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Short Communication

A single-base deletion in an ABC transporter gene causes white eyes, white eggs, and translucent larval skin in the silkworm *w-3*^{oe} mutant

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ABSTRACT

The *w-3*^{oe} silkworm mutant has white eyes and eggs due to the absence of ommochrome pigments in the eye pigment cells and serosa cells. The mutant is also characterized by translucent larval skin resulting from a deficiency in the transportation of uric acid, which acts as a white pigment in larval epidermal cells. A silkworm homolog of the fruitfly *white* gene, *Bmwh3*, a member of ATP-binding cassette transporter superfamily, was mapped on the *w-3* locus. The *w-3*^{oe} mutant has a single-base deletion in exon 2 and a premature stop codon at the 5′ end of exon 3. These results show that *w-3* is equivalent to *Bmwh3* and is responsible for the transportation of ommochrome precursors and uric acid into pigment granules and urate granules, respectively.

1. Introduction

Insect ommochrome and pteridine pigments are often accumulated in specific pigment granules of eye pigment cells and epidermal cells (Summers et al., 1982). In *Drosophila* eyes, three ATP-binding cassette (ABC) transporter genes, *white*, *scarlet*, and *brown*, are responsible for the accumulation of pigment precursors in pigment granules (Ewart and Howells, 1998). The ABC transporters are classified in two types: full transporters and half transporters. The full transporters, which function as monomers, have two transmembrane domains and two ATP-binding domains. The half transporters such as the White, Scarlet, and Brown proteins comprise only one transmembrane domain and one ATP-binding domain and work as dimers. The null mutants of *scarlet* and *brown* lack ommochrome and pteridine, respectively, while the *white* mutants are deficient in both pigments, thus resulting in white eyes (Ewart and Howells, 1998). The White protein forms a heterodimer with Scarlet to transport ommochrome precursors and with Brown for pteridine precursors. These transporter proteins are located in the membrane of pigment granules to transport pigment precursors from the cytoplasm into the granules (Mackenzie et al., 2000). In the silkworm *Bombyx mori*, eye pigment cells and diapausing egg serosa cells accumulate ommochrome in their pigment granules (Kikkawa, 1953). Pteridines do not contribute to the silkworm eye and egg colors. Null mutants of *w-3*, e.g., *w-3*^{oe} and *w-3*^{ol}, have white eyes and eggs because they lack ommochrome pigments (Goldsmith, 1995). They also exhibit the distinct phenotype of translucent larval skin. The epidermal cells of normal silkworms accumulate uric acid within urate granules, which make the skin white and opaque, while those of the *w-3* mutants lack this property (Tamura and Akai, 1990). The mutants exhibit normal xanthine dehydrogenase activity to produce uric acid mainly in fat body but are deficient in the ability to incorporate uric acid into the urate granules of the epidermal cells (Eguchi, 1961; Tamura and Sakate, 1983). These data suggest that the *w-3* gene encodes a transporter responsible for both uric acid and ommochrome precursors. The silkworm gene *Bmwh3*, which encodes an ABC half transporter homologous to the *Drosophila* White protein, is a candidate equivalent of *w-3* (Abraham et al., 2000). Both *Bmwh3* and *w-3* are found on chromosome 10, and *Bmwh3* mRNA is decreased in *w-3* mutants. Knocking out *Bmwh3* by embryonic RNAi causes translucent larval skin and white eggs (Quan et al., 2002). However, the full genomic region of *Bmwh3* must be sequenced and compared between normal and mutant silkworms to conclude that *Bmwh3* is indeed identical to *w-3*. Here

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we show that *Bmwh3* is equivalent to *w-3* by mapping both genes to the same locus and identifying a structural change in the *Bmwh3* gene that corresponds to the *w-3*^{oe} mutant.

2. Materials and methods

A silkworm normal strain, C108, was obtained from GenBank, National Institute of Agrobiological Sciences (Kobuchizawa, Japan). The e02 (*w-3*^{ol}/*w-3*^{ol}) and e03 (*w-3*^{oe}/*w-3*^{oe}) silkworm strains were provided by the National Bio-Resource Project (NBRP) of the Ministry of Education, Culture, Sports, Science and Technology of Japan. The linkage and recombination value between the *w-3* and *Bmwh3* genes was estimated by PCR product length polymorphism. A female moth of the C108 (+/+) strain was mated with a male moth of the e02 strain (*w-3*^{ol}/*w-3*^{ol}) to obtain F_1 offspring. To confirm that the two genes are on the same chromosome, an F_1 female moth was backcrossed to an e02 male to obtain normal and translucent larvae. To calculate the recombination value, e02 females were mated with F_1 males. Genomic DNA samples extracted from the moths and larvae (third instar) were then used as templates for PCR using the primers W10L02 (5′-GGT TCA ATC CGA TCA TGT AAT AAG C) and W10U04 (5′-TGA GAT TTG GAC CTT CTA CGT TAA GAC).

A silkworm BAC library was screened, and a *Bmwh3*-positive clone was kindly donated by Dr. Y. Yasukochi (NIAS, Japan). The BAC DNA was subcloned into pBluescript vectors and sequenced. Genomic DNA was extracted from the silk glands of fifth-instar larvae. mRNA was purified from 120-h embryos with a QuickPrep *Micro* mRNA Purification Kit (Amersham Biosciences) and used for cDNA synthesis with a First Strand cDNA Synthesis Kit (Roche) to obtain the full-length 5′ end of the *Bmwh3* cDNA with a GeneRacer Kit (Invitrogen). The *Bmwh3* gene of the *w-3* mutants was amplified by PCR from genomic DNA. All PCR products were sequenced directly or after cloning into a pGEM-T Easy vector (Promega). The nucleotide sequence was registered to DDBJ/EMBL/GenBank under accession number AB445460.

Amino acid sequences of ABC transporters were obtained from public databases and aligned using ClustalW (Thompson et al., 1994). After the removal of gap regions, phylogenetic trees were constructed using a maximum likelihood method with TREE-PUZZLE (Schmidt et al., 2002). Intron sites were compared on the aligned sequences considering the frame of translation.

3. Results and discussion

A previous study revealed the partial genomic structure of the *Bmwh3* gene (Abraham et al., 2000). Here we report its complete genomic sequence, including 13 exons spanning 56 kb from a BAC clone (Fig. 1A). The first exon reported by Abraham et al. (2000) was not found in the BAC clone. Screening of a BAC library showed that the exon is on a different chromosome, thus suggesting that the cDNA from the previous analysis was chimeric (data not shown). The transcription start sites estimated by an oligo-capping method were found slightly upstream of the exon previously designated as the second, which we now rename as exon 1. The translational start site was in exon 1, and the stop codon was in exon 13.

The phylogeny of ABC half transporters of subfamily G, which comprises the *white* proteins, was analyzed by comparing the amino acid sequences of insect and vertebrate proteins cloned previously or annotated in genomic studies. The relationship between insect and vertebrate ABC transporters was unclear because the insect genes were clustered independently of the

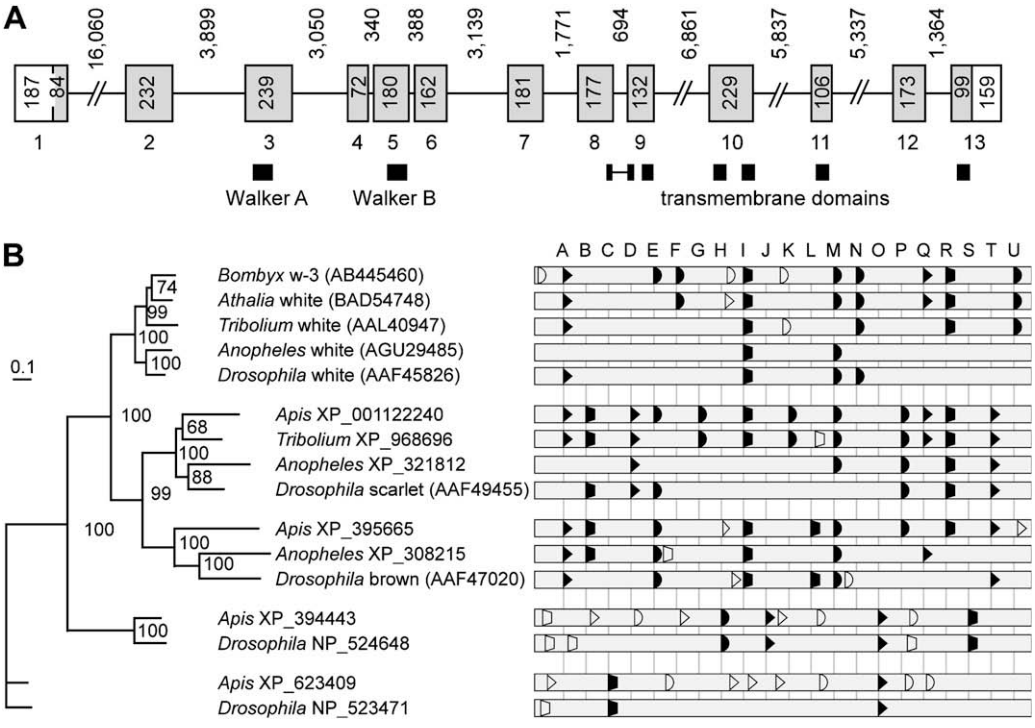

Fig. 1. The genomic structure and phylogenetic analysis of *Bmwh3*. (A) Schematic diagram of the complete genomic structure of *Bmwh3*. White and gray boxes indicate non-coding regions and open reading frames (ORFs), respectively. Horizontal lines indicate introns. Numbers in the boxes and over the lines indicate the length of corresponding exons and introns (bp). Exon numbers are shown below the boxes. (B) A phylogenetic tree and intron site comparison of *white*, *scarlet*, and *brown* as well as closely related members of ABC half transporter genes of subfamily G. Amino acid sequences were aligned using ClustalW (Thompson et al., 1994). After removing gap regions, the phylogenetic tree was constructed by a maximum likelihood method using TREE-PUZZLE (Schmidt et al., 2002). Numbers in the tree indicate bootstrap values (1000 replicates). Intron site comparison is shown on the right of the tree. Intron sites shared by two or more genes are indicated by solid marks and letters A–U, while non-conserved sites are shown with open marks. Half-circles indicate phase-0 introns (splicing between codons); triangles indicate phase-1 introns (splicing between the first and second nucleotides of codons); rectangles indicate phase-2 introns (splicing between the second and the third nucleotides of codons). Vertical lines show the conserved position of introns.