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Research paper

Unique synteny and alternate splicing of the chitin synthases in closely related heliothine moths☆

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ABSTRACT

Chitin is an extracellular biopolymer that contributes to the cuticular structural matrix in arthropods. As a consequence of its rigid structure, the chitinous cuticle must be shed and replaced to accommodate growth. Two chitin synthase genes that encode for chitin synthase A (*ChSA*), which produces cuticular exoskeleton, and chitin synthase B (*ChSB*), which produces peritrophic membrane, were characterized in the genomes of two heliothine moths: the corn earworm/cotton bollworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) and the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). In both moths, the two genes were arranged in tandem with the same orientation on the same strand with *ChSB* located 5′ of *ChSA*. Sequence comparisons showed that the coding sequences were highly conserved with homologues from other species but that the tandem juxtaposed genomic arrangement of the two genes was unique in these insects. The mechanism that has led to this arrangement is unclear but is most likely a recent recombinational event. Transcript mapping of *HzChSB* and *HzChSA* in *H. zea* demonstrated that both transcripts were differentially spliced in various tissues and larval stages. The identification of the *HzChSB*–E12b alternate spliced transcript is the first report of alternate splicing for the ChSB group. The importance of this splice form is not clear because the protein produced would lack any enzymatic activity but retain the membrane insertion motifs. As for other insects, these genes provide an important target for potential control through RNAi but also provide a subject for broad scale genomic recombinational events.

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1. Introduction

Chitin is assembled as stacked sheets of a straight-chained, water insoluble amino-sugar homopolymer of β-(1-4)-linked N-acetyl-D-glucosamine and is the major component of insect cuticle, peritrophic membrane, foregut lining, hindgut lining and trachea (Lehane, 1997; Cohen, 2010; Merzendorfer, 2011). This extracellular biopolymer provides the matrix that lends mechanical strength and forms to the exoskeleton (Kramer and Muthukrishnan, 2005; Merzendorfer, 2006; Moussian, 2010). In addition to comprising the major structural component of the integument, chitin contributes to establishing the environmental barriers necessary for physical containment and waterproofing as well as providing the physical form that facilitates muscle attachment and movement. Because the insect increases in size throughout development, the chitin exoskeleton must be repeatedly shed and regrown during molting (Merzendorfer and Zimoch, 2003). The presence of chitin is essential to the functions of the peritrophic membrane (Hegedus et al., 2009) especially in maintaining the barrier function by limiting permeability (Agrawal et al., 2014; Kelkenberg et al., 2015). Chitin is also critical during reproduction for the formation of the chorion, which influences egg hatch, fecundity and embryonic survival (Moreira et al., 2007; Arakane et al., 2008) and as a crucial component of the embryonic serosa, which is necessary for protection of the embryo against desiccation (Jacobs et al., 2013, 2015; Chaudhari et al., 2015).

Chitin synthases are central to the final enzymatic transfer of sugar moieties from activated sugar donors to specific acceptors by forming a glycosidic bond (Merzendorfer, 2011). Uracyl-dinucleotide phosphate-acetylglucosamine (UDP-GlcNAc) is the substrate for chitin synthases

Abbreviations: UDP-GlcNAc, Uracyl-dinucleotide phosphate-acetylglucosamine; *ChSA*, Chitin synthase A; *ChSB*, Chitin synthase B; *RT-qPCR*, Quantitative reverse transcription-PCR.

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(CHS, EC 2.4.1.16), a membrane-integral glycosyltransferase that transfers the sugar moiety of UDP-GlcNAc to the growing chitin chain (Merzendorfer, 2011). This reaction takes place on the outer surface of the epidermal cells catalyzed by the chitin synthase proteins, which are transmembrane components of the plasma membrane (Kramer and Muthukrishnan, 2005; Merzendorfer, 2006, 2011). In most insects the chitin synthases belong to the family 2 glycosyltransferases and have been segregated into two classes, A and B, each with specific functional and developmental activities (Merzendorfer, 2011). Class *ChSA* forms a well-defined clade and is expressed in the epidermal cells producing the exoskeleton and linings of the foregut and hindgut, while the class *ChSB* clade consists of a paraphyletic agglomeration that are expressed in the gut epithelial cells, which produce the chitin component of the peritrophic membrane (PM) (Lehane, 1997; Merzendorfer and Zimoch, 2003; Arakane et al., 2004, 2005; Hogenkamp et al., 2005; Zimoch et al., 2005; Ashfaq et al., 2007; Merzendorfer, 2011; Zakrzewski et al., 2014).

Forty five genetic loci that affected larval cuticular morphology were first identified on the third chromosome of *Drosophila melanogaster* using the elegant embryonic screens of Jürgens, Wieschaus and Nüsslein-Volhard (Jürgens et al., 1984). Histological examination of mutants for the *ChSA* (CS-1) locus in *D. melanogaster* demonstrated that chitin formation and differentiation are critical for stability of procuticle formation and for attachment of cuticle to epidermal cells (Moussian et al., 2005). It also established that cuticular sclerotization and pigmentation were dependent on normal activities of the *ChSA* locus.

The cDNA sequence of *ChSA* from the sheep blowfly, *Lucilia cuprina*, was the first chitin synthase described in insects (Tellam et al., 2000). Subsequently, there have been more than 45 additional *ChSA* and/or *ChSB* sequences published for insect species including the mosquitoes, *Aedes aegypti* (Ibrahim et al., 2000), *Anopheles gambiae* (Ibrahim et al., 2000; Zhang et al., 2012), *Anopheles quadrimaculatus* (Zhang and Zhu, 2006); the flies, *D. melanogaster* (Gagou et al., 2002; Ostrowski et al., 2002; Devine et al., 2005), *Bactrocera dorsalis* (Yang et al., 2013); the moths *Manduca sexta* (Zhu et al., 2002; Hogenkamp et al., 2005), *Ostrinia furnacalis* (Qu et al., 2011; Qu and Yang, 2011, 2012), *Plutella xylostella* (Ashfaq et al., 2007), *Spodoptera exigua* (Chen et al., 2007; Kumar et al., 2008), *Spodoptera frugiperda* (Bolognesi et al., 2005); the beetle, *Tribolium castaneum* (Arakane et al., 2004); the locust, *Locusta migratoria manilensis* (Zhang et al., 2010); and the hemipterans, *Aphis glycines* (Bansal et al., 2012), *Laodelphax striatellus* (Wang et al., 2012) and *Nilaparvata lugens* (Wang et al., 2012). However, in these three hemipterans only the *ChSA* was present while genes for *ChSB* were

Table 1
PCR Primers for sequence analysis of *ChSA* and *ChSB* from *H. zea*.

Primer name	Amplicon size (bp)	Sequence (5′–3′)
BAC library screening		
783HzChS-Ctg18925F	829	CCGCATGCGGGATGAGGAAT
784HzCSCtg18925R		GTCAGAGGGCTTGACATCCTTTGA
792HzCSCtg9554F1	942	AAGTAAGATTTTCTGACTGTTTATACGCGC
794HzCSCtg9554R1		TTTCCTCTGAACAGAGAGAAGCATCC
Direct PCR primers	Site of binding	Sequence (5′–3′)
HzChSAEx1F1347	5	GAGAGCCAGCCTCTCAAGTGTCAAGG
HzChSAEx1F-5 F	9	GCCAGCCTCTCAAGTGTCAA
HzChSAEx2F789	11,820	GAGGGTAGCGACAACTCCGACGA
HzChSAEx3R1348	19,112	CACCTTAAACAACCGCACCGTGAAT
HzChSAEx5F1350	20,223	ATCGCTGCACACGATAGGAATGGGC
HzChSAEx7F1450	21,093	TCGTCATTCCCTTGGTGGTC
HzChSAEx8F1264	21,351	GCAAACGTGGATAACTATACACATCTGG
HzChSAEx9F792	21,853	AAGTAAGATTTTCTGACTGTTTATACGCGC
HzChSAEx15F2960	23,179	GAGTCCCTACATCGCATACC
HzCChSAEx16F781	24,105	ATCCCAAAGGAAACGAGGAGAAAGT
HzCChSAEx19F1259	26,810	GGTTCTGTTCGAAGAAGGTTAGCAT
HzChSAEx19F1260	26,907	CGATGACTTATCTCAAGACGCTCTGC
HzChSAEx19F1358	26,703	AGTGTTCGTGTTCTTCTTCGCCTTGA
HzChSAEx19F4238	26,732	GTTCTTCTTCGCCTTGATTC
HzChSAEx19F1262	26,748	CAGCTATGTTGTTCCATCGATTTGG
HzChSAEx7R790	21,111	GACCACCAAGGGAATGACGAGATT
HzChSAEx7R1351	21,149	TCGCCATTTCTAATACCACATGCAGC
HzChSAEx8R1606	21,329	ATCCAAGCCATTTGACGGG
HzChSAEx10R1353	22,041	CGATTCACTTGGGAGTCGTCGTCACT
HzChSAEx13R1355	23,000	CGATAGCCACGTTGCAGTAATAGCGT
HzChSAEx15R3300	23,719	AAGCGGCTATGAAGAACG
HzChSAEx16R1357	24,014	GCAGCGATTTCTGTTTTGCAGATTTC
HzChSAEx18R782	24,617	GGGACTCAAAAAGTCTACTTCACCCTTT
HzChSAEx22R1359	27,374	CAAGGTCTCACGTCGCAATGTCATC
HzChSAEx23R4790	27,653	GGCAGTTGATGTTCTGTGC
HzChSAEx23R786	27,660	ATATGTTGGCAGTTGATGTTCTGTGC
HzChSBEx1F1360	600	ATGGCGACGAAACCCAAGACTCC
HzChSBEx3R1267	1739	CCCACCTCCGGAAAACTGAACATT
HzChSBEx4F1268	2706	GCGATGCTCGTTCTGATAATCCTGC
HzChSBEx4F1362	2747	TAAAGGCACAATGTTGATGAATGCGA
HzChSBEx4F1364	4282	AGTTCGTAGTACGCGAGATGTCGTGG
HzChSBEx9F1271	4862	TTTTCCGTCTCGACGAAGATCAGAGC
HzChSBEx11F1366	5587	CACCGTTTGATGGACTTGCCGA
HzChSBEx11R1365	5602	AGTCCATCAAACGGTGGCCGA
HzChSBEx18F-F48	5588	ACCGTTTGATGGACTTGCCG
HzChSBEx18F-F49	5619	TCGCAAGGGAGTTATCGCTGAG
HzChSBEx18F-F73	8760	AGAGTGATGAAACCAGCGGCTC
HzChSBEx18F-F2	8781	GAGACAGAGCCGTTGAATCG
HzChSBEx4R1361	2830	TATCGGCGCTCGTTTCGGTCTC
HzChSBEx7R1363	4333	TGGGAGACAATCCACAGCAACCAAA
HzChSBEx18R-R96	8333	GCAGCAGATGTAAGTCTTCCTTGTG
HzChSBEx18R-R2	8893	GGTACATCGCTGGATACATCG
HzChSBEx19R1369	9949	CCACTTGAAGTGCAGTTGATCCTGGT
HzChSBEx19R1370	9834	CGGACTTGAAGAATCTTCGCGACA
HzChSBEx22aR109	11,987	TGAATGGTCTTCCTCCTGCTCAC
HzChSBEx22aR110	12,018	CTGCTTAGTATCCTTCCCTTTCTCC
HzChSBEx23R1277	13,330	ATCGACGCCCGACTGCCAAGAT
HzChSBEx23R1371	13,364	CGCTGGCTCGTAACCACGATTCA
RT-qPCR primers		Sequence (5′–3′)
28SiF		GGGGAGGAAAAGAAACTAAC
28SiR		CAACTTTCCCTTACGGTACT
HzChSBF2		GAGACAGAGCCGTTGAATCG
HzChSBR2		GGTACATCGCTGGATACATCG
HzChSBF48		ACCGTTTGATGGACTTGCCG
HzChSBR48		TGAATGGTCTTCCTCCTGCTCAC

Table 2
Direct PCR Primers for sequence analysis of *ChSA* and *ChSB* from *H. armigera*.

Primer name	Site of binding	Sequence (5′–3′)
HaChSB-F1	−4824	GAAGAAAAGATGCTTGAACCG
HaChSB-R1	49	CTGTCATCTCCCAAACCTGTGAA
HaChSB-F2	1	ATGGCGACGAAACCCAAGACTCC
HaChSB-R2	2197	GGAGCCCTGGCACGAAGCACA
HaChSB-F3	2151	TAAAGGCACAATGTTGATGAATGCGA
HaChSB-R3	10,131	GTAACATTGAACACCAAAATTGC
HaChSB-F6	9313	TCGTAATGCTGAACTCTTTGTTCGT
HaChSB-R6	10,892	AGTGAAGTACCAGTTGAGCTGAACAT
HaChSB-F7	10,063	ACCGTACACATCGTGATCGTTC
HaChSB-R7	12,816	GGAGGTAGTGATCGGTATCACGC
HaChSA-F1	−12,668	CACGCATAGTCCAACACACTGAA
HaChSA-R1	−7684	CCACGTAGTCTTGTCTGCCCT
HaChSA-F2	−7663	AGGGCAGACAAGACTACGTGG
HaChSA-R2	57	GCTCRTCGTCGGRSTYGYCGC
HaChSA-F3	37	GCGRCRASYCCGACGAYGAGC
HaChSA-R3	4946	TGGCAATCTTTGAGAGTAGGCA
HaChSA-F4	4925	TGCCTACTCTCAAAGATTGCCA
HaChSA-R4	6327	CAACGAAGGTGGTAGGTGACC
HaChSA-F5	6292	CGCAACAGTTAATTGGGTCAC
HaChSA-R5	9814	CGATTCACTTGGGAGTCGTCGTCACT
HaChSA-F6	9752	CATTTTCTTGGACGACGCATTCG
HaChSA-R6	15,572	TCTACCCTGGAAGGAAACTTGATT
HaChSA-F7	14,575	GGTTCTGTTCGAAGAAGGTTAGCAT
HaChSA-R7	16,026	CATTAGGTTAATGCCGTAGCTTCTG