Am. J. Trop. Med. Hyg., 98(3), 2018, pp. 710–716
doi:10.4269/ajtmh.17-0404

cDNA Isolation and Expression of Nicotinamide Adenine Dinucleotide Phosphate–Dependent Cytochrome P450 Reductase Gene in the Chagas Disease Vector *Triatoma infestans*

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Abstract. Pyrethroid resistance has been detected in *Triatoma infestans* (Hemiptera: Reduviidae), which was atributed to target site insensitivity and increased oxidative metabolism of the insecticide by cytochrome P450s. Nicotinamide adenine dinucleotide phosphate (NADPH) cytochrome P450 reductase (CPR) plays an essential role in transferring electrons from NADPH to the P450-substrate complex. In this study, the full length *CPR* cDNA of *T. infestans* was isolated and gene expression was determined by quantitative polymerase chain reaction. The open reading frame is 2,046 bp long, encoding a protein of 682 amino acids. Amino acid sequence analysis indicates that the *T. infestans* CPR and the putative *Rhodnius prolixus* and *Triatoma dimidiata* CPRs present conserved ligand-binding domains. Congruent with a previous study of our laboratory, in which the expression of three cytochrome *P450* genes (*CYP4EM7*, *CYP3085B1*, and *CYP3092A6* genes) was induced by deltamethrin, the levels of *T. infestans CPR* mRNA were upregulated in the fat body of fifth instar nymphs after topical application of deltamethrin. Besides, as it was observed in the *CYP4EM7* gene, it was detected overexpression of the *CPR* gene in the most resistant strain of *T. infestans* included in the study. These results suggest that CPR plays an essential role in P450-mediated resistance of *T. infestans* to insecticides.

INTRODUCTION

Chagas disease, also known as American trypanosomiasis, is caused by the parasite *Trypanosoma cruzi*, which is transmitted to humans by vectors of the subfamily Triatominae (Hemiptera: Reduviidae). The disease and its vectors are extensively distributed from southern United States of America to southern Argentina and Chile (latitude 42°N to latitude 46°S). Chagas disease is a serious public health problem in Latin America, where about eight million people are estimated to be infected with *T. cruzi* and more than 25 million people are at risk of contracting the infection.[1,2]

Triatoma infestans is the main vector of Chagas disease in the Southern Cone of Latin America between latitudes 10°S and 46°S, where it is primarily restricted to domestic and peridomestic environments. Pyrethroid insecticides have been the major means to control the vector populations. However, vector control has proven to be difficult because of the variability and extension of endemic areas, and the difficulties to implement sustained entomological vigilance to prevent the recovery of treated bug populations.[3] In addition, during the past years, pyrethroid resistance in the vector insect has been reported as one of the main explanations of the unsatisfactory control observed.[4] Analyses of genes involved in insecticide resistance in insect vectors of diseases as *T. infestans* are of considerable interest. Particularly, the importance of studying the expression patterns of genes involved in their resistance lies in its potential for exploitation in novel insect control strategies.

Results of a work focused on detecting the frequency of two knockdown resistance (Kdr) mutations in different *T. infestans* populations[5] suggested that Kdr mutations and other insecticide resistance mechanism as enhanced metabolism and reduced penetrance would be involved in pyrethroid resistance in *T. infestans*.[6,7] The single base-pair substitutions in the sodium channel gene can reduce the binding of pyrethroid insecticides in the target site of the voltage-gated sodium channel on the insect nervous system. However, in some insects as in the malaria vector *Anopheles gambiae*, pyrethroid resistance has been attributed to a combination of target site insensitivity and increased oxidative metabolism of the insecticide, catalyzed by cytochrome P450s.[8]

Cytochrome P450 monooxygenases (cytochrome P450s) are involved in the oxidative metabolism of various endogenous and exogenous substrates.[9–11] They play a predominant role in the metabolism of insecticides, which often results in the development of insecticide resistance in insect populations.[12] Increases of expression of cytochrome *P450* genes at transcriptional level are often considered responsible for increasing the metabolism of insecticides and seems to be a common phenomenon in the evolution of resistance development in insects.[13–17] In a previous work carried out in our laboratory, the cDNA sequences of three cytochrome *P450* genes (*CYP4EM7*, *CYP3085B1*, and *CYP3092A6*) were identified in *T. infestans*. The mRNA levels of the cytochrome *P450* genes identified were obtained from total RNA extracted from pools of fat body collected from individuals of different resistant and susceptible strains of *T. infestans*, and at different interval times after the topical application of the lethal doses 50% (LD_{50}) of deltamethrin on the ventral abdomen of insects belonging to the different populations analyzed. The expression of the three cytochrome *P450* genes isolated was induced by deltamethrin in the susceptible and resistant populations included in this study and overexpression of the *CYP4EM7* gene was detected in the most resistant strain of *T. infestans*.[18] The constitutively increased expression and induction of P450s are thought to be responsible for increased levels of detoxification of insecticides in insects and would be involved in the development of insecticide resistance.[10,14,17,19–24] The results suggested that the *CYP4EM7*, *CYP3085B1*, and *CYP3092A6* genes would be involved in the detoxification of deltamethrin and the evolution of resistance to this insecticide in *T. infestans* populations.

Cytochrome P450s constitute a large superfamily of hemcontaining monooxygenases. All P450 monooxygenation

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reactions occurring in the endoplasmic reticulum require electrons supplied by nicotinamide adenine dinucleotide phosphate (NADPH) cytochrome P450 reductase (CPR), a diflavin enzyme that contains flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) cofactors that shuttle electrons from the reduced form of NADPH through a series of redox-coupled reactions to P450.[25] In addition to cytochrome P450s, CPR also serves as the electron donor protein for several oxygenase enzymes found in the endoplasmic reticulum of most eukaryotic cells.[26–29]

Although multiple *P450* genes have been found in the genomes of insects, typically only one *CPR* gene exists in each insect genome.[30] Because the identification and analysis of *P450* genes involved in insecticide resistance can be complex, it is important to analyze the *CPR* gene, which encodes for an enzyme essential for the activity of cytochrome P450s and is considered to be a vital part of P450-mediated insecticide resistance. The role of CPR in P450-mediated insecticide metabolism has been shown in some insects, but CPR has not been identified and characterized in *T. infestans*.[31–33]

In this study, we report the cDNA nucleotide sequence and deduced amino acid sequence for CPR in *T. infestans*, and we explore the expression pattern at the mRNA level of the *CPR* gene at different interval times after the application of deltamethrin. We also perform a comparative analysis of transcriptional expression of this gene in susceptible and resistant strains of *T. infestans*.

MATERIALS AND METHODS

Insects. Four laboratory strains of *T. infestans* were analyzed. The insects from two of these strains were provided by the Centro de Referencia de Vectores of the Servicio Nacional de Chagas de Córdoba (Córdoba province, Argentina). One belonging to a colony susceptible to deltamethrin (Centro de Referencia de Vectores [CRV]-susceptible strain) that was originated in 2006 from insects collected in the locality of Chuña (Department of Ischilín, Córdoba province, Argentina) (30°28′S, 64°40′W) and other belonging to a second generation of a colony resistant to deltamethrin (CRV-resistant strain) that was originated from individuals collected in the locality of Mataral (Department of Santa Cruz, Bolivia) (18°06′S, 64°12′W). The insects from the other two strains were provided by the Centro de Investigaciones de Plagas e Insecticidas (Buenos Aires province, Argentina). They consisted of the first laboratory generation of a colony susceptible to deltamethrin (Centro de Investigaciones de Plagas e Insecticidas [CIP]-susceptible strain) that was originated from insects collected in the locality of Los Quirquinchos (25°07′S, 61°22′W) and of a colony resistant to deltamethrin (CIP-resistant strain) that was originated from specimens collected in the locality of La Esperanza (26°03′S, 60°27′W), both localities belonging to the Department of General Güemes (Chaco province, Argentina). The laboratory colonies were reared at 28°C ± 1°C at a relative humidity of 60–70% with a 6-hour light/18-hour dark cycle and fed once every 2 weeks on restrained chickens.

For the *CPR* cDNA sequence identification, fifth instar nymphs from the CRV-susceptible strain were fed after 7 days of moulting and their fat bodies were extracted 7 days later. For expression analysis of the *CPR* gene, fifth instar nymph fat bodies from the CRV-susceptible, CRV-resistant, CIP-susceptible, and CIP-resistant strains were extracted under the same conditions. Each sample was a pool of tissue from three specimens. Besides, fifth instar nymphs from the CRV-susceptible strain were fed after 7 days of moulting and 7 days later was performed a topical application of 1 μL of the LD_{50} of deltamethrin on the ventral abdomen of insects.[18] Subsequently, pools of fat bodies from three fifth instar nymphs were collected at different time intervals after the application of insecticide. For control, topical applications with 1 μL of acetone were performed. In all cases, the topical application was carried out at the same time of day, and the tissue was dissected under aseptic conditions and stored in liquid nitrogen until used for RNA extraction.

Isolation of total RNA and cDNA synthesis. Extraction of total RNA and cDNA synthesis were carried out as indicated in previous works.[18,34–36] For *CPR* cDNA identification, total RNA was extracted from pools of fat bodies from five fifth instar nymphs and for quantitative polymerase chain reaction (qPCR), total RNA was isolated from pools of fat bodies from three fifth instar nymphs.

Amplification and sequencing of *CPR* gene. Partial *CPR* cDNA sequence was amplified by the polymerase chain reaction (PCR), using unspecific primers (F3CPR and R3CPR) (Table 1) designed from the *CPR* gene coding region that was identified in the genome of *Rhodnius prolixus* (GenBank accession number ACPB03018078.1). The PCR was performed as described elsewhere.[18,34–36] To obtain the complete open reading frame (ORF) of CPR, the rapid amplification of cDNA ends (RACE) method was performed by using the commercial kit ExactSTART Eukaryotic mRNA 5′- & 3′-RACE (Epicentre, Madison, WI).[34] Specific primers for isolation of 3′ cDNA end and 5′ cDNA end are shown in Table 1. After the PCR products were electrophoresed, bands corresponding to the expected sizes were excised from the agarose gel and purified using QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany). The PCR products were then cloned into the pCR4-TOPO TA cloning vector (Invitrogen, Carlsbad, CA) before being sequenced in an ABI 3130XL automated DNA sequencer (Applied Biosystems, Foster City, CA).

cDNA sequence analysis. The *CPR* cDNA sequence of *T. infestans* was compared with those of other insects deposited in GenBank using the "BLAST-N" tool available on the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nhi.gov) and the program Clustal W2 (http://www.ebi.ac.uk/Tools/clustalw2/index.html). The *T. infestans* amino acid CPR sequence was deduced from the corresponding cDNA using the translation tool from the ExPASy Proteomics website (http://www.expasy.org/tools/dna.html).

Phylogenetic relationship. Phylogenetic analysis was conducted to investigate evolutionary relationships among the putative CPR protein identified in *T. infestans* and other selected sequences reported in the literature from *Anopheles funestus* (ABO77954.1), *A. gambiae* (AAO24765.1), *Apis mellifera* (XP_006569769.1, XP_016769012.1), *Atta cephalotes* (XP_012055902.1), *Bactrocera dorsalis* (NP_001306717.1), *Bemisia tabaci* (AGT15701.1), *Bombus impatiens* (XP_012246140.1, XP_012246141.1, XP_012246143.1), *Bombus terrestris* (XP_012173161.1), *Cerapachys biroi* (EZA61651.1), *Cimex lectularius* (NP_001303631.1), *Culex quinquefasciatus* (XP_001865801.1), *Dendroctonus armandi* (ALC78506.1), *Dendroctonus ponderosae* (AFI45002.1), *Drosophila melanogaster* (NP_477158.1, NP_723173.1, NP_001260128.1, NP_001260129.1), *Drosophila mettleri* (AAB48964.1), *Fopius arisanus* (XP_011306347.1, XP_011306348.1), *Helicoverpa armigera* (ADK25060.1), *Laodelphax striatella* (AHM93010.1), *Monomorium pharaonis*

TABLE 1
Sequences of all primers used in this study

Name	Sequences	Function
Unspecific primers (for RT-PCR)		
F3CPR	5′-GACAATTGAAGGATCTAAAATGCG-3′	Sense
R3CPR	5′-CTTTCTGCATCCAAAATATAGG-3′	Antisense
RACE primers (for 3′ cDNA isolation)		
CPR3F	5′-GTGTGGCTACATCATGGTTAGC-3′	Sense
CPR3F1	5′-GACCTGGTACAGGTTTAGCTCC-3′	Sense
PCR-Primer2	5′-TAGACTTAGAAATTAATACGACTCACTATAGGCGCGCCACCG-3′	Antisense
RACE primers (for 5′ cDNA isolation)		
PCR-Primer1	5′-TCATACACATACGATTTAGGTGACACTATAGAGCGGCCGCCTGCAGGAAA-3′	Sense
CPR5R	5′-TTAACTCCGAGTAGCGTGCC-3′	Antisense
CPR5RN	5′-CCGAGTAGCGTGCCAAACT-3′	Antisense
Cloning		
M13F	5′-GTAAAACGACGGCCAG-3′	Sense
M13R	5′-CAGGAAACAGCTATGAC-3′	Antisense
qPCR primers (for real time PCR)		
CPRRTF	5′-AACACAGATGAGGATTCGAGTAAAAA-3′	Sense
CPRRTR	5′-GTGTGCGTGGATTGGATGTTAT-3′	Antisense
QβactinaF	5′-CCCCTTTCAGTGAGGATCTTCA-3′	Sense
QβactinaR	5′-CGCCATCCTTCGATTGGA-3′	Antisense

PCR = polymerase chain reaction; qPCR = quantitative PCR; RACE = rapid amplification of cDNA end; RT-PCR = reverse transcription PCR.

(XP_012541364.1), *Musca domestica* (NP_001273818.1), *Nilaparvata lugens* (AHB59865.1), *Panonychus citri* (AHZ12899.1), *Papilio polytes* (XP_013146229.1, XP_013146230.1), *Papilio xuthus* (XP_013182134.1), *Pogonomyrmex barbatus* (XP_011643152.1, XP_011643153.1), *Spodoptera exigua* (ADX95746.1), *Spodoptera littoralis* (AFP20584.1), *Tenebrio molitor* (AKZ17715.1), and *Tribolium castaneum* (XP_008197839.1). Multiple alignment of sequences was performed using the multiple alignment program Clustal W in MEGA version 6.06.[37] Tree construction was performed using the maximum likelihood method, using MEGA version 6.06 software.[37] The reliability of the trees was tested by the bootstrap procedure with 1,000 replications.

qPCR. The transcript levels of the *CPR* gene identified in *T. infestans* were measured by using qPCR as described in previous works carried out in our laboratory.[18,34–36] Specific primers (Table 1) and a Taqman probe were designed from the *CPR* cDNA sequence using Primer Express program (Applied Biosystems). The reaction conditions were of 15 minutes at 95°C, and 40 cycles of 15 seconds at 95°C and 60 seconds at 60°C. The relative copy number of *CPR* mRNA was calculated according to $2^{-\Delta\Delta CT}$.[38] The threshold cycle value difference ΔCT between *CPR* mRNA and β-*actin* mRNA of each reaction was used to normalize the level of total RNA.

Statistical analyses. For the studies involving gene expression analysis by qPCR, two independent experiments were performed and data for each point were registered by triplicate to account for intra-experimental variation. Graphs and statistical tests were performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA). One way analysis of variance with Bonferroni posttest was used for comparisons. The results were presented as mean ± standard deviation and a P value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Identification and sequence analysis of *CPR* gene. A fragment of 746 bp of *CPR* cDNA of *T. infestans* was amplified by reverse transcription PCR using unspecific primers designed from the *CPR* gene coding region that was identified in the genome of *R. prolixus* (GenBank accession number ACPB03018078.1). The procedure of rapid amplification of 3′ cDNA end (3′-RACE) allowed obtaining a segment of 452 bp, which overlapped with that fragment and contained the 3′ cDNA end of the gene. Subsequent rapid amplification of cDNA 5′ end (5′-RACE) resulted in a segment of 1,040 bp, which overlapped with the fragment of 746 bp and contained the 5′ cDNA end of the *CPR* gene. The comparative analysis of the cDNA fragments of *T. infestans* with cDNA sequences of the *CPR* gene of other insect species revealed that they were part of the *CPR* gene. A total of 2,049 bp of cDNA corresponding to CPR of *T. infestans* was sequenced, which comprises an ORF of 2,046 nucleotides encoding a protein of 682 amino acids and the stop codon thymine, adenine, and adenine (GenBank accession number KY350179). This cDNA allowed to identify in the genome of *R. prolixus*, in the reverse complementary chain between the nucleotide positions 12,036 and 26,719 of the Scaffold341 (GenBank accession number KQ034397.1), a total of 14,687 bp corresponding to the *CPR* gene that involve 14 exons encoding a protein of 682 amino acids,

FIGURE 1. Schematic representation of the *CPR* gene in *Rhodnius prolixus*. Exons are shown as shaded boxes and introns as horizontal lines. Exons and introns are labeled (5′ to 3′) with arabic and roman numerals, respectively. Their sizes in base pairs are indicated in the box (exons) and in parentheses (introns).

13 introns, and the stop codon TAA (Figure 1). Moreover, in the present work, the deduced 682 amino acid sequence from the Transcriptome Shotgun Assembly of *Triatoma dimidiata* (GenBank accession number GECL01003769.1) identified as a "putative NADP-dependent flavoprotein reductase" (GenBank accession number JAP02355.1) was recognized as *T. dimidiata* CPR.

The alignment of the deduced amino acid sequences from *CPR* cDNA of the three Chagas disease vectors of the subfamily Triatominae (*T. infestans*, *T. dimidiata*, and *R. prolixus*) is shown in the Figure 2. All functional domains involved in the binding of cofactors FMN, FAD, and NADPH were identified in the predicted CPR protein primary structure.

The deduced amino acid sequence of CPR from *T. infestans*, *T. dimidiata*, and *R. prolixus* was compared with the corresponding sequences of other hemipteran CPRs. Sequence comparisons revealed that *T. infestans* CPR was most similar to CPR from *T. dimidiata* (95.31% identity), followed by *R. prolixus* (93.24%), *C. lectularius* (82.92%), *L. striatella* (77.12%), *N. lugens* (76.79%), and *B. tabaci* (74.44%).

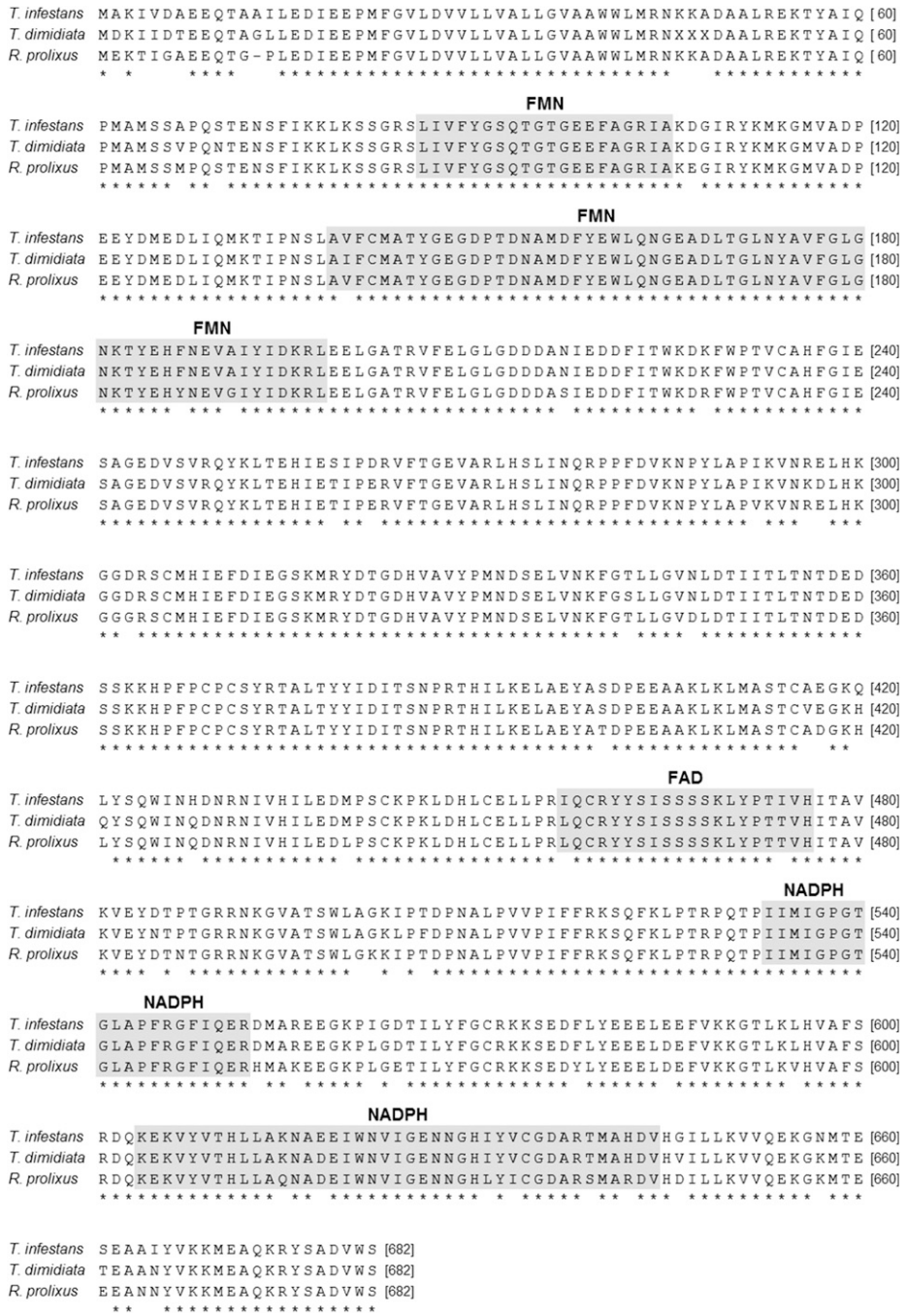

FIGURE 2. Alignment of amino acid sequences of cytochrome P450 reductases from *Triatoma infestans*, *Triatoma dimidiata*, and *Rhodnius prolixus*. Identical residues among the three sequences are marked by asterisks. The residues constituting the flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD), and nicotinamide adenine dinucleotide phosphate (NADPH) binding sites are boxed.

A phylogenetic analysis was performed based on the complete amino acid sequences of CPR of the hemipteran *T. infestans*, *T. dimidiata*, *R. prolixus*, *C. lectularius*, *L. striatella*, *N. lugens*, and *B. tabaci*, and of 24 representative species of the orders Diptera, Lepidoptera, Coleoptera, and Hymenoptera. As expected, insect CPRs from the same insect order were grouped together with significant bootstrap support (Figure 3). Within Hemiptera, *T. infestans* clustered with *T. dimidiata* with high bootstrap support (91%), as well as these two species with *R. prolixus* (100% bootstrap value). These three members of the subfamily Triatominae clustered with strong support with *C. lectularius* (100% bootstrap value), and *N. lugens* was also closely related to *L. striatella* (100% bootstrap value).

***CPR* gene expression.** The expression at transcriptional level of the *CPR* gene was analyzed in two deltamethrin susceptible strains (CRV-susceptible and CIP-susceptible strains) and two deltamethrin-resistant strains (CRV-resistant and CIP-resistant strains). The CRV-resistant strain, which was originated from individuals collected in Mataral (Department of Santa Cruz) showed a lower resistance ratio (RR = 17.38) than the CIP-resistant strain (RR = 233.42) that was originated from specimens of La Esperanza (Department of General Güemes).[39,40] Comparative analysis of transcriptional expression of the *CPR* gene of groups of fifth instar nymphs from the different strains considered for this study revealed that the mRNA levels of the *CPR* gene in individuals of the CIP-resistant strain were significantly higher than in insects of the CRV-susceptible and CRV-resistant strains (Figure 4). This result agrees with a recent finding in our laboratory of a high level of constitutive expression of a cytochrome *P450* gene (*CYP4EM7*) in the CIP-resistant strain.[18] However, the highly resistant individuals from the CIP-resistant strain did not show mRNA levels of the *CPR* gene significantly higher than in insects from the CIP-susceptible strain. The CIP-susceptible and CIP-resistant colonies were originated from specimens collected in two localities of the same geographical area, Los Quirquinchos and La Esperanza (Department of General Güemes), respectively. The constitutive expression of the *CPR* gene detected in the CIP-susceptible and CIP-resistant strains agrees with the high level of constitutive expression of the *CYP4EM7* gene observed in both strains[18] and would support the hypothesis stating that in this area could exist naturally tolerant populations to pyrethroids, explaining the presence of many localities with very low susceptibility.[4]

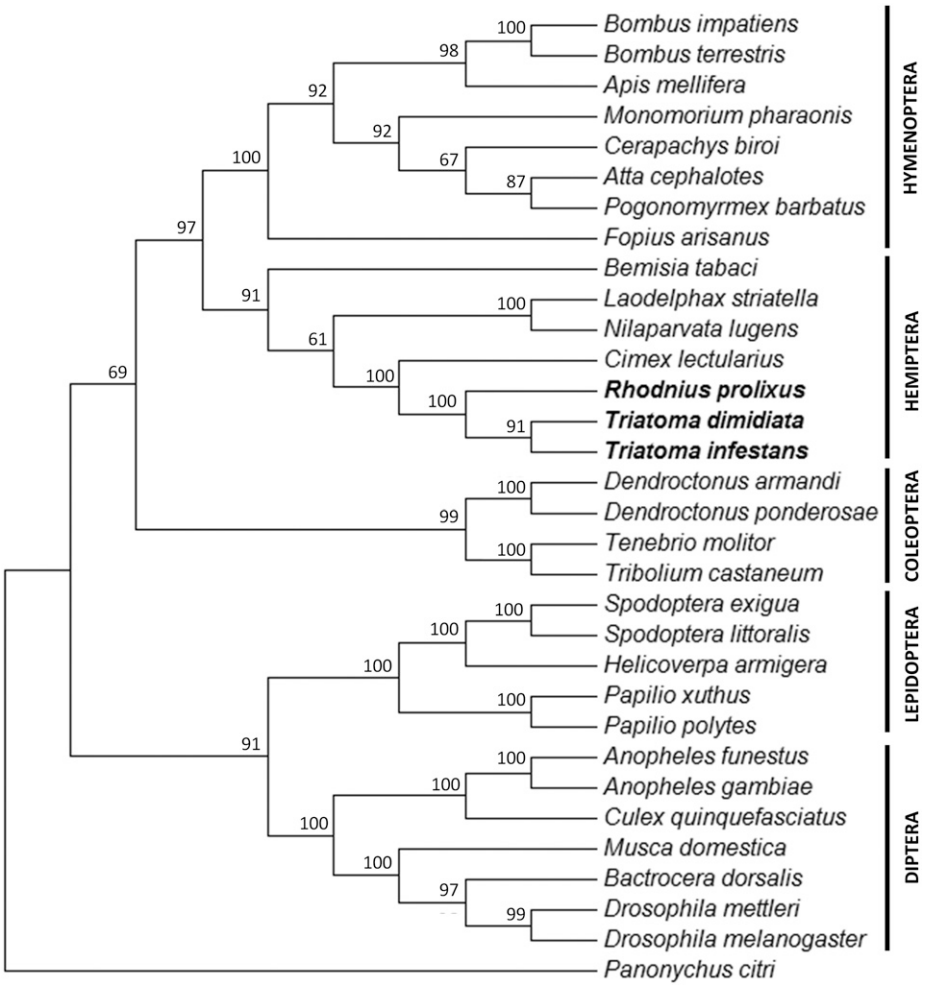

FIGURE 3. Maximum likelihood phylogeny of *Triatoma infestans*, *Triatoma dimidiata*, and *Rhodnius prolixus* cytochrome P450 reductases (CPRs) deduced amino acid sequences (in bold), selected CPRs from other hemipteran species, and representative species of the orders Diptera, Lepidoptera, Coleoptera, and Hymenoptera. Bootstrap values next to nodes represent the percentage of 1,000 replicate trees that preserved the corresponding clade.

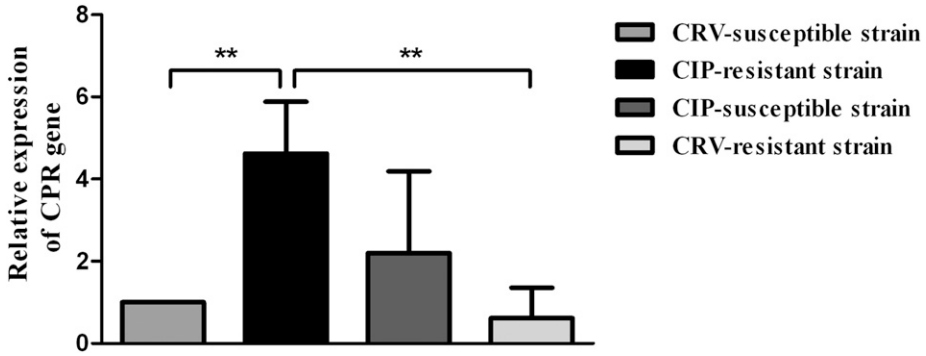

FIGURE 4. Relative expression (mRNA) of the *CPR* gene in the fat body of *Triatoma infestans* nymphs from the CRV-susceptible, CRV-resistant, CIP-susceptible, and CIP-resistant strains. The error bars represent the standard deviation of the mean. Two asterisks indicate significant difference between the mean of insects from the CIP-resistant strain and the mean of the CRV-susceptible and CRV-resistant strains at $P < 0.01$.

Although substantially less than in the CIP-resistant strain, resistance to deltamethrin was detected in the CRV-resistant strain originated from specimens collected in the locality of Mataral (Bolivia). However, the transcript level of the *CPR* gene detected in individuals of the CRV-resistant strain was not significantly higher than in individuals of the CRV-susceptible strain (Figure 4). Therefore, as it was also observed for the *CYP4EM7* gene, the constitutive expression of the *CPR* gene in the CRV-resistant strain was not significant.[18] This may arise in response to different insecticides applied in the places of origin of each population and/or genetic variations between Argentinian and Bolivian populations of *T. infestans*. Vector control of Chagas disease in Argentina is mainly based on the application of deltamethrin, whereas in Bolivia other pyrethroids are more frequently used. Differences in treatments with insecticides could have selected different mechanisms that confer resistance in the insect vector.[4]

The mRNA levels of the *CPR* gene were also determined at different interval times after the application of deltamethrin in fifth instar nymphs from the CRV-susceptible strain (Figure 5). The results show that the levels of *CPR* mRNAs were increased significantly (7-fold) 1 hour after insecticide application

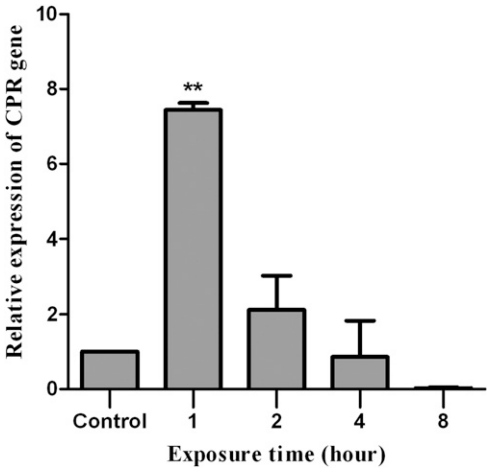

FIGURE 5. Relative expression (mRNA) of the *CPR* gene in the fat body of *Triatoma infestans* nymphs of the CRV-susceptible strain at different times after treatment with deltamethrin at LD_{50} concentration. The error bars represent the standard deviation of the mean. Two asterisks on the standard error bar indicate significant difference between the mean of the treatment with the insecticide and the mean of the control at $P < 0.01$.

in relation to those detected in individuals not exposed to deltamethrin (control). Coincidently, the expression at transcriptional level of the three cytochrome *P450* genes previously isolated (*CYP4EM7*, *CYP3085B1*, and *CYP3092A6*) was induced by deltamethrin in the CRV-susceptible, CRV-resistant, CIP-susceptible, and CIP-resistant strains.[18] Such an induction could lead to an elevated tolerance to these insecticides, which consequently contributes to a difficulty in controlling *T. infestans* populations in the field. Because the *CPR* gene encodes for an enzyme essential for the activity of cytochrome P450s and considered to be a vital part of P450-mediated insecticide resistance, the overexpression of the *CPR* gene detected in the CIP-resistant strain and the deltamethrin-inducibility of that gene in the CRV-susceptible strain is consistent with the hypothesis that postulates the involvement of *P450* genes in the development of resistance to pyrethroid insecticide in *T. infestans*.

CONCLUSIONS

This study is the first report of the *CPR* gene in triatomines. Consistent with the overexpression of the *CYP4EM7* gene in the CIP-resistant strain of *T. infestans* and the deltamethrin-inducibility of the *CYP4EM7*, *CYP3085B1*, and *CYP3092A6* genes in all the strains analyzed in a previous work,[18] it was observed overexpression of the *CPR* gene in the CIP-resistant strain and induction of that gene in the CRV-susceptible strain. These results suggest that the P450-mediated metabolism detoxification of xenobiotics might be an important mechanism for deltamethrin resistance in *T. infestans*.

Received May 23, 2017. Accepted for publication December 2, 2017.

Published online January 22, 2018.

Acknowledgments: We thank the Centro de Referencia de Vectores, Servicio Nacional de Chagas de Córdoba (Córdoba, Argentina) and the Centro de Investigaciones de Plagas e Insecticidas (Buenos Aires, Argentina) for providing insects used in our studies.

Financial support: This research was supported by grants from Agencia Nacional de Promoción Científica y Tecnológica (FONCyT-Argentina), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET-Argentina), and Secretaría de Ciencia y Tecnología, Universidad Nacional de Córdoba (SECyT-UNC-Argentina). M. M. S. and B. A. G. are Career Investigators of CONICET.

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