Ovarian- and Somatic-specific Transcripts of the Mosquito Clathrin Heavy Chain Gene Generated by Alternative 5'-Exon Splicing and Polyadenylation*

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Insect oocytes are extraordinarily specialized for receptor-mediated endocytosis of yolk protein precursors. The clathrin heavy chain (CHC) is the major structural protein of coated vesicles, the principal organelles of receptor-mediated endocytosis. To understand the role of clathrin in the development of the oocyte's powerful endocytic machinery we determined the structure of the mosquito chc gene. The gene spans approximately 45 kilobases and its coding region is divided into seven exons, five of which encode the protein. Three distinct mature transcripts of this gene were identified in mosquito tissues. Two of them code isoforms of the CHC polypeptide differing in their NH2-terminal sequences, and are specifically expressed in female germ-line cells. The third transcript has a 3'-untranslated region about 1 kilobase longer than the other variants, and is found only in the somatic cells. Tissue-specific 5'-exon splicing and alternative polyadenylation of the primary transcript combine to give rise to these mRNAs. We identified two alternative promoters, distal and proximal, separated by approximately 10 kilobases involved in tissue-specific regulation of mosquito chc gene expression. Our data provide the first molecular evidence for complex structure and regulation of a chc gene, in this case occurring at both the transcriptional and post-transcriptional levels.

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The abbreviations used are: CHC, clathrin heavy chain; CLC, clathrin light chain; hp, base pair(s); kb, kilobase(s); PCR, polymerase chain reaction; RT-PCR, reverse transcription-polymerase chain reaction; RACE, rapid amplification of cDNA ends; UTR, untranslated region; PBM, post blood meal.
ease transmission are intimately tied in anautogenous mosquitoes through the requirement of a blood meal. Recently, one of the major components of this vesicular transport system, the mosquito vitellogenin receptor, was characterized (30, 31) and its cDNA isolated and sequenced (32). This paper reports the molecular characterization of the gene coding another major component of coated vesicles, the CHC. Our results demonstrate that the A. aegypti chc gene is regulated at both transcriptional and post-transcriptional levels. Combinations of alternative 5′-exon splicing and alternative polyadenylation generate three distinct mature CHC transcripts, which exhibit tissue-, stage-, and sex-specific expression. Our data also suggest that two alternative promoter regions are involved in A. aegypti chc gene regulation resulting in two CHC isoforms in ovarian germ-line cells. In addition, whole mount in situ hybridization experiments with mosquito ovaries have revealed that the A. aegypti chc gene is expressed very early during previtellogenic development of the oocyte and that the ovarian CHC transcripts are found only in germ-line cells.

MATERIALS AND METHODS

Insects—Mosquitoes, A. aegypti, were maintained in laboratory culture as described elsewhere (33). Vitellogenesis was initiated 3–5 days after eclosion with a blood meal on rats.

Cloning and Characterization of Mosquito CHC cDNA and Genomic Sequences—A cDNA fragment of the A. aegypti chc gene was first amplified from 20 μg of ovarian total RNA by reverse transcriptase-polymerase chain reaction (RT-PCR), using a degenerate forward primer, TCGGCTCTAGATGTCGAC-3′ and a reverse primer, ATTCTAGATCA,C,G,TGTATAGAACAT-(A,C,G,T)CCCTAAGT/G,TG, which were designed based on CHC sequences conserved in Drosophila and rat (12, 15). To facilitate cloning of PCR products, an anchor sequence (italics) containing an XbaI restriction site (underlined) was added to the 5′-end of the primers. A 650-bp cDNA fragment was generated and subcloned into the pUC119 vector, and its identity verified by double-strand sequencing (34).

Seven independent cDNA clones and four partially overlapping genomic DNA clones, comprising the entire coding region of the A. aegypti chc gene, were subsequently isolated by hybridization screening of a λ Zap II cDNA library generated from previtellogenic female mosquitoes, and of a λFix II genomic library prepared from adult mosquito whole bodies (35). In addition, two cDNA clones, representing the 5′-most end of CHC transcripts, were generated by the rapid amplification of cDNA ends (RACE) technique (36). For this, 1 μg of poly(A)+ RNA isolated from vitellogenic ovaries 6 h post-blood meal (PBM) was reverse transcribed using random hexamer primers. The RNA/DNA hybrid was digested with RNase H, and the single stranded cDNA was then amplified using a 5′-anchor primer (5′-AGGAATTCCTCCCCCCCCCCCCC-3′) and a gene-specific reverse primer R3 (5′-TGCGGATCCGTCGATCAG-3′) designed from the genomic sequence of clone ACHC N1. Two different PCR-generated fragments of 0.34 and 0.68 kb were obtained, subcloned, and sequenced.

The nucleotide and deduced amino acid sequences of the putative A. aegypti CHC clones were analyzed using the FASTA and GAP programs (University of Wisconsin, Genetics Computer Group software).

Primer Extension and S1 Nuclease Mapping—Primer extension experiments were performed following the method described by Sambrook et al. (34). A 30-base oligonucleotide (5′-TTTGGACGTTTGCCTCTCAT- GAATTCGAGGCTCTCCCGATTC-3′) was end-labeled with [γ-32P]ATP (3000 Ci/mmol, DuPont New) by T4 polynucleotide kinase (Boehringer Mannheim) and used in primer extension reactions with 10 μg of total ovarian RNA isolated from previtellogenic females and 10 μg of tRNA (as a control). The products were fractionated on a DNA sequencing gel. Bacteriophage M13mp18 DNA was sequenced as a marker using M13 primer from a Sequenase version 2.0 kit (U.S. Biochemical Corp.).

S1 nuclease mapping was performed with a 522-bp genomic SacI-SalI fragment derived from phage clone ACIC 3D. The 5′-end of the SacI site of this fragment was dephosphorylated with calf intestine alkaline phosphatase (Boehringer Mannheim) and 32P-labeled with T4 polynucleotide kinase. After hybridization of the probe with 10 μg of total ovarian RNA and 10 μg of tRNA (as a control), and subsequent digestion with S1 nuclease as described by Sambrook et al. (34), the reaction products were processed in the same manner as in primer extension analyses.
of the long 3'-UTR (1-kb fragment) was used as a probe, hybridization was observed to the somatic mRNA only (Fig. 1B), indicating that the major difference between the two mRNAs was the length of the 3'-UTR.

Because the largest cDNA clone contained only the 3'-half of the CHC protein-coding region, a fragment from its 5'-end was used to screen cDNA libraries (constructed from previtellogenic females and vitellogenic ovaries) and a genomic library in an attempt to isolate the full CHC coding sequence. A 14-kb clone containing the beginning of the coding region of the CHC was isolated from the genomic library. This genomic clone, \( \lambda \)CHC1N, was characterized by restriction mapping, hybridization to cDNAs, and extensive sequence analysis (Fig. 2). Based on comparisons with other clathrin sequences, we estimated that it contained 99% of the CHC protein-coding region, including four exons (3, 4, 5, and 6; Fig. 2); the last of these also coded the first 148 bp of the 3'-UTR. However, sequences coding for the 5'-UTR, the very beginning of the translated region (24 amino acids), and the majority of the 3'-UTR were unrepresented in this clone.

It was not known how distant the remaining short protein-coding region at the 5'-end was from the beginning of exon 3, and sequencing over 1 kb into the intron failed to reveal it. The strategy we adopted was to determine the sequence of this region from 5'-RACE PCR-generated cDNA, which would allow us to design probes from its sequence to isolate genomic clones containing it. Surprisingly, sequencing of the RACE-PCR products revealed two classes of cDNA (0.34- and 0.68-kb) coding CHC isoforms, \( A. \) aegypti CHCa and \( A. \) aegypti CHCb, differing in their 5'-UTRs and NH\(_2\)-terminal portions of the protein (Fig. 3). Two isoforms of \( A. \) aegypti CHC are coded by alternative exons.

![Fig. 2. Organization of the mosquito chc gene. A, position of the genomic clones. B, intron-exon organization of the gene. C, restriction map of the \( A. \) aegypti chc gene. The map of the gene is continuous except for a gap (\( \triangledown \)) in intron 6. The length of intron 6 is about 15 kb, as determined by Southern blotting of genomic DNA.](image)

![Fig. 3. Amino acid sequence comparison of two isoforms of mosquito CHC with the NH\(_2\)-terminal regions of CHCs from other species. NH\(_2\)-terminal sequences of two mosquito CHC isoforms, \( A. \) aegypti CHCa and \( A. \) aegypti CHCb, were aligned with those of \( D. \) melanogaster CHC; \( D. \) melanogaster CHC; \( R. \) norvegicus (rat) (15), \( R. \) norvegicus CHC; \( C. \) elegans (nematode) (26), \( C. \) elegans CHC; \( D. \) discoideum CHC; and \( S. \) cerevisiae (yeast) (19), \( S. \) cerevisiae CHC. Two isoforms of \( A. \) aegypti CHC are coded by alternative exons. Arrow indicates the position of the intron which separates tissue-specific alternative exons 1a (codes for \( A. \) aegypti CHCa) and 2b (codes for \( A. \) aegypti CHCb) from common exon 3. Amino acid residues that are conserved in all seven of the aligned sequences, or at least six of seven are in bold.](image)

\( M A S L L K L T S T I L V Y G N G T K L Q L T N I N P S S S S F I N L T M E S D R E F I \) \( A a C H C a \)
\( M S Q L P I F R F Q E H L Q L T N I N P S S S S F I N L T M E S D R E F I \) \( A a C H C b \)
\( M Q I L P I F R F Q E H L Q L T N I N P S S S S F I N L T M E S D R E F I \) \( D e C H C \)
\( M A Q I L P I F R F Q E H L Q L T N I N P S S S S F I N L T M E S D R E F I \) \( R n C H C \)
\( M A L P I K F H E H L Q L P N A G I R V P M I T S N V I T M E S D K N I \) \( C e C H C \)
\( M T N I P I R F Q E H L Q L T N I N G S N I S I O T S N T M E S E K Y I \) \( D d C H C \)
\( M S I L P I E T E L V D L M S G I S Q P Q L P R S T P F S D H F V \) \( S c C H C \)

\( A. \) aegypti CHCb is generated by splicing exons 1b, 2b, and 3 (Fig. 2; Table I). Exon 1a is 182 bp long, is located \(-15\) kb upstream of exon 3, and codes the 5'-UTR and the beginning of the NH\(_2\)-terminal sequence of \( A. \) aegypti CHCa. Exons 1b and 2b are separated by a small intron of 53 bp and are located \(-5\) kb upstream of exon 3. Exon 1b codes part of the 5'-UTR and exon 2b codes the remainder of the 5'-UTR and the NH\(_2\)-terminal sequence of \( A. \) aegypti CHCb.

To isolate a clone containing the 3'-end of the gene, the unique 1-kb fragment of the long 3'-UTR cDNA was used as a probe. This screening successfully yielded the remainder of the 3'-UTR: the isolated genomic clone, \( \lambda \)CHC5-1, coded the last exon (exon 7; Fig. 2). Because the clones \( \lambda \)CHC5-1 and \( \lambda \)CHC1N did not overlap, the size of the intron separating
exons 6 and 7 was gauged by Southern blot analysis of mosquito genomic DNA. The minimum size of this intron was estimated to be ~15 kb (data not shown). The structure of exon 7 was determined by sequencing a 7.1-kb genomic EcoRI-EcoRI fragment derived from ACHC5-1 (Fig. 2). This genomic sequence was compared to the cDNA sequences of the long 1.4-kb and short 0.35-kb 3'-UTRs (Fig. 4). The genomic sequence from phage ACHC5-1 was identical to the long cDNA sequences, and no splice variants were observed in this region.

The sequence of the 3'-UTR of the A. aegypti chc gene (Fig. 4) is coded by the last 148 nucleotides of exon 6 and exon 7, about 1.3-kb. Several putative consensus sites, ATTATA, which are thought to increase instability of mRNA, are located in this region. Two of these sites were identified in the nucleotide sequence of the ovarian transcript (short 3'-UTR), and seven sites were found in the somatic transcript sequence (long 3'-UTR) (Fig. 4). A nonamer consensus sequence, TTATTAGTAA, containing repeat number 5 of the somatic transcript sequence and the tandemly arranged repeats numbers 6 and 7 are two motifs recently shown to significantly increase mRNA instability in many cases (43).

Analysis of exon-intron organization of the A. aegypti chc gene (Table I) revealed that all splice sites conform to the GT/AG consensus (44); the introns start with a GT dinucleotide and end with an AG dinucleotide. Examination of exon phase indicates that introns 1a, 1b, and 2 are positioned between codons, while all other introns which interrupt the protein-coding region (3, 4, and 5) are situated between the first and second nucleotide of a codon.

**Transcription Start Site Mapping—** Primer extension and S1 nuclease protection experiments were conducted to determine the transcription start site of the ovarian mRNA-coding isoform, A. aegypti CHCb. A 30-base oligonucleotide primer, complementary to the sequence of putative exon 1b (position 91–121 of the A. aegypti CHCb cDNA generated by 5'-RACE PCR) (GenBank accession number GSDB:S:1076487), was hybridized to total previtellogenic ovarian RNA and extended using SuperScript II reverse transcriptase. This resulted in a 552-bp extension product (Fig. 5A), placing the transcription start site 429 bp upstream of the available cDNA.

We performed S1 nuclease protection experiments to confirm that this position is the genuine start site of chc gene transcription, and that the extension product was not prematurely terminated due to mRNA secondary structure. When a 522-nucleotide DNA probe, corresponding to a genomic fragment containing most of putative exon 1b and extending upstream, was hybridized to total ovarian RNA, one strong S1-resistant product of 502 bp was detected (Fig. 5B). Thus, the size of exon 1b was calculated to be 692 bp, and the inferred start site position corresponds well with that predicted from the primer extension experiments.

**Tissue, Sex, and Stage Specificity of Alternative Exon Splicing—** Tissue specificity of alternative 5'-end exon splicing, and the possible correspondence of the ovarian transcript to the sequence of putative exon 1b (position 91–121 of the A. aegypti chc gene (Table I) revealed that all splice sites conform to the GT/AG consensus (44): the introns start with a GT-dinucleotide and end with an AG-dinucleotide. Examination of exon phase indicates that introns 1a, 1b, and 2 are positioned between codons, while all other introns which interrupt the protein-coding region (3, 4, and 5) are situated between the first and second nucleotide of a codon.

| Exon | Size (bp) | 5'-Donor site | 3'-Acceptor site |
|------|----------|----------------|-----------------|
| 1a   | 182      | ATC CAGgtatat...ttcacaagCTC ACC | 1a >15 |
| 1b   | 692      | TCTGTAgtataa...ctgttcatagCTCGTA | 1b 0.053 |
| 2b   | 259      | TTGA CAGaat...ttcacaagCTC ACC | 2b >5  |
| 3    | 208      | Leu Gin       | Leu Tre          |
| 4    | 256      | Lys A         | Val G            |
| 5    | 4041     | A. CHC5-1     | CHC5-1           |
| 6    | 620      | ACTGgtatg...ctttcagATCATT | 6 >15  |
| 7(o) | 201      | 7.5-kb ovarian mRNA | 7.5-kb ovarian mRNA |
| 7(i) | 1274     | 7.5-kb somatic mRNA | 7.5-kb somatic mRNA |

**FIG. 4. Nucleotide sequence of the 3'-untranslated region of the A. aegypti chc gene.** The untranslated sequences of exons 6 and 7 are in uppercase and the genomic sequence downstream of exon 7 is in lowercase. The nucleotide sequence of the CHC cDNA comprising the 3'-UTR of the 6.5-kb ovarian mRNA is in boldface, and the sequence specific to the 7.5-kb somatic mRNA is in standard type. The sequence is numbered beginning from the starting ATG of the A. aegypti CHCb transcript. Two alternative polyadenylation signals are enclosed in

**thick boxes**. The sequences ATTATA, which confer instability to mRNA, are located in this region. Two of these sites were identified in the nucleotide sequence of the ovarian transcript (short 3'-UTR), and seven sites were found in the somatic transcript sequence (long 3'-UTR) (Fig. 4). A nonamer consensus sequence, TTATTAGTAA, containing repeat number 5 of the somatic transcript sequence and the tandemly arranged repeats numbers 6 and 7 are two motifs recently shown to significantly increase mRNA instability in many cases (43).

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female carcases, males, and early embryos. Three PCR primers were used: the reverse primer RP3 (containing a sequence from exon 3 common to both the \textit{A. aegypti} CHCa and A. aegypti CHCb isoforms), and two forward primers, FP1a (specific to the \textit{A. aegypti} CHCa isoform, derived from the exon 1a sequence) and FP2b (specific to the \textit{A. aegypti} CHCb isoform, derived from the exon 2b sequence). PCR amplification using exon 1a- and 3-specific primers produced an intense band of the expected size (203 bp) from ovaries of previtellogenic females (1 and 4 days after eclosion) and early vitellogenic females (6 h PBM), and a less intense band from ovaries of later vitellogenic females (18 h PBM) (Fig. 6). No bands were observed using RNA from ovaries of late vitellogenic females (24 and 48 h PBM), female carcases, males, or early embryos (Fig. 6). In contrast, PCR amplification using primers specific to exons 2b and 3 yielded intense bands of the expected size (177 bp) for the ovaries of previtellogenic and vitellogenic females, as well as for female carcases, males, and early embryos (Fig. 6).

**Localization of CHC Transcripts in the Ovary**—We examined the spatial distribution of the AaCHC transcripts in the ovary by whole mount \textit{in situ} hybridization. The hybridization signal was observed in the oocytes of developing primary follicles during early previtellogenic stages and later in their nurse cells (Fig. 7). There was no apparent accumulation in the somatic follicle cells. Interestingly, \textit{A. aegypti} CHC RNA could be detected even in oocytes of the undifferentiated ovary, 0–1 day after eclosion (Fig. 7A). It dramatically increased in abundance by 3–4 days after eclosion (Fig. 7B).

During the vitellogenic period, \textit{A. aegypti} CHC mRNA was present in both oocytes and nurse cells from 3 to 12 h PBM (Fig. 7C) but virtually disappeared from the primary follicles by 24 h PBM. It is noteworthy that, at midvitellogenesis, the accumulation of \textit{A. aegypti} CHC transcripts could also be clearly detected in the oocytes of secondary follicles (not shown). Significantly, no hybridization was observed to a probe derived from the long 3'-UTR, which specifically recognizes the 7.5-kb somatic transcript (not shown), suggesting that only the 6.5-kb ovarian transcript is synthesized in germ-line derived cells during pre- and vitellogenic stages. Finally, control (sense) DNA probes never produced hybridization signals (not shown), confirming the specificity of the \textit{in situ} hybridization technique used in this study.

**DISCUSSION**

The \textit{A. aegypti chc} gene spans \(\sim 45\) kb and is composed of 7 exons, five of which encode the protein (Fig. 2). The protein-coding sequence of the \textit{A. aegypti chc} gene is highly conserved evolutionarily, at both the nucleotide and amino acid levels. Although the protein-coding sequences of CHCs are well characterized in several organisms, until now no detailed molecular characterization of a chc gene has been reported. Hybridization analyses demonstrated that the \textit{Drosophila} CHC transcription unit is not less than 10-kb; and P element-mediated transformation has shown that a 13-kb genomic DNA fragment, including the transcription unit, is sufficient to rescue lethal CHC.
mutations (12). Thus, the length of the Drosophila CHC locus should be approximately three times less than that of the mosquito, consistent with the 5-fold larger size of the A. aegypti genome (45).

Two CHC isoforms, A. aegypti CHCa and A. aegypti CHCb, were identified when 5'-RACE PCR products were sequenced (Fig. 3), and analyses of overlapping genomic clones revealed that these isoforms are coded by distinct transcripts generated as a result of alternative splicing of exons at the 5'-end of the gene (Fig. 2). The transcription start site was identified for the A. aegypti CHCb mRNA (Fig. 5), but has not yet been determined for the A. aegypti CHCa transcript. Preliminary experiments suggest that there may be another exon upstream of exon 1a containing an untranslated sequence that has yet to be isolated. Nevertheless, it is clear that transcription of the two A. aegypti CHC mRNA variants is regulated by two alternative promoters, which we designate distal (for A. aegypti CHCa) and proximal (for A. aegypti CHCb).

Employment of isoform-specific primers in RT-PCR showed that A. aegypti CHCa transcripts are present in ovarian tissue only, but that A. aegypti CHCb transcripts can also be detected in somatic tissues of females and males (Fig. 6). These results strongly suggest that there is tissue- and sex-specific usage of exon 1a, but not of exons 1b and 2b.

Differential stage-specific expression is characteristic of the splice variants as well. Both the A. aegypti CHCa transcript, regulated by a distal promoter, and the A. aegypti CHCb transcript, regulated by a proximal promoter, exhibit similar kinetics, peaking in the late previtellogenic and early vitellogenic stage (Fig. 6). However, the distal ovarian transcript (A. aegypti CHCa) is expressed only before 24 h PBM (peak of vitellogenesis), and is not detectable later. In contrast, the proximal ovarian transcript (A. aegypti CHCb) is present throughout the entire vitellogenic period, persisting until the later stages of egg development and even in newly-laid eggs (Fig. 6). We suggest that the distal ovarian transcript is required for the receptor-mediated machinery of developing oocytes; it is uniquely germ-line specific and is expressed prior to or during yolk protein endocytosis, disappearing at the time of uptake termination. The proximal ovarian transcript with its persistent presence in fully-developed eggs indicates that this mRNA may be produced to meet the future needs of the mosquito embryo.

In addition to the two types of transcripts generated through alternative splicing, two tissue-specific size classes of A. aegypti CHC transcripts (6.5-kb ovarian and 7.5-kb somatic) were detected by Northern hybridization experiments (Fig. 1). Sequencing of cDNA clones revealed that the ~1-kb difference in size is accounted for by a corresponding difference in the lengths of the 3'-UTRs. Comparison of the genomic sequence of this region with those of the cDNA clones (Fig. 4) indicated that the size variants do not arise through alternative splicing of exons in the 3'-region. Instead, alternative polyadenylation is the probable mechanism generating the observed size variants of A. aegypti CHC mRNAs (Fig. 1). Two putative alternative polyadenylation signals were identified at positions 5357 and 6426 (Fig. 4). It is thus likely that alternative usage of these polyadenylation signals results in expression of two different transcripts in somatic and female reproductive tissues.

The deduced alternative splicing and transcription termination events occurring in the transcripts derived from the A. aegypti chc gene are presented in Fig. 8. The dual mechanisms of alternative splicing in the 5'-region and alternative polyadenylation in the 3'-region combine to produce at least three types of mature CHC transcripts in mosquito tissues, which we designate A. aegypti CHCa-O (ovarian), A. aegypti CHCb-O (ovarian), and A. aegypti CHCb-S (somatic) (Fig. 8). Thus, the A. aegypti chc gene is apparently regulated at both the tran-
scriptional and post-transcriptional levels in a tissue-, sex-, and stage-specific manner.

In contrast to A. aegypti CHCa-O and A. aegypti CHCb-O, the A. aegypti CHCb-S transcript is found only in female somatic tissues (Fig. 1). Both A. aegypti CHCa-O and A. aegypti CHCb-S are regulated by the proximal promoter (Figs. 2 and 8), and alternative polyadenylation results in unique sequences in the 3′-UTR. Eukaryotic gene expression is partly controlled by rate of mRNA degradation, which is generally a function of regulatory sequences in the 3′-UTR (46). The longer 3′-UTR of the A. aegypti CHCb-S transcript contains more and more potent consensus sequences (42, 43) that promote mRNA decay than does the shorter 3′-UTR of the A. aegypti CHCa-O and CHCb-O transcripts (Fig. 4). These data suggest that the ovarian A. aegypti CHC transcripts are more stable than the somatic one. Such stability would facilitate accumulation of A. aegypti CHC mRNA in developing oocytes, permitting large-scale production of clathrin protein during vitellogenesis when endocytosis of massive amounts of vitellogenin is occurring. Ovarian AochC transcripts with short 3′-UTR begin to accumulate very early in oocyte differentiation (Fig. 7A) and resistance to decay would be of obvious advantage.

Clathrin is widely distributed in both germ-line and somatic cells of eukaryotes, playing an extremely important role in the universally critical processes of receptor-mediated endocytosis and secretion. It is likely that clathrin exhibits specialized functions in various tissues, implying a need for specific regulation of chc gene expression during the development of multicellular organisms. Genetic studies in yeast and Drosophila demonstrated that different functions of clathrin are genetically distinguishable (12, 19). Yeast CHC with a carboxyl-terminal deletion can rescue viability defects of null mutations but fails to complement defects in α-factor processing (19). Three of four lethal mutations of the CHC locus in Drosophila block development late in embryogenesis probably affecting the neuromotor function (12). Individuals with the fourth allele occasionally survive to adulthood but are characterized by variable male sterility, pointing to a specialized role for clathrin in spermatogenesis.

The results presented in this paper provide the direct molecular evidence for complex regulation of a chc gene in higher organisms. Two promoter regions have been identified in the A. aegypti chc gene. Alternative usage of these promoters clearly affects tissue-, sex-, and stage-specific characteristics. One would expect that mutations associated with these sequences will effect different functions and lead to various phenotypes. In addition, alternative polyadenylation has been observed at the 3′-end of the A. aegypti CHC primary transcript, which may differentially effect the stability, and perhaps translation efficiency, of A. aegypti CHC mRNA in germ-line and somatic cells. The combination of all these complex genetic processes provides a putative basis from which a diversity of CHC transcripts is derived, a diversity which may be essential for specialized functions of clathrin in eukaryotic cells.

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