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Short Communication

Characterization of a novel chromodomain-containing gene from the silkworm, *Bombyx mori*

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ABSTRACT

Heterochromatin protein 1 (HP1) is an evolutionarily conserved protein across different eukaryotic species, and is crucial in the establishment and maintenance of heterochromatin. HP1 proteins have two distinct functional domains, an N-terminal chromodomain (CD) and a C-terminal chromoshadow domain (CSD), which are required for the selective binding of HP1 proteins to modified histones. During our screen for HP1-like proteins in the *Bombyx mori* genome, we found a novel silkworm gene, *Bombyx mori chromodomain protein 1* (*BmCdp1*), encoding a putative chromobox protein with only two CDs. The BmCdp1 family proteins are closely related to the HP1 proteins, and most of them belong to insect lineages. qRT-PCR analysis indicated that *BmCdp1* mRNA was most abundantly expressed in early embryos, and relatively higher expression was observed in larval testes, hemocytes, and pupal ovaries. Western blot and immunostaining experiments showed that BmCdp1 was localized mainly in the nucleus of BmN4 cells. We searched BmCdp1-bound loci in the *Bombyx* genome by ChIP-seq analysis using Flag-tagged BmCdp1-expressing BmN4 cells. Combined with ChIP-qPCR experiments, we identified two reliable BmCdp1-bound loci in the genome. siRNA-mediated knockdown of *BmCdp1* in BmN4 cells and early embryos did not affect the expression of the gene located close to the BmCdp1-bound locus.

1. Introduction

A chromodomain (CD) is a conserved region of approximately 60 amino acids that was first identified in *Drosophila* modifiers of variegation (Paro and Hogness, 1991). Proteins containing the CD can be divided into three classes. The first class includes proteins such as heterochromatin protein 1 (HP1) that harbor an N-terminal CD followed by a chromoshadow domain (CSD) with weak, but significant sequence similarity to CD (Messmer et al., 1992; Platero et al., 1995). The second class includes proteins with only a single CD, e.g. *Drosophila* Polycomb protein (Pc). In the third class, such as mammalian DNA-binding/helicase proteins CHD1–4 and yeast protein CHD1, paired tandem CDs are found (Aasland and Stewart, 1995).

Members of the HP1 family have important roles in heterochromatin organization (Grewal and Elgin, 2002). The CD of HP1 proteins is shown to be crucial for heterochromatin recognition. Di- and trimethylated histone H3 lysine 9 (H3K9me2 and H3K9me3, respectively) are prominent binding partners for the CD of HP1a (Bannister et al., 2001). The CSD is a unique motif found in the HP1 family, and is involved in the dimerization and interaction of HP1 with diverse HP1-associating proteins (Brasher et al., 2000; Smothers and Henikoff, 2000). Truncated HP1a that contains CD, but not CSD was abundant in the heterochromatic chromocenter (Platero et al., 1995). In addition, a chimeric molecule in which the HP1's CD was replaced with the Pc's CD localized to both HP1 and Pc binding loci using endogenous CSD and exogenous CD, respectively (Platero et al., 1995). These results demonstrate that both domains can independently bind to the heterochromatin.

The silkworm, *Bombyx mori*, possesses two functional HP1 homologs, BmHP1a and BmHP1b (Mitsunobu et al., 2012). Biochemical experiments revealed that BmHP1s form homo- and heterodimers, and interacted with BmSu(var)3–9, which is a H3K9 methyltransferase (Mitsunobu et al., 2012). In this study, we identified a novel HP1-like CD-containing protein in the *Bombyx* genome, and characterized its gene structure, expression profile, cellular localization, and potential genomic targets.

2. Materials and methods

2.1. cDNA cloning of BmCdp1

We screened the *B. mori* EST databases, and identified three cDNA clones as showing significant homology to *Drosophila melanogaster*

Abbreviation: CD, chromo domain; CSD, chromo shadow domain; ChIP, chromatin immunoprecipitation; EST, expressed sequence tag; HP1, heterochromatin protein 1.

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HP1a. Two of the three cDNAs were *BmHP1a* (fdpeP27_F06) and *BmHP1b* (fdpeP18_G11) (Kawaoka et al., 2013; Mitsunobu et al., 2012) and the other (fdpeP15_A03) was a novel gene that we named *BmCdp1*. This cDNA clone was sequenced using an ABI Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) and an ABI Prism 3100 DNA Sequencer (Applied Biosystems).

2.2. Alignment and phylogenetic analysis

We obtained the amino acid sequences of proteins homologous to BmCdp1 (E-value of <1E−10) using the BLASTP program in the NCBI RefSeq protein database. To investigate ancestral relationships, we used the Molecular Evolutionary Genetic Algorithm (MEGA 5) (Tamura et al., 2011). A neighbor-joining tree was constructed and the reliability of the tree was tested by bootstrap analysis with 1000 replications.

2.3. Cells and insects

BmN4 cells were cultured at 27 °C in IPL-41 medium (Applichem) supplemented with 10% fetal bovine serum. The cell line stably expressing BmCdp1 with a FLAG-tag at the N-terminus was generated as previously described (Kawaoka et al., 2009). The non-diapause silkworm strain N4 was maintained as described previously (Wang et al., 2013).

2.4. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

Total RNA was prepared using TRIzol reagent (Invitrogen) according to the manufacturer's protocol and subjected to reverse transcription using avian myeloblastosis virus (AMV) reverse transcriptase with oligo-dT primer (TaKaRa). qRT-PCR analyses were performed with KAPA™ SYBR FAST qPCR kit (Kapa Biosystems) using specific primers listed in Table S1.

2.5. Biochemical fractionation and Western blotting

Biochemical fractionation of BmN4 cells expressing FLAG-tagged BmCdp1 was performed using the Subcellular Protein Fractionation Kit for Cultured Cells (Thermo scientific) according to the manufacturer's protocol. Subcellular localization was determined by Western blot with anti-Flag antibody (Sigma, F-1804, 1:1000) and anti-Actin antibody (Santa Cruz, sc-1616-R, 1:2000) as described previously (Kawaoka et al., 2009).

2.6. Immunostaining

We cultured BmN4 cells on cover slips in a Treated Cell Culture Dish (20 mm) (Corning) for 12 h. The cells were washed twice using 1 ml phosphate-buffered saline (PBS buffer; 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4) and fixed with 3.7% paraformaldehyde in PBS buffer for 10 min. After washing the cells three times using PBS buffer containing 0.1% bovine serum albumin (BSA), the cells were permeabilized with PBS buffer containing 0.5% Triton X-100 and 0.1% BSA for 10 min, and then incubated with a primary antibody (Mouse anti-Flag antibody (Sigma), 1:100) overnight at 4 °C. After incubation, the cells were washed three times with PBS buffer containing 0.1% BSA and incubated with anti-mouse IgG-Alexa Flour 546 (Invitrogen,

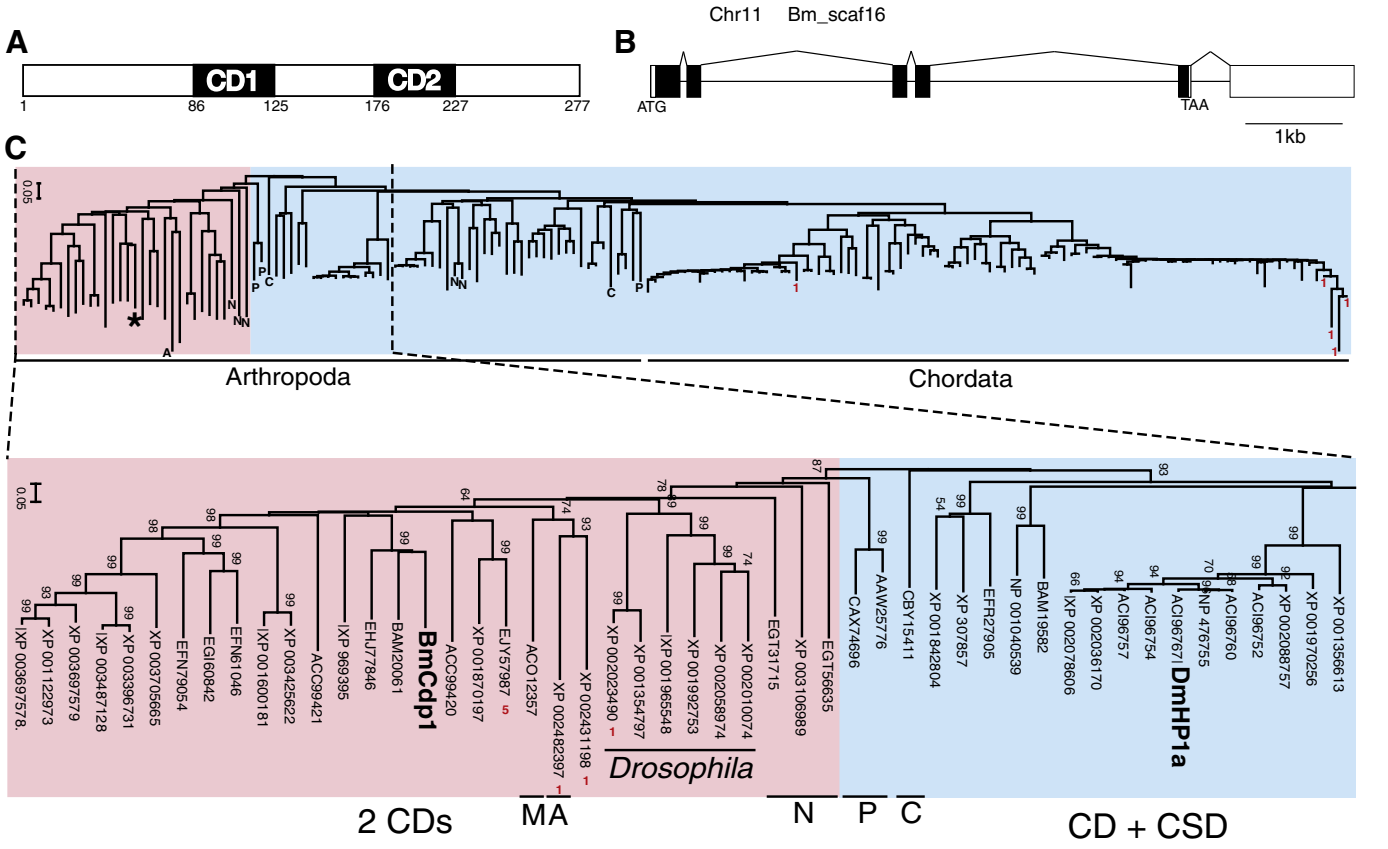

Fig. 1. Characterization of *BmCdp1*. (A) Domain structure of BmCdp1. BmCdp1 has two chromodomains (CD1 and CD2) but no chromoshadow domain. (B) Genomic and mRNA structure of *BmCdp1*. *BmCdp1* is located on chromosome 11 of *B. mori* (Bm_scaf16). Full and open boxes indicate ORF and non-coding regions, respectively. (C) Phylogenetic analysis of BmCdp1. Neighbor-joining tree was generated using the amino acid sequences of proteins showing homology to BmCdp1 (*E*-value of <1*E*−10). Although most BmCdp1 family proteins belong to insect lineages, few are from other phyla (N, Nematoda; P, Platyhelminthes; C, Chordata; A, Ascomycota; M, Maxillopoda). Proteins with either one or more than two CDs are indicated by red numbers. The asterisk in the upper tree indicates the position of BmCdp1. Bootstrap values of 1000 replicates are also indicated.