

# Transferrin in a cockroach: Molecular cloning, characterization, and suppression by juvenile hormone

(insect/fat body/hemolymph/iron transport/molecular evolution)

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**ABSTRACT** In a study of juvenile hormone-regulated gene expression, we isolated an anonymous cDNA representing a message that was strongly suppressed by juvenile hormone in the fat body of the cockroach *Blaberus discoidalis*. The protein deduced from the cDNA sequence showed compelling resemblance in sequence to the transferrins, a superfamily of internally duplicated, 80-kDa iron-binding/transport proteins characterized from several vertebrates and, to date, one insect (the tobacco hornworm, *Manduca sexta*). We isolated a 78-kDa protein from cockroach hemolymph, verified its congruence with the cloned cDNA, and found that it did bind iron. The cockroach protein is a member of the transferrin superfamily based on several features, including 32–46% amino acid positional identity with transferrins whose sequences are known, internal homology, positioning of cysteine residues, and iron binding. Whereas the previously characterized insect transferrin binds one atom of iron per protein molecule, *B. discoidalis* transferrin binds two iron atoms as do the vertebrate transferrins. The diferric property of cockroach transferrin is consistent with presence of two sets of residues positioned appropriately for iron binding. Juvenile hormone suppressed transferrin mRNA levels drastically in the adult female cockroach.

The transferrins constitute a superfamily of single-chain, 80-kDa glycoproteins noted for their remarkably tight ( $K \leq 10^{-20}$  M) but readily reversible iron-binding properties (1–3). Serum transferrin is the main iron transport protein of vertebrates. It absorbs iron at the gut, shuttles it between peripheral sites of storage and use, and maintains the metal at circulating concentrations sufficient to support cells having special demand for iron. Other members of the transferrin superfamily include lactoferrin and ovotransferrin (= conalbumin). These are generally at low levels in circulation but are prominent in secreted fluids such as milk, tears, and egg white, where by avid binding of free iron they act as bacteriostats. Finally, membrane-anchored melanotransferrin (antigen p97), though found at trace amounts in several tissues, is dramatically overexpressed in melanomas, where it may support rapid cellular proliferation.

Complete sequences for several vertebrate transferrins are known (4–12). They share >35% amino acid positional identity with one another. Within each transferrin there is significant sequence identity between the N- and C-terminal halves, a feature suggestive of an ancestral intragenic duplication that has been confirmed by structural gene analysis (13). In harmony with their internal homology and bilobed tertiary structure, all of the vertebrate transferrins appropriately analyzed (with exception of melanotransferrin; ref. 14) have the capacity to bind two ferric ions per protein molecule. Crystallographic studies (15, 16) suggest that the two

cleft lobes of the transferrins have identical sets of residues that serve as ligands to iron. Positions of cysteine residues (34 average per polypeptide) are highly conserved.

Although iron transport in vertebrates has been investigated intensively over recent decades, the subject has received little attention in studies of invertebrate animals. Circulating iron-binding proteins have been described from a tunicate (17), a crab (18), and a spider (19), but to date the only invertebrate transferrin that has been characterized and reported as a primary structure is from the sphinx moth, *Manduca sexta* (20, 21). The insect glycoprotein (77 kDa, a molecular mass consistent with the transferrin superfamily) has significant (25–30%) global sequence identity with the vertebrate transferrins, with most notable similarity around the N-terminal iron-binding sites and in the positioning of cysteine residues. Its CD spectrum is highly reminiscent of human transferrin. *M. sexta* transferrin circulates and donates iron (20), indicating a primary role in iron transport. These features suggest that, despite having the capacity to bind only one ferric ion instead of two (reflected by absence of a complete iron-binding motif in the C-terminal half), the moth transferrin is evolutionarily related and functionally similar to the transferrins of vertebrates. The fat body (the main source of hemolymph proteins in insects) is a site of transferrin synthesis in *M. sexta*.

This paper represents a dovetail of our interests in iron metabolism and hormonal control of gene expression in insects. It originated with isolation of an anonymous cDNA to a cockroach (*Blaberus discoidalis*) fat body mRNA that was, in the adult female, markedly suppressed by the sesquiterpenoid juvenile hormone (JH), a principal regulator of insect development and reproduction (22, 23). DNA sequence analysis indicated that the JH-regulated cockroach message codes for a protein homologous to the vertebrate transferrins and transferrin from *M. sexta*, a distantly related insect. We isolated the protein from cockroach circulation and showed that it bound iron with a stoichiometry of two metal ions per protein molecule, a feature consistent with its similarity to the vertebrate sequences but notably in contrast to the monoferric moth transferrin. We conclude that the transferrin superfamily is widely represented among insects. We do not know the significance of the vivid regulation of transferrin by JH.<sup>§</sup>

## MATERIALS AND METHODS

**Insects.** Adult female *B. discoidalis* were maintained communally on Purina Dog Chow and water at 27°C under a 12-hr light:12-hr dark cycle.

Abbreviation: JH, juvenile hormone.

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§The sequence reported in this paper has been deposited in the GenBank data base (accession no. L05340).

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**Hormone Replacement.** Cockroaches were decapitated on the day of the adult molt (= day 0) to remove the glandular corpora allata (source of JH). On days 3, 6, 9, and 12, 40  $\mu$ g of JH III (racemic mixture; Sigma) in 5  $\mu$ l of mineral oil were injected into the hemocoel through an abdominal intersegmental membrane. Control decapitated insects received mineral oil alone on the same days.

**Isolation of cDNA to a JH-Suppressed Message.** A cDNA library, selected to contain  $\geq 250$ -nucleotide inserts by Sephacryl S400 (Pharmacia) gel filtration of double-stranded cDNA, was constructed in bacteriophage  $\lambda$ ZAP (Stratagene) using fat body poly(A)<sup>+</sup> RNA obtained from day-15 normal females. Plaque lifts in duplicate were screened with total fat body [<sup>32</sup>P]cDNA (24) from decapitated control cockroaches versus total fat body [<sup>32</sup>P]cDNA from decapitated, JH-treated insects. Hybridization was as described (25). Recombinants that visibly hybridized with cDNA from control insects, but not with cDNA from JH-treated cockroaches, were selected as representing JH-suppressed fat body mRNAs and propagated for phagemid excision *in vitro*. One recombinant had a cDNA insert of 2380 nucleotides, the largest of 10 examined. The DNA sequence of the 2380-nucleotide insert was determined with an exonuclease III deletion mutagenesis system (Promega) and Sequenase 2.0 (United States Biochemical).

**Northern Hybridization.** Total fat body RNA was isolated (26), denatured with methylmercuric hydroxide (27), separated in 1.2% agarose, and transferred to a nylon membrane using 10 mM NaOH as transfer medium. The membrane was hybridized overnight as described (25) with the 2380-nucleotide cloned cDNA that had been labeled by nick-translation. Hybridization was visualized with autoradiography.

**Protein Isolation.** Hemolymph was collected from day-12 decapitated females by a flushing-out method (28) with phosphate-buffered saline (50 mM sodium phosphate, pH 7.0/0.15 M NaCl) containing 30 mM trisodium citrate, 10 mM spermine, 2 mM phenylmethylsulfonyl fluoride, 1 mM EDTA, 40  $\mu$ M phenylthiourea, 0.2 mg of *N*-tosyl-L-phenylalanine chloromethyl ketone per ml, and 4  $\mu$ g each of aprotinin, benzamidine, leupeptin, and pepstatin A per ml. After immediate centrifugation (12,000  $\times$  g, 5 min) to remove hemocytes and clotted material, diluted hemolymph from 10–15 cockroaches was applied to a Sephacryl S300 column (130  $\times$  2.5 cm) equilibrated with 0.1 M Tris-HCl, pH 7.6/0.2 M NaCl, and 3-ml fractions were collected at a 15 ml/hr flow rate. Fractions were tentatively designated as containing transferrin according to SDS/PAGE and Ferene S (Diagnostic Chemicals, Oxford, CT) staining. Transferrin-containing fractions from 63 insects were pooled, reduced in volume by ultrafiltration, and again subjected to Sephacryl S300 chromatography. Appropriate fractions were pooled, concentrated, and applied to a nickel-dipicolylamine metal ion affinity column prepared from epichlorohydrin-activated Sepharose 6B (29). The column had been equilibrated with 0.1 M sodium phosphate, pH 8.0/0.6 M potassium sulfate. After removal of contaminating proteins with equilibration buffer containing 0.1 M imidazole, transferrin bound to the column was eluted with 50 mM EDTA (pH 9.8).

Homogeneity of transferrin preparations was assessed with SDS/PAGE and Coomassie blue R. Amino acid composition was determined with a Beckman 7300 amino acid analyzer after 24 hr of hydrolysis with 6 M HCl at 110°C under reduced pressure. N-terminal residues were determined by automated Edman degradation and a Beckman 890M sequencer. The individual phenylthiohydantoin derivatives were analyzed on a Beckman 110 HPLC system fitted with a C<sub>18</sub> reversed-phase column.

**Iron-Binding Studies.** Iron was removed from *M. sexta* transferrin, human serum transferrin (Sigma), and *B. discoidalis*

putative transferrin under acidic conditions (30). Resulting apoprotein solutions were incubated for 10 days with 0.25–4.0 molar equivalents of [<sup>59</sup>Fe]nitrilotriacetic acid freshly prepared from <sup>59</sup>FeCl<sub>3</sub> (24.28 mCi/mg; 1 Ci = 37 GBq; New England Nuclear). Samples were diluted 10-fold and small solutes were removed using a Centricon 30 filtration unit (Amicon). The retentate was diluted and filtered twice more. The distribution of radiolabeled iron between retentate and combined filtrates was measured with an LKB 1282 scintillation spectrometer.

## RESULTS AND DISCUSSION

**cDNA Characterization.** Of 5000 fat body recombinant cDNAs screened with total fat body cDNA preparations from control decapitated cockroaches and JH-treated decapitated insects, 10 hybridized only with cDNA from decapitated controls. Among these, the largest (2380 nucleotides) was selected for further study.

The sequence of the 2380-nucleotide cDNA and the primary structure of the protein we infer it to represent are displayed in Fig. 1. The longest open reading frame begins with nucleotide position 10 and ends with position 2187, encoding a putative primary translation product of 726 amino acid residues. The method of von Heijne (31) predicts a secretory signal peptide consisting of 16 residues (supported by protein analysis below) although, unusually, no basic residues closely follow Met-1. The calculated molecular mass of the mature protein is 78,444 Da. There are four potential N-linked glycosylation sites.

The sequences of the cockroach cDNA and deduced protein (Fig. 1) were used to search the GenBank (release 73.1) and SwissProt (release 23.0) data bases, respectively, using the WORDSEARCH program of the Genetics Computer Group. The searches selected transferrin cDNAs and proteins as most closely resembling the *B. discoidalis* sequences. This led to our attempt to isolate an 80-kDa iron-binding protein from cockroach hemolymph.

**Isolation and Characterization of the Cockroach Iron-Binding Protein.** *B. discoidalis* hemolymph rapidly clots upon exposure, and use of the bleeding solution (*Materials and Methods*) followed by immediate centrifugation was critical in obtaining a satisfactory protein yield. Two rounds of gel filtration largely separated the iron-binding protein from other major hemolymph proteins (storage hexamerins, lipophorin). A homogeneous preparation was obtained with the nickel-dipicolylamine metal ion affinity column, where imidazole removed minor contaminants while the iron-binding protein remained adsorbed.

The N-terminal sequence of the cockroach protein (15 residues determined by automated Edman degradation) was identical to that deduced from cDNA sequence analysis (Fig. 1), and the observed amino acid composition of the protein closely conformed to the predicted composition (Table 1).

Equilibrium-binding of iron to human serum transferrin, *M. sexta* transferrin, and the cockroach protein showed them to bind (three assays each)  $1.69 \pm 0.08$ ,  $0.82 \pm 0.04$ , and  $1.92 \pm 0.06$  mol of Fe per mol of protein, respectively. These results contrast the *B. discoidalis* protein with *M. sexta* transferrin by indicating that the cockroach polypeptide, like the vertebrate protein, has two iron sites.

**Insect and Vertebrate Transferrins.** To explore further possible functional and evolutionary relationships between the cockroach iron-binding protein and the transferrin superfamily, we compared the deduced *B. discoidalis* sequence with those of nine vertebrate transferrins (4–12) and *M. sexta* transferrin (21), using the method of Needleman and Wunsch (32). Amino acid positional identities were 32–34% with the vertebrate proteins and 46% with the insect sequence, as compared to values of 13–17% obtained when the sequences

t g c t g c a a g A T G C T G C T C T G C C T C A C G C T C C T G T T C T C A G C T T C T G C T G T C G C C A T G C C T A C C C C C G A G G G T T C A C C A C C C A G A A T A T A C A A G G T G T G T C C C A 111  
 1 M L L C L T L L F S A S A V L A M P T P E G S P H H E I Y K V C V P  
 G A A G G A C C C T A G A A T C G T G T C A T C G C A T G T C T C A G G A G A G T A T C T G C A T A T G A C G T G T G T A G C T G C C A G A G A C A G A A T T G A G T G T C T G G A C A A G A T A A G C A C A G A G A G 222  
 35 E G A L E S C H R M S Q E S D L H M T C V A A R D R I E C L D K I K H R E  
 G C A G A C T C G C G C T G T A G A C C C T G A G G A C A T G T A C G T G G C C G C A A G A T C C C C G A G C A A G C T T C A T C A T T T C A A G G A G A T C A G G A T A A G G A G G C C T G A T G A G G A G 333  
 72 A D F A V P D P E D M Y V A A K I P Q Q D F I F K E I R T K E P D E E  
 T T C A G A T A C A G G C A G T G T G C G T G A T T C A A A G G A T C T C G A C A T T A C G A G C A T C C A C G G T G T C A A A T C T G C C A C A C G G G T A G T G A G A A C G T T G G C T A C 444  
 109 F R Y E A V C V I H K D L D I T S I H G L Q G L K S C H T G V G R N V G Y  
 A A G A T A C C C A T A A C A A G T T G A G G C A T A T G G G A G T G C T G G G T C C A C T T A A C A A T A G T G A C T G A C G C C A A G G G A A A C G A A T T G C A C G C A C T T T C A T C T G T T C A G C G A A 555  
 146 K I P I T K L R H M G V L G P L [N N S] D L T P R E N E L H A L S H L F S E  
 G C A T G T C T G T G G G T A A A T G G C C A C C G G A T C C T G C A C A A A C C A G G C A C T T A A G G C G A A G T A C C C C A A C T T G T G T G C T C T A T G C G A A C C C C G A G A T T T G T G A C T A C C C T 666  
 183 A C L V G K W A P D P A Q N Q A L K A Y P N L C A L C E H P E I C D Y P  
 G A C A A T A T T C A G G A T G A T G A G G C G T C G G T G T C T G G C A G A A C A T G A G G T C A G G T C G C C T G G A C C A A G G T C T A T T A T G T C A A G A A C A C T T T G G A A T G C C T A T C G G A 777  
 220 D K Y S G Y D G A L R C L A E H G G Q V A W T K V Y Y V K K H F G M A I G  
 G C T G G A G A A G C A G T A C C A C A G G A C A A A C C C T G A T G A C T A T G C T A C C T G T G T C C T G A T G C T A C C A A G A A C C A T C A C G G G T A A C C A T G T A T C T G G G C A G T A G G C C A 888  
 257 A G E A V P T G Q N P D D Y A Y L C P D A T K K P I T G K P C I W A A R P  
 T G C A A G G G T A T A T G G C C A A T C A G A C T T G A C A A T A T A G C T G A T C G A G G G C T A A G A T T T C T G G C T G A C A C T A T T G G G G A G A C A G A A A T G C T G A C T G G C T A T C C 999  
 294 W Q G Y M A N H D L D N D I A D L R A K I S L A D T I G E T E N A D W L S  
 A A G G T A C T C G A C C T G A A C A A A A C C A T T C C T A T A G A T A A T C A G G G A C C A T A C C C C G G A A C T A T C T T A A C A A G C T A A C T A C A C A G A C G T G A T A G A G A G A T A C C 1110  
 331 K V R E A G L N [N K T] I P I D L N [N K T] I P I D L N [N K A [N Y T] D V I E R D T  
 G G A G C T C C A C A T C G G C C G T C A G G T T T T G T G T G A C A T C A G A C G C A G A A C T G G A A A A G T G C A G A G T C T T G A A G C G C G C C G C A T A C T C C A G A G A T A T T C G T C C C G C C T C G A C 1221  
 368 G A P H R P V R F C V T S D A E L E K C R V L K R A A Y S R D I R P A F D  
 T G T G T C C G G A A G C G G G T C A T A C T G A G T G T C T T A G A A C G G T T A G A G A C A T G G C C C G A C G T A T C A C T C T G G A C G C G G T G A A G T C T C G T T G C A C A G A G G C A G T A T A C 1332  
 405 C V R E A G L H E C L R I T V R D D G A D V I T L D G G E V F V A Q R Y N  
 C T C A A A C C T A T A G T A G C A G A A C A G T A T G G C G A A C C G G C A G C C T C T A T A T G C A G T A G C A G T G G T C A G A A G G A C T C C A C T T A C C A G T C C A T C G A G G A T C C C G A G G G C C 1443  
 442 L K P I V A E Q Y G E H G S Q A Y A V A V V R K D S T Y Q S I E D L R G A  
 A A G T T T G T C A C A C G G T A C G G T A G A A C G C A G G T G G A A C G T G C C A C T A T A C A G C T A T T G A G C A A G G A A C T G A T C A G C A A G A A C A G T T G C C A T A T T C A G T C A G T C T G 1554  
 479 K S C H T G Y G R N A G W N V P L Y T L L S K E L I S K N S C P Y S S A L  
 T C T T C A T A C T T C C G G A G G T A G T T G C G T T C C T G G A G C T A A T T G C C G G A A A A T A A C C C C G A A A T C A G A A C C C G A T T C A C T T G C A G A T C T G C C C G G A A A C C T C G C A 1665  
 516 S S Y F S G G S C V P G A Q L P E N N P A N Q N P D S L C S I C A G N L D  
 G C T C C A A C A A T G A C C C A G A T G G A A T G T T C T G C T A G C A C G C A G C A T T T T C G G A T T A G C A G T G A G C T T T C A G G T G T C T G C C A T C C G G T A G G G C A A G T G G C T T T C 1776  
 553 A P N N D P A W K C S A S N D E S F F G Y S G A F R C L A S G E G Q V A F  
 G T G A A C A T A C C A G T A C C A G A A T A C A G A T G G C A T A A T C A A G C T G C T T G G A C C G T G G G C T G C G A T C C C A G G A C T T C G A A C T G T T A T G T C G T A G G A G T G C T G T C 1887  
 590 V K H T T V P E N T D G H N Q A A W T A G L R S E D F E L L C A D G G R A  
 T C T A T C A A T G A A T T C T A G A T G C A T C T G G C A G A A G T G C C A C C T C A C A T G G T T G T A C A A G C A A C G A T A A A C A G A T A T T C A A C T C A A C G A A T C A G A C A T G C T A T T C T G 1998  
 627 S I N E Y S R C H L A E V P P H M V V T S N D K T D I Q L N E I R H A I L  
 G C T G C T G G A G A C C T C T A T A G C A G A C T C C A G A C C T C T T C A A G C T C T T T G G T G A C T T T G G C G G C A A A G G A C C T T C T A T T C A A G A A C T G C A A C T G G G C T A T T G T C G G T G 2109  
 664 A A G D L F S R R P D L F K L F G D F G G T K D L L F K N S A T F C A T L S V  
 G A G A C G G A T C C C G C T A T G C A G A T A C T C A G A G A T T C T G G A G G T A T T A G A C A T G C G A G A A C C A G C C A A C G C C T a a a t a a c t t t c c a t a t g g a g t t g c a c t t c c a a 2220  
 701 E [N G S] P L M Q R Y S E I L E V I R A C E N Q P T P end  
 g t a c a t a t g t a g t g g a a a g a t a c g t g a t t g a a t g t g c a t t g c t g t g g t a t g t g a c a t c g a g t g c g a a a c g c a a a t c a t g a a a t t g a a t g t g t a g t t t a g t a t g 2331  
 t g a a t t g t g a t a a t t t t g t c a a t a a a a t a a a a t t t a c a t t t t (a)<sub>n</sub> 2380

FIG. 1. cDNA sequence representing a JH-suppressed cockroach fat body message and deduced 726-residue translation product. Lowercase nucleotide symbols indicate noncoding regions. The doubly underlined sequence, following a predicted 16-residue signal peptide, was confirmed by N-terminal sequence analysis of the protein purified from *Blaberus hemolymp*. Four possible N-linked glycosylation sites (positions 162, 337, 358, 702) are enclosed in brackets. The calculated molecular mass of the mature protein is 78,444 Da.

were repeatedly made random and aligned. An alignment (33) of the cockroach sequence with those of *M. sexta* transferrin

Table 1. Composition of cockroach iron-binding protein (78.4 kDa)

Residue	Mol %	
	From cDNA	Experimental
Asx	12.1	13.6
Glx	9.6	9.9
Ala	9.2	10.0
Leu	8.2	8.3
Gly	7.2	8.5
Pro	6.2	6.4
Ser	6.2	6.2
Val	5.5	5.0
Lys	5.2	4.8
Arg	4.9	5.3
Ile	4.9	3.9
Thr	4.4	4.4
Tyr	4.2	4.0
Cys	3.8	ND
His	3.4	3.0
Phe	2.7	2.7
Met	1.3	2.0
Trp	1.1	ND

ND, not determined.

and human serum transferrin (5) is displayed in Fig. 2. One-hundred twenty-one residues are conserved in the three proteins, with the most striking global feature being identity of cysteine residues at 19 positions (conserved among all transferrins; not shown). The high level of cysteinyl identity suggests similarity of tertiary structure, although vertebrate transferrins can accommodate more disulfide bonds than can the insect proteins. There are 28–38 Cys residues in vertebrate transferrins, compared with 24 and 26 in the insect iron-binding proteins. The CD spectra of the moth transferrin (21) and the *B. discoidalis* protein (not shown) indicate secondary structures low in  $\alpha$ -helix and high in  $\beta$ -sheet, a feature also seen in vertebrate transferrins (34).

At the nucleic acid level, the *B. discoidalis* coding region was found to be 54% identical with *M. sexta* transferrin and 45% identical with human serum transferrin. Comparison of the moth and human coding regions showed 42% nucleotide positional identity.

Each of the two domains in the vertebrate transferrins has four strictly conserved residues that serve collectively as an iron-binding site, based on crystallographic studies of two superfamily members (15, 16) and sequence analysis of others. In human serum transferrin these are, in the N-terminal lobe, likely Asp-63, Tyr-95, Tyr-188, and His-249 (Fig. 2). Three of the four residues appear conserved in the insect proteins. His-249 (positions 279 and 274 in the cockroach and moth polypeptides, respectively) is not represented in the insect proteins, although we note possibly functional His

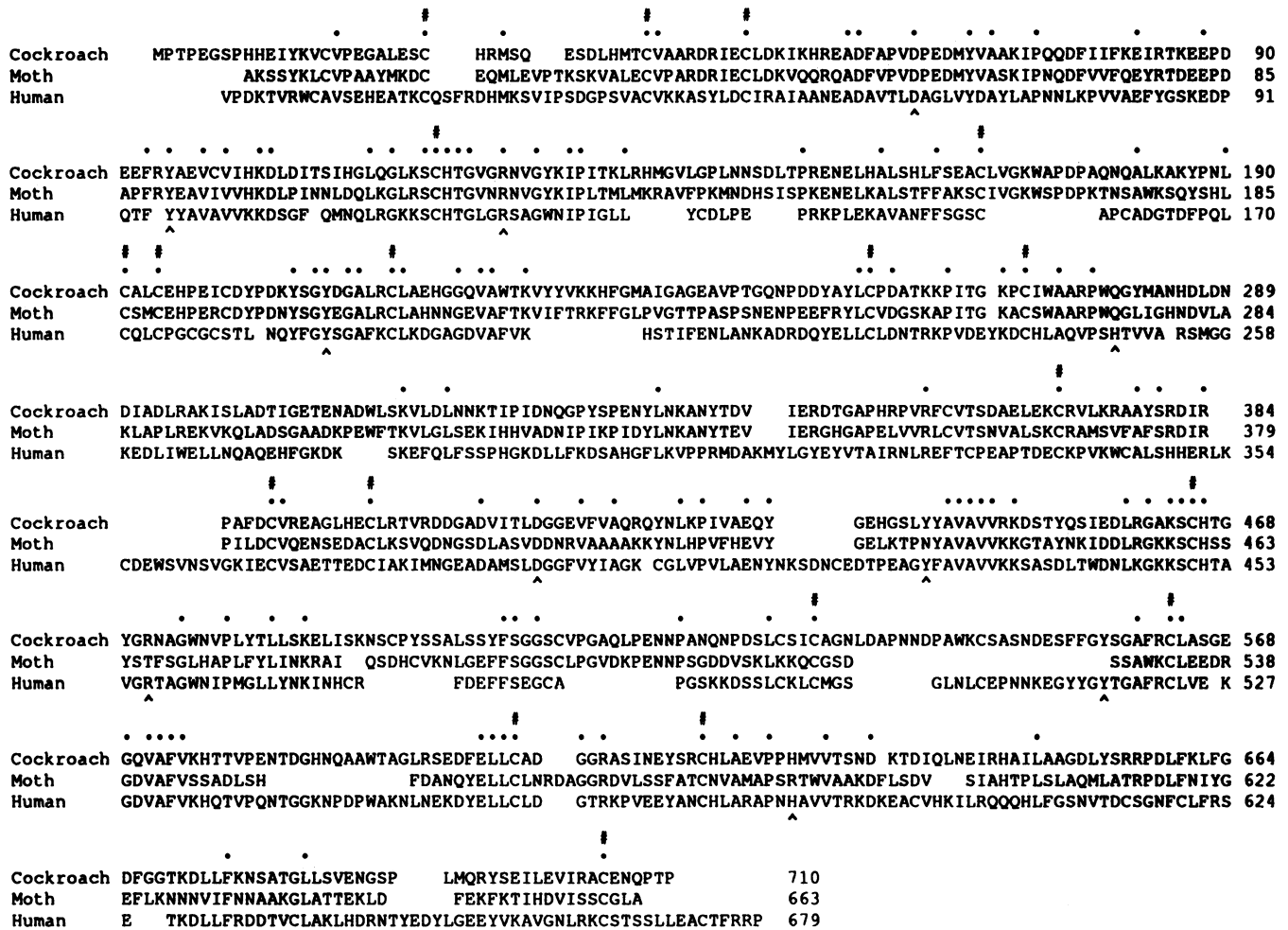


FIG. 2. Progressive, gapped alignment of the cockroach iron-binding protein, *Manduca* transferrin (21), and human serum transferrin (5). Identical residues are indicated by dots; # indicates conserved cysteine residues; ^ indicates residues in the human protein likely involved in iron binding (D, Y, R, Y, H interspersed in each half of the polypeptide).

residues positioned in close proximity. In the C-terminal lobe of vertebrate transferrins, ligands to iron (human protein as reference) are likely Asp-387, Tyr-426, Tyr-517, and His-585. All are maintained in the cockroach sequence but, notably, Tyr-517 and His-585 are not represented in the monoferric *M. sexta* transferrin. In vertebrate transferrins, iron binding requires binding of an anion (one anion, usually bicarbonate, per lobe). The anion-binding function is mediated by basic residues (Arg-124 and Arg-456 in human serum transferrin) that are mirrored by Arg-125 and Arg-471 in the cockroach protein. The second Arg residue has been replaced by a neutral residue in *M. sexta* transferrin, a further likely reflection of loss of an iron-binding site in the moth protein.

Levels of internal identity in the cockroach and moth iron-binding proteins are 28% and 26%, respectively, substantially lower than values of 37–48% seen among vertebrate transferrins, yet they suggest that the insect proteins were, as in the vertebrates, produced by intragenic duplication. The argument for an ancestral duplication event is strengthened by our observation that the cockroach polypeptide persists as a diferric protein. Given that the duplication did occur, the N-terminal and C-terminal halves of the insect sequences have diverged from one another, to the point of loss of the C-terminal iron-binding site in *M. sexta* and perhaps other species. The physiological significance of one- versus two-sided iron-binding proteins in insects remains undetermined.

Many features of the cockroach protein described in this paper argue that it be assigned to the transferrin superfamily.

We suggest that the insect and vertebrate proteins descend from a sequence that was present in the last common ancestor of vertebrates and arthropods, about 600 million years ago (35, 36). Our data, taken with documentation of transferrin in a moth (20, 21), suggest as well that transferrins are maintained in many insects, since the progenitor of cockroaches and moths (~300 million years ago) gave rise to most of the present-day insect orders (37).

**JH-Regulated Transferrin Expression.** The dramatic suppression of the transferrin message by JH is illustrated in Fig. 3. Lane A shows hybridization of the cloned transferrin cDNA to total fat body RNA from day-15 normal adult

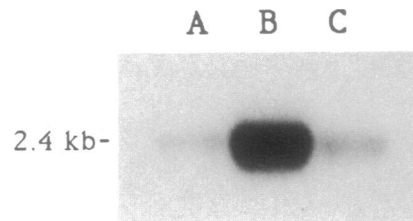


FIG. 3. Suppression of cockroach transferrin mRNA by JH. Total fat body RNA was separated in agarose, transferred to nylon, and hybridized with cloned transferrin cDNA. Each lane contained 5 µg of RNA pooled from six insects. Lane A, Normal females 15 days after the adult molt; lane B, day-15 females that had been decapitated on day 0; lane C, as in lane B and treated with 40 µg of JH III on days 3, 6, 9, and 12.

females, whose corpora allata are producing JH actively (G. Bhaskaran, personal communication). The hybridization signal approximates that from day-15 females that had been decapitated on day 0 and treated repeatedly with JH (lane C) but is much weaker than the intense signal seen from control decapitated females (lane B). By dot-blot titration (not shown) we estimate that transferrin mRNA was 50 times more abundant in the absence of JH than in its presence. In an earlier study (25) we showed that 2.4-kilobase fat body messages are remarkably abundant in JH absence and that 80-kDa polypeptides are the dominant secreted products of the JH-deprived female fat body.

We do not yet know the significance of the striking hormonal regulation of transferrin gene expression in *B. discoidalis*. In adult female cockroaches and many other insects, the principal function of JH is stimulation of ovarian maturation, a process requiring deposition, in many eggs contemporaneously, of iron in amounts sufficient to sustain embryonic growth. Perhaps iron reserves (in the form of ferritin; see ref. 38) are drawn upon at this time. Many nonoverlapping cycles of JH-dependent egg development take place in female *B. discoidalis* over her 2 years of adult life. Between these the JH titer is, presumably, low. Perhaps between cycles the transferrin gene is derepressed, for maximal capture of iron from the diet and replenishment of stores.

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