGTTTGAATTCACTGGAGATAATCTCGTATCACAGCCTGCTCTTTTCTTTACTGCTGGCTTTGAAACAAATGCA  1052
301   D  K  D  S  H  L  F  E  F  T  G  D  N  L  V  S  Q  P  A  L  F  F  T  A  G  F  E  T  N  A

     ACAACATTGAGCTTCACATTGTATGAGTTGTCACTCCAACCTGACCTCCAGAACCGTCTTAGATCTGAGATTGCAGGAGTCATGAAAACA  1142
331   T  T  L  S  F  T  L  Y  E  L  S  L  Q  P  D  L  Q  N  R  L  R  S  E  I  A  G  V  M  K  T

     AGTAATGGTAAACCTACATATGAAGATGTGTTTGGTATGCCATACTTGCATATGGTTGTGTCGGAAACACTACGGAAGTACCCTCCTCTG  1232
361   S  N  G  K  P  T  Y  E  D  V  F  G  M  P  Y  L  H  M  V  V  S  E  T  L  R  K  Y  P  P  L

     CCACTATTGGACAGAGTGTGTCTGCAAGACTACAAAGTACCTGGAACAGACCTCATAATAGAGCGAGATACCCCAGTGTTCATTGCACTG  1322
391   P  L  L  D  R  V  C  L  Q  D  Y  K  V  P  G  T  D  L  I  I  E  R  D  T  P  V  F  I  A  L

     CTTGGTTTACATCGTGATCCACAGTATTATCCTAACCCCGAACGCTATGATCCAGAGCGATTCTCAGAGGAAAACAAAAGACAAAGGAAG  1412
421   L  G  L  H  R  D  P  Q  Y  Y  P  N  P  E  R  Y  D  P  E  R  F  S  E  E  N  K  R  Q  R  K

     GCCTATACGTATTTGCCCTTTGGAGAGGGTCCCCATAACTGCATTGGACTCCGATTTGGATACATGGCTGTAAAGACTGCTTTGGTCCAC  1502
451   A  Y  T  Y  L  P  F  G  E  G  P  H  N  C  I  G  L  R  F  G  Y  M  A  V  K  T  A  L  V  H

     ATGTTGGCAGAATTTGAAGTGAAGCCATGCAAGGATACACCAATACCATTGGAACTCAGTACTAGATCGAGTGTATTGGCGACAACATCA  1592
481   M  L  A  E  F  E  V  K  P  C  K  D  T  P  I  P  L  E  L  S  T  R  S  S  V  L  A  T  T  S

     GGGATACCACTCACTTTTGTAAAATCAACAGCACAAGTTTCTTgagaagttaaaatgcctttcattgcataaataatgtaatattagggc  1682
511   G  I  P  L  T  F  V  K  S  T  A  Q  V  S ***
     cttgtatttcttgccttgtttccatcctcatatacagtggaagtcataaaattcataattagtgtagagaaagtgttgggtggattatc  1772
     acatacatttttgtttgttaactctgattcccaataattattctgaagcctattttcaaacaatttcccacaatttaatttcataatcca  1862
     tttttctttatctcaaatgccctgaatattattaaacctaattccaaaaaatataaatcttaaaaatttaagaaacatggatgtgttgtg  1952
     tgaagtttgaaaattatctgtaataatttccatttcatttactatagttactacagatacaataaatagtatttgaaaatgtt  (poly A)  2035
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Figure 1. Full length cDNA sequence of CYP6K1 (accesssion number: AF281328) and its deduced amino acid sequence. Amino acids (AA) are numbered on the left and nucleotides (NT) on the right. Primers (Table 1) are thickly underlined and amino acids in conserved regions thinly underlined.

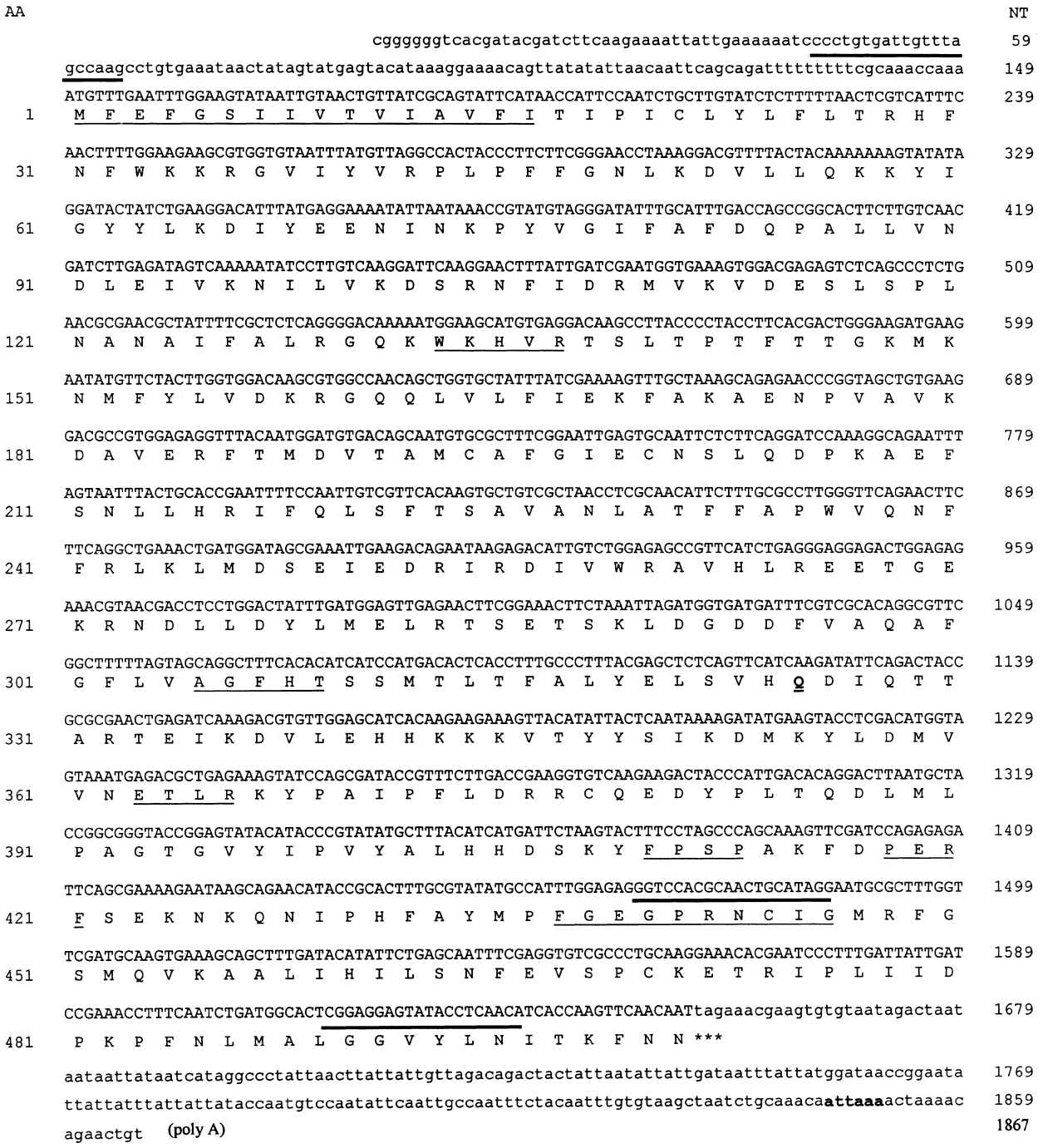

Figure 2. Full length cDNA sequence of CYP6J1 (accession number: AF281325) and its deduced amino acid sequence. Amino acids (AA) are numbered on the left and nucleotides (NT) on the right. Primers (Table 1) are thickly underlined and amino acids in conserved regions thinly underlined. The putative polyadenylation signal is indicated by bold font.

	B. germanica			*D. melanogaster*				*M. domestica*		*L. migratoria*
CYP	6K1	6J1	6L1	6A2	6A4	6A17	6T3	6A1	6D1	6H1
6K1	–	37.1	38.2	37.7	37.1	34.9	26.7	32.0	32.4	43.3
6J1	37.1	–	32.2	35.3	33.5	36.1	23.2	30.3	28.7	35.9

Table 1. Per cent identity of CYP6K1 and CYP6J1 deduced amino acid sequences from German cockroaches with other insect P450s

Table 2. Comparison of the deduced amino acid sequences in the heme binding region of some family 6 P450s

CYP	AA sequences	References
6A2	CIG**MRFGQMQ**	(Waters *et al.*, 1992)
6A13	CIG**ERFGKLQ**	(Adams *et al.*, 2000)
6D1	CIA**QRMGVIN**	(Tomita & Scott, 1995)
6D4	CIA**QRMGRIN**	(Adams *et al.*, 2000)
6G1	CIG**SRIGLLQ**	(Adams *et al.*, 2000)
6H1	CIG**MRFGLMS**	(Winter *et al.*, 1999)
6L1	CIG**LRLGLMS**	(Wen & Scott, 2000)
6K1	CIA**LRFGYMA**	Figure 1
6J1	CIA**MRFGSMQ**	Figure 2

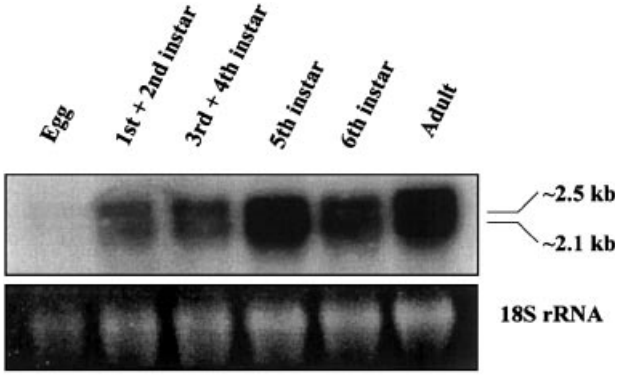

Figure 3. Expression of CYP6K1 at different life stages. Total RNA was prepared from ~1 g mixed sexes for all the life stages and 10 μg of total RNA were loaded in each lane. Northern hybridization with the CYP6K1 cDNA probe is shown in the top panel. RNA loading was standardized by ethidium bromide staining of 18S rRNA (bottom panel).

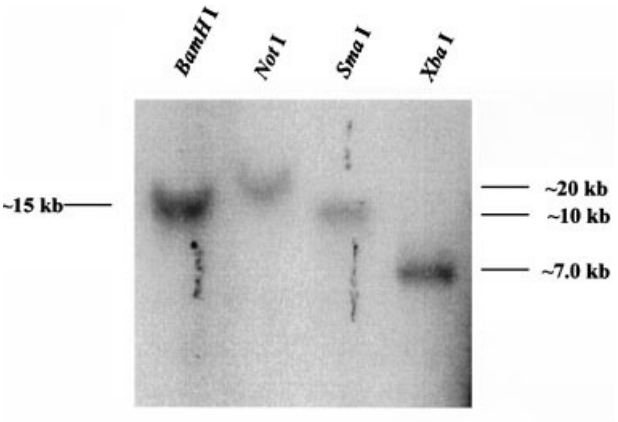

Figure 4. Southern blot analysis of *CYP6K1*. Genomic DNA (2.5 μg) was digested with four restriction enzymes.

Conversely, CYP6J1 mRNA could not be detected in any life stage. This suggests that the CYP6J1 transcript must be quite rare. By comparison, CYP6L1 mRNA, which is expressed only in the reproductive system of adult male cockroaches (Wen & Scott, 2001), could be readily detected in pools of mixed sex adults. Thus, even though mRNA was utilized as the starting material, this method is apparently not limited to the cloning of abundant P450s. Future studies employing either a suitable expression system and/or the use of isoform specific antisera will be necessary to determine the substrates for CYP6K1 and CYP6J1.

Table 3. Primers used for cloning cDNAs of CYP6K1 (MCHB) and CYP6J1 (MCHC) from German cockroaches

CYP	Primer name	Sequences (5′–3′)	Positions
6K1	MCHBS2	AGTTCTTGTGAAACACTGGC	7/26
	MCHBA1	GAGTGGTATCCCTGATGTTG	1585/1604
	MCHBA7	CAAGGCAAGAAATACAAGGC	1681/1700
6J1	MCHCS3	CCCTGTGATTGTTTAGCCAAG	45/65
	MCHCA1	TGTTGAGGTATACTCCTCCG	1616/1635

Experimental procedures

Cockroaches, RNA isolation and cDNA synthesis

The Kenly strain of German cockroach (Scott *et al.*, 1990) was selected with propoxur by residue exposure in 1988 and 1995 and was renamed Baygon-R. This strain has been subsequently reared (on Purina dog chow and water at 28 °C) without insecticide exposure.

Total RNA was isolated from German cockroaches (Baygon-R strain) using 5 M guanidine thiocyanate solution (Chirgwin *et al.*, 1979). mRNA was isolated from total RNA using Oligotex suspension (QIAGEN) or directly from tissues using a QuickPrep mRNA purification kit (Pharmacia) as described by the manufacturers.

Superscript II (Gibco/BRL) was used to synthesize the first strand cDNA following the manufacturer's instructions. For the first strand cDNA synthesis, C3PT (Danielson *et al.*, 1997) was used as the primer and ~500 ng of mRNA was used as template. After RNAase H (2 units) treatment at 37 °C for 30 min, the first strand cDNA was isolated using a QIAquick PCR purification kit (QIAGEN) to remove the primers and short cDNA (eluted with 100 μl H_2O). The purified cDNA was used as template for 3′ and 5′ RACEs (Frohman *et al.*, 1988) as described below.

cDNA cloning

3′ RACE using a primer based on the heme binding region of P450s led to the isolation of six clones, MCH2, MCH4, MCH5, MCH9, MCH18 and MCH29, which encoded the C-terminus of two putative P450s, MCHB (MCH2, 4, 5, 18 and 29) and MCHC (MCH9), respectively. Based on the 3′ sequence information of MCHB, a gene specific antisense primer MCHBA1 (Table 3 and Fig. 1) was designed. Similarly, MCHCA1 (Table 3 and Fig. 2) was designed based on the 3′ sequence information of MCHC. The two gene specific primers were used to clone the 5′ cDNA sequences of MCHB and MCHC after two rounds of 5′ RACEs. All the adjacent sequences obtained by 3′ and 5′ RACEs had at least 150 bp overlap with each other. By merging the overlapping sequences from the 3′ and 5′ RACEs, two putative full-length cDNA sequences MCHBMER (for MCHB) and MCHCMER (for MCHC) were generated. More details about the procedures used for cDNA isolation using 3′ and 5′ RACE techniques were given previously (Wen & Scott, 2001).

To ensure that the fragments used to generate MCHBMER were from the same gene, a gene specific primer set (MCHBS2/MCHA7, Table 3 and Fig. 1) flanking the open reading frame of

MCHBMER was used to PCR amplify the full length cDNA of MCHB using first strand cDNA as template. Following denaturation at 95 °C for 3 min, the PCR conditions were set for the first ten cycles in the order of 94 °C for 1 min, 45 °C for 1.5 min and 72 °C for 5 min. The next twenty-five cycles followed immediately using the same conditions except that the annealing temperature was increased to 55 °C. The PCR reaction ended with a final extension for 15 min at 72 °C. A single PCR product (1.7 kb) was observed following agarose gel electrophoresis. The band was cut and DNA purified and cloned into pCR®2.1 vector (Invitrogen). Two positive clones (FB2 and FB6) were identified by colony PCR (Gussow & Clackson, 1989) using the MCHBS2/MCHBA7 primer set. The inserts of FB2 and FB6 were entirely sequenced.

Nearly the whole open reading frame of MCHC was cloned after two rounds of PCR amplifications. The products of the first round PCR using MCHCA1/AAP (Wen & Scott, 2001) as the primer set and first strand cDNA as template served as templates for the second round PCR using MCHCA1/MCHCS3 (Table 1 and Fig. 2) as the primer set. The PCR conditions were the same as described for cloning CYP4C21 (Wen, 2000). Four positive clones (FC6, FC12, FC13 and FC15) with inserts of 1.6 kb were identified by colony PCR using the MCHCA1/MCHCS3 primer set. The inserts of these positive clones were entirely sequenced.

Northern and Southern blot analyses

Using MCHBS2/MCHBA7 as the primer set and plasmids from FB2 clone as template a 1.7 kb cDNA fragment of CYP6K1 was PCR amplified. Similarly, a 1.6 kb cDNA fragment of CYP6J1 was PCR amplified using MCHCS3/MCHCA1 as the primer set with plasmids from FC6 clone as template. These fragments were used as templates to prepare probes for Northern and Southern hybridizations (Wen & Scott, 2000) as described below.

For Northern blots, 10 μg of total RNA was fractionated on 1% denaturing formaldehyde agarose gel containing ethidium bromide. After washing in distilled water for about 3–4 h with several changes, the RNA was transferred to a GeneScreen Plus® hybridization transfer membrane (NEN™ Life Science Products, Inc.). Equal loading was monitored by comparing the density of the 18S ribosomal RNA (rRNA) band (Savonet *et al*., 1997; Spiess & Ivell, 1998) on the agarose gel before transfer and/or on the membrane after transfer under UV. The above mentioned PCR fragments were labelled with [α-^{32}P] dCTP using the RadPrame labelling system (Gibco/BRL), and used as a hybridization probe. The membrane was hybridized to the probe in QuickHyb solution (Stratagene) at 68 °C. Washing was done at high stringency (i.e. three 15 min washes with 2 × SSC + 0.1% SDS at room temperature, followed by a 30-min wash with 0.2 × SSC + 0.1% SDS at 60 °C). The membrane was air dried and exposed to a BioMax MR film (Eastman Kodak). All Northern analyses were repeated at least three times with independent preparations of RNA.

Genomic DNA was isolated from the abdomens of male adult German cockroaches of Baygon-R strain using standard methods (Sambrook *et al*., 1989). Southern blots were performed by standard methods (Sambrook *et al*., 1989) with some modifications. Four restriction enzymes (*Bam*HI, *Not*I, *Sma*I and *Xho*I), having no cutting site within the cDNA sequence were used to digest 2.5 μg of genomic DNA overnight as described by the manufacturer (Gibco/BRL). Fifty units of each enzyme were used for each reaction in a volume of 100 μl. The digested DNA was then ethanol precipitated and fractionated by electrophoresis on 1% agarose gel containing ethidium bromide. After electrophoresis, the gel was placed in 0.25 M HCl with gentle shaking until 10 min after the dyes had changed colour. After being rinsed in H_2O, the gel was denatured in a solution containing 1.5 M NaCl and 0.5 M NaOH with gentle shaking for 30 min. The gel was then rinsed with H_2O and neutralized in a solution containing 1.5 M NaCl, 0.5 M Tris-HCl (pH 7.2) and 0.001 M EDTA. After rinsing in H_2O, the DNA was transferred to a GeneScreen Plus® membrane (NEN™ Life Science Products, Inc.). Probe preparation, membrane hybridization and film exposure were performed exactly as described above. Southern analyses were repeated three times.

Sequence analyses

Sequences analyses were carried out using the Clustal method of MEGALIGN program (DNASTAR Inc., Madison, Wisconsin).

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References

Adams, M.D. *et al.* (2000) The genome sequence of *Drosophila melanogaster*. *Science* **287**: 2185–2195.

Agosin, M. (1985) Role of microsomal oxidations in insecticide degradation. *Comprehensive Insect Physiology, Biochemistry, and Pharmacology* (Kerkut, G.A. and Gilbert, L.I., eds), pp. 647–712. Pergamon, New York.

Agosin, M., Michaeli, D., Miskus, R., Nagasawa, S. and Hoskins, W.M. (1961) A new DDT-metabolizing enzyme in the German cockroach. *J Econ Entomol* **54**: 340–342.

Appel, A.G. (1995) *Blattella* and related species. *Understanding and Controlling the German Cockroach* (Rust, M.K., Owens, J.M. and Reierson, D.A., eds), pp. 1–19. Oxford Press, New York.

Berenbaum, M.R. (1999) Animal–plant warfare: molecular basis for cytochrome P450-mediated natural adaptation. *Molecular Biology of the Toxic Response* (Puga, A. and Wallace, K.B., eds), pp. 553–571. Taylor & Francis, Philadelphia.

Bradfield, J., Lee, Y. and Keeley, L. (1991) Cytochrome P450 family 4 in a cockroach: molecular cloning and regulation by hypertrehalosemic hormone. *Proc Natl Acad Sci* **88**: 4558–4562.

Brenner, R.J. (1995) Economics and medical importance of German cockroaches. *Understanding and Controlling the German Cockroach* (Rust, M.K., Owens, J.M. and Reierson, D.A., eds), pp. 77–92. Oxford Press, New York.

Chapman, M.D., Vailes, L.D., Hayden, M.L., Platts-Mills, T.A.E. and Arruda, L.K. (1997) Cockroach allergens and their role in asthma. *Allergy and Alleric Diseases* (Kay, A.B., ed.), pp. 942–951. Blackwell Science Ltd, London, UK.

Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J. and Rutter, W.J. (1979) Isolation of biologically active ribonucleic acid from sources enriched in ribonucease. *Biochem* **18**: 5294–5299.

Consortium, C. *elegans*. (1998) Genome sequence of the nematode *C. elegans*: a platform for investigating biology. *Science* **282**: 2012–2018.

Danielson, P.B., MacIntyre, R.J. and Fogleman, J.C. (1997) Molecular cloning of a family of xenobiotic-inducible drosophilid cytochrome P450s: evidence for involvement in host-plant allelochemical resistance. *Proc Natl Acad Sci USA* **94**: 10797–10802.

Feyereisen, R. (1999) Insect P450 enzymes. *Ann Rev Entomol* **44**: 507–533.

Fogleman, J.C., Danielson, P.B. and MacIntyre, R.J. (1997) The molecular basis of adaptation in *Drosophila.* The role of cytochrome P450s. *Evol Biol* **30**: 15–77.

Frohman, M.A., Dush, M.K. and Martin, G.R. (1988) Rapid production of full-length cDNAs from rare transcripts: amplification using a single gene-specific oligonucleotide primer. *Proc Natl Acad Sci (USA)* **85**: 8998–9002.

Gussow, D. and Clackson, T. (1989) Direct clone characterization from plaques and colonies by the polymerase chain reaction. *Nucl Acids Res* **17**: 4000.

Hemingway, J., Dunbar, S.J., Monro, A.G. and Small, G.J. (1993a) Pyrethroid resistance in German cockroaches (Dictyoptera: Blattelidae): resistance levels and underlying mechanisms. *J Econ Entomol* **86**: 1631–1638.

Hemingway, J., Small, G.J. and Monro, A.G. (1993b) Possible mechanisms of organophosphorus and carbamate insecticide resistance in German cockroaches (Dictyoptera: Blattelidae) from different geographical areas. *J Econ Entomol* **86**: 1623–1630.

Herrick, G.W. (1914) *Insects Injurious to the Household*. The MacMillan Company, New York.

Hodgson, E. (1985) Microsomal mono-oxygenases. *Comprehensive Insect Physiology Biochemistry and Pharmacology* (Kerkut, G.A. and Gilbert, L.C., eds), pp. 647–712. Pergamon Press, Oxford.

Khan, M.A.Q. and Matsumura, F. (1972) Induction of mixed-function oxidase and protein synthesis by DDT and dieldrin in German and American cockroaches. *Pestic Biochem Physiol* **2**: 236–243.

Lee, C., Yap, H. and Chong, N. (1996) Insecticide resistance and synergism in field collected German cockroaches (Dictyoptera: Blattellidae) in Peninsular Malaysia. *Bull Entomol Res* **86**: 675–682.

Mahmood, T.E.F.H.Y., Kasai, Y. and Motoyama, N. (1993) *In vitro* studies on the mechanism of pyrethroid resistance in the German cockroach. *J Pestic Sci* **18**: 253–261.

Matsumura, F., Telford, J.N. and Hayashi, M. (1967) Effect of sesamex upon dieldrin resistance in the german cockroach. *J Econ Entomol* **60**: 942–944.

Nelson, D.R. (1998) Cytochrome P450 nomenclature. *Meth Molec Biol* **107**: 15–24.

Porter, T.D. and Coon, M.J. (1991) Cytochrome P450: multiplicity of isoforms, substrates, and catalytic and regulatory mechanisms. *J Biol Chem* **266**: 13469–13472.

Ross, M.H. and Cochran, D.G. (1975) The German cockroach, *Blattella germanica*. *Handbook of Genetics* (King, R.C., ed.), pp. 35–62. Plenum Press, New York.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: a Laboratory Manual*. Cold Spring Harbor Laboratory Press, New York.

Savonet, V., Maenhaut, C., Miot, F. and Pirson, I. (1997) Pitfalls in the use of several 'housekeeping' genes as standards for quantitation of RNA: the example of thyroid cells. *Anal Biochem* **247**: 165–167.

Scharf, M., Kaakeh, W. and Bennett, G. (1997) Changes in an insecticide-resistant field population of German cockroach (Dictyoptera: Blattellidae) after exposure to an insecticide mixture. *J Econ Entomol* **90**: 38–48.

Scharf, M.E., Neal, J.J., Marcus, C.B. and Bennett, G.W. (1998) Cytochrome P450 purification and immunological detection in an insecticide resistant strain of German cockroach (*Blattella germanica* L.). *Insect Biochem Molec Biol* **28**: 1–9.

Scott, J.G. (1999) Molecular basis of insecticide resistance: cytochromes P450. *Insect Biochem Molec Biol* **29**: 757–777.

Scott, J.G., Cochran, D.G. and Siegfried, B.D. (1990) Insecticide toxicity, synergism, and resistance in the German cockroach, *Blattella germanica* (Dictyoptera: Blattellidae). *J Econ Entomol* **83**: 1698–1703.

Scott, J.G., Liu, N. and Wen, Z. (1998) Insect cytochromes P450: Diversity, insecticide resistance and tolerance to plant toxins. *Comp Biochem Physiol* **121C**: 147–155.

Scott, J.G. and Wen, Z. (1997) Toxicity of fipronil to susceptible and resistant strains of German cockroaches (Dictyoptera: Blattellidae) and house flies (Diptera: Muscidae). *J Econ Entomol* **90**: 1152–1156.

Siegfried, B.D. and Scott, J.G. (1991) Mechanisms responsible for propoxur resistance in the German cockroach, *Blattella germanica* (L.). *Pestic Sci* **33**: 133–146.

Siegfried, B.D., Scott, J.G., Roush, R.T. and Zeichner, B.C. (1990) Biochemistry and genetics of chlorpyrifos resistance in the German cockroach, *Blattella germanica*. *Pestic Biochem Physiol* **38**: 110–121.

Spiess, A.-N. and Ivell, R. (1998) Normalization of RNA hybridization signals by means of SYBR Green II-stained 28S or 18S ribosomal RNA and phosphor imager. *Biotechniques* **26**: 46–50.

Tomita, T. and Scott, J.G. (1995) cDNA and deduced protein sequence of *CYP6D1*: the putative gene for a cytochrome P450 responsible for pyrethroid resistance in house fly. *Insect Biochem Molec Biol* **25**: 275–283.

Valles, S. (1998) Toxicological and biochemical studies with field populations of the German cockroach, *Blattella germanica*. *Pestic Biochem Physiol* **62**: 190–200.

Valles, S.M., Yu, S.J. and Koehler, P.G. (1994) Detoxifying enzymes in adults and nymphs of the German cockroach: evidence for differnet microsomal monooxygenase systems. *Pestic Biochem Physiol* **49**: 183–190.

Waters, L.C., Zelhof, A.C., Shaw, B.J. and Ch'ang, L.Y. (1992) Possible involvement of the long terminal repeat of transposable element 17.6 in regulating expression of an insecticide resistance-associated P450 gene in *Drosophila*. *Proc Natl Acad Sci USA* **89**: 4855–4859.

Wen, Z. (2000) *Cloning of five novel cytochrome P450s and related pseudogenes from German cockroaches,* Blattella germanica*: implications for function, nomenclature, expression studies and molecular evolution.* PhD Dissertation, Cornell University, Ithaca, NY.

Wen, Z. and Scott, J.G. (1997) Cross-resistance to imidacloprid in strains of German cockroach (*Blattella germanica*) and house fly (*Musca domestica*). *Pestic Sci* **49**: 367–371.

Wen, Z. and Scott, J.G. (2001) Cytochrome P450 CYP6L1 is specifically expressed in the reproductive tissues of adult male German cockroaches, *Blattella germanica* (L.). *Insect Biochem Molec Biol* in press.

Winter, J., Bilbe, G., Richener, H., Sehringer, B. and Kayser, H. (1999) Cloning of a cDNA encoding a novel cytochrome P450 from the insect *Locusta migratoria*: CYP6H1, a putative ecdysone 20-hydroxylase. *Biochem Biophys Res Comm* **259**: 305–310.

Wu, D., Scharf, M.E., Neal, J.J., Suiter, D.R. and Bennett, G.W. (1998) Mechanisms of fenvalerate resistance in the German cockroach, *Blattella germanica* (L.). *Pestic Biochem Physiol* **61**: 53–62.