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A 25 bp-long insertional mutation in the *BmVarp* gene causes the waxy translucent skin of the silkworm, *Bombyx mori*

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ABSTRACT

In *Bombyx mori*, there are more than 35 mutant strains whose larval skin color is transparent. The waxy translucent strain *ow* is one of the oily mutants which lack accumulation of uric acid in the epidermis. Here we performed positional cloning of the *ow* gene using the *Bombyx* draft genome sequence. For fine structure mapping, we succeeded to narrow the *ow* linked region to approximately 150 kb, and identified the *ow* candidate gene by annotation analysis and DNA sequencing. The complete cDNA sequences of the *ow* gene from wild-type strains were 3501 bp-long and potentially encoded a protein of 920 amino acids. We found a 25 bp-long insertion in this gene in the *ow* mutant strain, resulting in a frame-shift mutation and generation of a premature stop codon. A BLAST search revealed that this protein had high homology to Varp, a recently identified protein containing a vacuolar sorting protein 9 domain and ankyrin repeats, and we termed the silkworm protein BmVarp. Varp has been shown to regulate endosome dynamics, suggesting that BmVarp may play an important role in the incorporation and/or accumulation of uric acid in the epidermis.

1. Introduction

The larval skin color of the silkworm, *Bombyx mori*, is generally white and opaque. However, more than 35 mutant strains have a transparent skin color, which makes them look like oily paper (Fujii et al., 1998). The transparent skin color concerns the inability to synthesize uric acid and the lack of accumulation of uric acid in the epidermis. Tamura and Sakate showed that these oily mutants are classified into two types (Tamura and Sakate, 1983). Three mutants, *oq*, *og*, and og^t, at two loci are caused by low or no xanthine dehydrogenase activity and thus they synthesize very little or no uric acid (Tamura and Sakate, 1983). Other mutants, most of which are of the same type and whose oily genes are located in more than 10 loci, result from the lack of incorporation and accumulation of uric acid from haemolymph to the hypodermal cells (Tamura and Sakate, 1983). Tamura and Akai (1990) compared the morphological change of the hypodermal cells between normal silkworms and the various oily mutants using ultra-thin sections for electron microscopy and found that the urate granules in oily mutants were abnormal in shape and distribution. These results suggest that incorporation and accumulation of uric acid are differently controlled by each oily mutation.

The waxy translucent strain, *ow*, is one of the oily mutants that lack accumulation of uric acid in the epidermis (Fig. 1). Electron microscopy revealed that the *ow* mutant was an intermediately transparent silkworm whose integument contains 10–20% of the uric acid of the normal strain, and whose hypodermal cells have spindle-shaped and disrupted granules (Tamura and Akai, 1990). The *ow* mutation has been reported only once by Chikushi (1960), suggesting that multiple alleles of the *ow* gene do not exist. The *ow* gene was mapped at 36.4 cM on silkworm genetic linkage group 17. This linkage group contains the following 7 loci: Black moth (*Bm*; 17-0.0; gene affecting pigmentation of the imago (black)), Wild wing spot (*Ws*; 17-14.7; black spot on the apex of the wing), non-susceptibility to DNV-2 (*nsd-2*; 17-24.5; controls non-susceptibility to infection by BmDNV-2), brown head and tail spot (*bts*; 17-30.1; head cuticle and anal plates pigmented reddish brown after 3rd or 4th instar), Non-infectious to densonucleosis virus (*Nid-1*; 17-31.1; controls non-susceptibility to infection by BmDNV-1), waxy translucent (*ow*; 17-36.4; larval skin moderately translucent.), and non-molting glossy (*nm-g*; 17-39.4; homozygotes survive about ten days as 1st instar larvae with lustrous skin, most die without molting) (Fujii et al., 1998). Recently, we have reported the isolation and identification of the mutant gene, *nsd-2*, on this linkage group

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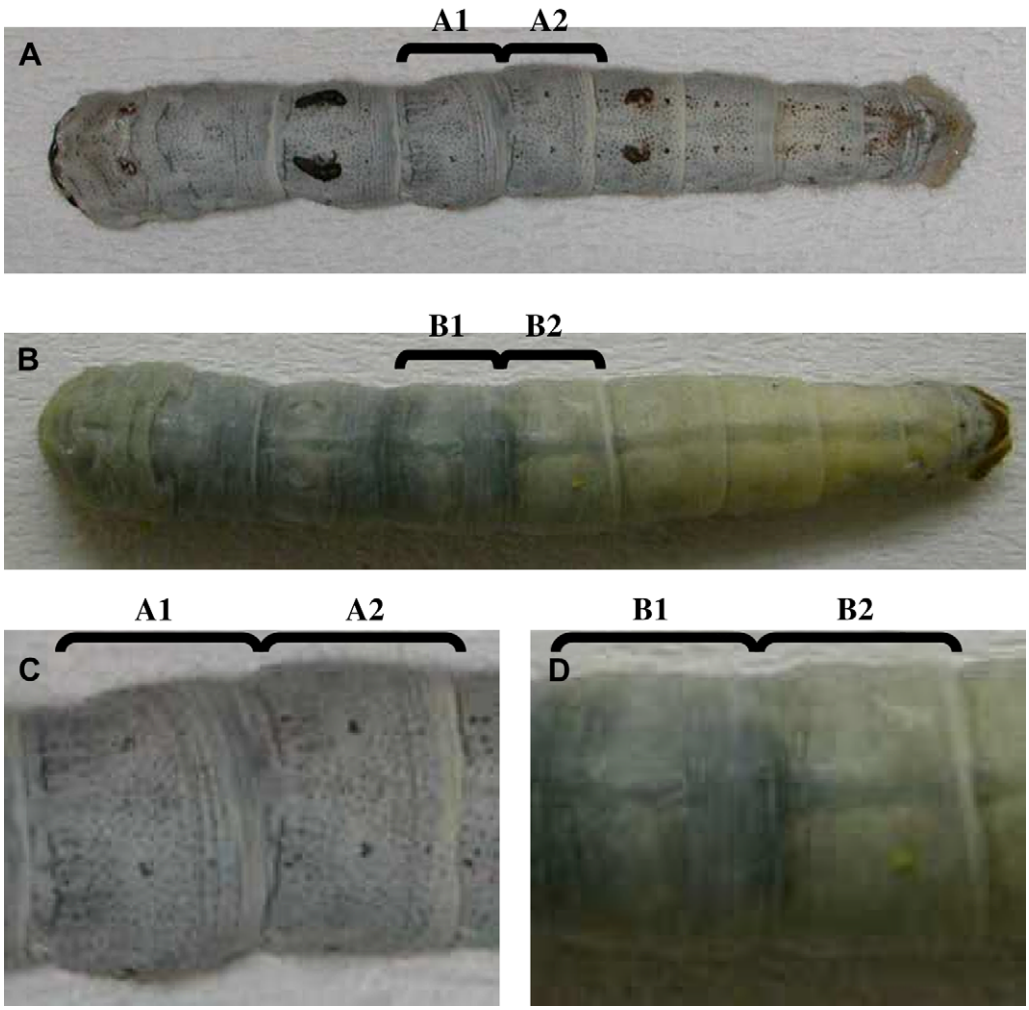

Fig. 1. Wild-type and *ow* mutant strains. (A) Wild-type strain ($+^{ow}/+^{ow}$), p50T. (B) *ow* mutant strain (*ow*/*ow*), w17. (C) Wild-type, segments A1 and A2. The larval skin color is white. (D) The phenotype of the *ow* mutant, segments B1 and B2. The larval skin color is transparent, and the dorsal vein is visible beneath the surface.

by positional cloning (Ito et al., 2008). Using many genetic and molecular markers on linkage group 17, we are attempting to isolate other genes mapped on this linkage group.

In this study, we report the isolation and identification of the *ow* gene by positional cloning. The *ow* gene encodes a Varp homolog, which we termed *BmVarp*. Varp is a recently identified protein which has been shown to regulate endosome dynamics (Zhang et al., 2006). Based on the collective data, we conclude that *BmVarp* may be involved in the incorporation and/or accumulation of uric acid in the epidermis of *B. mori*.

2. Materials and methods

2.1. Silkworm strains

The *ow* mutant strain (*ow*/*ow*) was w17 (Kyushu University); the wild-type strains ($+^{ow}/+^{ow}$) were p50T, C108T (The University of Tokyo), u42, u49, and w43 (Kyushu University). A single-pair cross between a female (p50T) and a male (w17) produced the F_1 offspring. For linkage and recombination analysis, F_2 progeny from the cross (p50T × w17) × (p50T × w17), and BC_1 progeny from w17 × (p50T × w17) were used. All silkworm larvae were reared on mulberry leaves at 25 °C.

2.2. Positional cloning

Positional cloning of the *ow* candidate gene was performed as described previously (Ito et al., 2008). Polymerase chain reaction (PCR) and single nucleotide polymorphism (SNP) markers were generated at each position on linkage group 17, and the markers that showed polymorphism between the parents were used for the genetic analysis of 91 F_2 and 380 BC_1 individuals with the *ow* phenotype (Tables 1 and 2).

2.3. Isolation of genomic DNA and total RNA

Genomic DNA was isolated from a small portion of the body from 3rd or 4th instar larvae using DNAzol (Invitrogen) according to the manufacturer's protocol. Total RNA was extracted from day 0 3rd instar larvae. Day 3 5th instar larvae were used for the isolation of total RNA from 16 tissues, including brain, prothoracic gland, salivary gland, fat body, trachea, hemocyte, testis, ovary, anterior silk gland, middle silk gland, posterior silk gland, foregut, midgut, hindgut, Malpighian tubule, and integument. Total RNA was also isolated from individuals of different stages, including eggs of day 0, 4, and 8, whole body from day 0, 1, 2, and 3 of 1st instar, day 0, 1, and 2 of 2nd instar, and day 0 of 3rd and 4th instar