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CHD1 Assumes a Central Role during Follicle Development

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During *Bombyx mori* follicle development, fine-tuning of chorion gene expression is under the control of bidirectional promoters. In this work, we show that the silkmoth chromo-helicase/ATPase-DNA binding protein 1 (CHD1) ortholog is responsible for repositioning of nucleosomes on chorion promoters, where the factor binds specifically. Chorion genes, occupying a single chromosomal locus, rely on an almost identical set of *cis* elements for their differential expression. As a direct consequence of remodeling, interaction of C/EBP and TFIID with promoter elements is facilitated and ultimately leads to initiation of transcription. Appending of methylation marks to H3K4 in a temporal-specific manner is dependent on CHD1 binding to cognate *cis* elements and signifies gene activation. Overall, CHD1 is a critical factor for proper development of the follicular epithelium in terms of whole-cell chromatin arrangement.

Keywords: choriogenesis; chromatin remodeling; transcriptional regulation; *Bombyx mori*; H3K4 methylation

Introduction

The developing silkmoth ovariole serves as a model system for studying differential gene expression. The follicular epithelium develops alongside the oocyte and comprises the sole locus of chorion gene expression.[1] So far, our work has indicated a group of transcription factors that contribute in activation and repression of chorion genes via interaction with specific promoter *cis* elements.[2–6] Recent genomic studies by Yamamoto *et al.* revealed that chorion genes occupy a continuous locus on silkmoth chromosome 2.[7] Thus, genes differentially expressed during follicle development are situated in neighboring sites. This raises a reasonable question: how can genes controlled by promoters bearing highly similar *cis* elements be regulated in a temporal-specific manner? Chorion genes may presumably be differentially 'marked' as active or inactive by modifications on histone tails. Nucleosome remodeling may accordingly boost or attenuate transcription. Under this assumption, the participation of factors recognizing chorion gene promoters and repositioning nucleosomes in an ATP-dependent manner would be a prerequisite.

In this study, we propose that the silkmoth ortholog of chromo-helicase/ATPase-DNA binding protein 1 (CHD1) may assume this role. CHD1 proteins possess antipodal features: (i) a double chromodomain initially thought to participate in chromatin compaction but now recognized as an interaction surface for various chromatin components,[8,9] (ii) a domain bearing consecutive helicase motifs that consume ATP in order to unwind DNA,[10,11] (iii) a composite DNA-binding domain (DBD) composed of an AT hook similar to those of the HMGA family[12] and an HMG box, commonly found in HMGB proteins.[13] CHD1 orthologs have been identified in a wide spectrum of organisms.[10,14–16] They are considered able to act as monomers[17] and have been associated with transcriptionally active loci,[15,17] transcriptional elongation,[18,19] establishment of large-scale nucleosomal positioning,[17,20,21] recognition of chromatin modifications, and consequent remodeling.[20,22,23] However, only the mouse CHD1-DBD has been characterized so

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Abbreviations used: CHD1, chromo-helicase/ATPase-DNA binding protein 1; DBD, DNA-binding domain; ChIP, chromatin immunoprecipitation; BB, binding buffer.

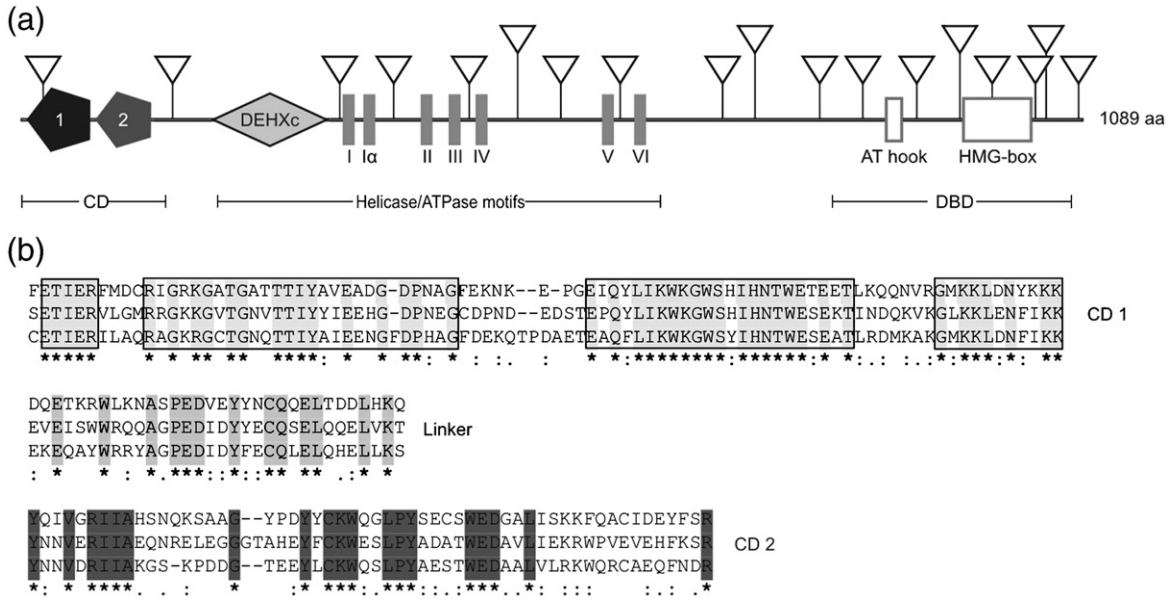

Fig. 1. Silkmoth CHD1 structural features. (a) Schematic representation of the CHD1 coding region. The two N-terminal chromodomains (CD1 and 2) are shown as polygons; the helicase/ATPase region is composed of a DEHXc motif (gray rhomb) and seven consecutive helicase motifs (gray boxes I-VI). In the C-terminal DBD, the characteristic AT hook and HMG-box motifs are shown as open boxes. The 18 introns of the coding region are represented by triangles and relative positions are shown. (b) Comparison of the CD amino acid sequences from hCHD1 (top), BmCHD1 (middle), and DmCHD1 (bottom). The first row shows comparison of the first CD sequences; the second row, that of the linker sequence; and the third row, that of the second CD. Asterisks denote identity and dots indicate physicochemical similarity of residues.

far.[14] Given that *Drosophila* CHD1 is functionally important for proper wing development and for female fertility,[24] we investigated the role of the silkmoth ortholog in follicular development. We present data that support its specific binding to chorion gene promoter elements, its involvement in modulating nucleosome positioning, in the deployment of specific protein–protein interaction, and, finally, its correlation to a hallmark of transcriptional activation,[25,26] the di-/trimethylation of H3 lysine 4.

Results

Isolation and structural characterization of the silkmoth Chd1 ortholog

The uncharacterized BmMCBP4 cDNA clone (3.9 kb)[1] was sequenced, and specific primers were used for PCR amplification of a 480-bp fragment from an early choriogenic cDNA library (constructed by Sourmeli *et al.*[3]). Sequencing confirmed identity of the product to BmMCBP4, which was, in turn, used as probe for screening of the cDNA library. Screening resulted in a single cDNA clone (~5 kb), which seems to be rare in the population. This clone contained a 1089-amino-acid open reading frame, which coded for a putative polypeptide highly similar to CHD1 proteins; the clone was accordingly named BmChd1 (GenBank accession no. DQ402511). The deduced amino acid sequence contained several conserved motifs (Fig. 1a); in the N-terminus, two tandem chromodomains (CD1–2) characteristic of HP1 proteins preceded the helicase/ATPase region (H/A), which contained a DEXHc box[27] and seven helicase motifs.[11] Close to the C-terminus lay the composite DBD containing an AT hook motif (R-K-R-G-R-P; core consensus) and an HMG box. The amino acid sequence was used for generating neighbor-joining cladograms after comparison to CHD1 proteins from various species. In cladograms, mammals, insects, plants, and fungi formed distinct outgroups under significant bootstrap values (data not shown). These features consented towards classification of the silkmoth factor in the CHD1 subfamily.

Unlike the yeast ortholog (yCHD1) or HP1 and Polycomb that use single chromodomains,[28–30] both human and *Drosophila* CHD1 double chromodomains assume a three-dimensional configuration, which allows interaction with methylated H3K4 tails.[31] Alignment of human and *Drosophila* CD1–2 sequences, as well as the linker sequence, with silkmoth counterparts revealed significant identity (Fig. 1b): 64%, 62%, and 50% identity between silkmoth and *Drosophila* and 59%, 41%, and 38% identity between silkmoth and human, respectively. In accordance, the silkmoth CHD1 chromodomains should confer similar properties to those of higher metazoans.

Use of the full-length BmChd1cDNA clone as probe for *in silico* screening of the *Bombyx mori* genomic sequence (Silkworm Genome Project†) and Expressed Sequence Tags (EST-SilkBase‡) databases showed that BmChd1 exists as a single copy gene carrying 19 intronic sequences. Introns (shown in

† http://sgp.dna.affrc.go.jp/
‡ http://www.ab.a.u-tokyo.ac.jp/silkbase/