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Genetics

CHD1 is concentrated in interbands and puffed regions of *Drosophila* polytene chromosomes

[chromatin/transcription/chromo domain/ATPase (helicase)]

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ABSTRACT **Previously, we reported on the discovery and characterization of a mammalian chromatin-associated protein, CHD1 (chromo-ATPase/helicase-DNA-binding domain), with features that led us to suspect that it might have an important role in the modification of chromatin structure. We now report on the characterization of the *Drosophila melanogaster* CHD1 homologue (dCHD1) and its localization on polytene chromosomes. A set of overlapping cDNAs encodes an 1883-aa open reading frame that is 50% identical and 68% similar to the mouse CHD1 sequence, including conservation of the three signature domains for which the protein was named. When the chromo and ATPase/helicase domain sequences in various CHD1 homologues were compared with the corresponding sequences in other proteins, certain distinctive features of the CHD1 chromo and ATPase/helicase domains were revealed. The dCHD1 gene was mapped to position 23C-24A on chromosome 2L. Western blot analyses with antibodies raised against a dCHD1 fusion protein specifically recognized a ≈210-kDa protein in nuclear extracts from *Drosophila* embryos and cultured cells. Most interestingly, these antibodies revealed that dCHD1 localizes to sites of extended chromatin (interbands) and regions associated with high transcriptional activity (puffs) on polytene chromosomes from salivary glands of third instar larvae. These observations strongly support the idea that CHD1 functions to alter chromatin structure in a way that facilitates gene expression.**

It has become increasingly apparent that eukaryotic cells have evolved elaborate systems to enable gene expression to occur in the context of chromatin. Alteration of chromatin structure in the form of compaction or extension is associated with many inducible genes and with the heritable expression patterns of genes involved in development. Proteins that implement these changes in chromatin structure and their mechanisms of action are beginning to be elucidated. However, there is still much to be learned about the process of chromatin remodeling and the interactions of chromatin constituents with other components of the gene expression machinery.

Our interest in this problem arose when we discovered a novel mouse protein, called CHD1 (chromo-ATPase/helicase-DNA-binding protein 1), which contains three identifiable domains that are present in proteins known to be determinants of chromatin structure (1). One of these domains, the 52-aa chromo (C) domain, is present in proteins that have been implicated in the process of chromatin compaction and the repression of gene expression (reviewed in refs. 2 and 3). Among these proteins are HP1, a 25-kDa protein that is mainly associated with pericentric/constitutive heterochromatin in *Drosophila* (2) and mammalian cells (4), and Polycomb, a 44-kDa protein, which has been implicated in the repression of homeotic genes in *Drosophila*, presumably by participating in locus-specific chromatin condensation or gene sequestration (5).

Another domain in CHD1 is a ≈500-aa ATPase/helicase (H) domain, which is present in a huge superfamily of proteins with a broad range of biochemical functions including transcriptional control, DNA recombination/repair, translation, and RNA processing. The CHD1 H domain is most closely related to the SNF2 family of proteins. Genetic and biochemical studies in yeast, *Drosophila*, and mammalian cells have implicated the SNF2 family of proteins as pleiotropic activators of transcription, presumably via their effect on chromatin structure (reviewed in refs. 6 and 7). These proteins are found in large multiprotein complexes with a combined molecular mass of $>10^6$ Da (8, 9). Recent evidence has indicated that these multiprotein complexes can elicit an ATP-dependent alteration in the nucleosomal organization of certain promoters, thereby facilitating the entry of both gene-specific and general transcription factors (10–12).

The third signature of CHD1 is its DNA-binding (D) domain, which has been localized to a 229-aa segment on the C terminal side of the H domain in mouse CHD1 (mCHD1; ref. 13). We have shown that CHD1 preferentially binds via minor groove interactions to DNA that contains (A+T)-rich tracts, including those in a matrix attachment region. The D domain contains sequence motifs known to be essential for the (A+T)-minor groove binding of other chromatin-associated proteins, such as histone H1, HMGI/Y, D1, and datin (14, 15).

Given the established or inferred functions of proteins that share one or another of the CHD1 signatures, it seems reasonable to assume that CHD1 plays an equally important role in the organization of chromatin structure and the regulation of gene activity. To study the function of CHD1 in a genetically pliable organism, we have isolated cDNA clones that encode the *Drosophila melanogaster* CHD1 homologue (dCHD1). When antibodies raised against a portion of dCHD1 were used to investigate the distribution of the protein on polytene chromosomes, we observed that CHD1 is concentrated in transcriptionally active regions of the genome.

MATERIALS AND METHODS

cDNA Cloning and Sequencing. A set of five overlapping cDNA clones encoding dCHD1 was isolated from a *D. melanogaster* 3- to 12-hr embryonic library obtained from T. Kornberg (University of California, San Francisco) and from 4- to 8-hr and 12- to 24-hr embryonic libraries constructed by Brown and Kafatos (16). The library screens were initiated with a fortuitously cloned 494-bp cDNA fragment (generously provided by Jonathan M. Rothberg, Yale University), which has strong sequence similarity to a region of the H domain of

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Abbreviations: C, chromo; H, ATPase/helicase; D, DNA-binding; dCHD1, *Drosophila* CHD1; mCHD1, mouse CHD1.

Data deposition: The sequence reported in this paper has been deposited in the GenBank data base (accession no. L77907).

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mCHD1, and completed with probes derived from subsequently isolated clones. In total, the clones span a region of 6242 bp. All of the clones were sequenced on both strands by a combination of manual and automated protocols [Sequenase 7-deaza-GTP kit (United States Biochemical); and ABI Prism 377 DNA sequencer (Perkin–Elmer)]. Sequence analysis was performed with the Applied Biosystems 373A DNA Sequencer Analysis Program and the Wisconsin Sequence Analysis Package (Genetics Computer Group, Madison, WI).

Fusion Protein Production and Antibody Purification. Rabbit polyclonal antibodies were raised against a glutathione *S*-transferase-*Cla*I-*Eco*RI fragment fusion protein (GST-CE) containing amino acid residues 1419–1644 of dCHD1. The fusion protein was produced as described previously (13) and purified by SDS/PAGE. Affinity-purified antibodies were isolated from the polyclonal antisera as described (13) with a column containing GST-CE fusion protein. GST-CE fusion protein (20 mg), purified by standard procedures (17) and according to manufacturer's specifications (Pharmacia), was combined with CnBr-activated Sepharose-4B (Pharmacia) to make the antigen column.

Extracts, Immunoblotting, and Immunolocalization of Proteins on Polytene Chromosomes. Nuclei were isolated from Oregon-R 0- to 24-hr *Drosophila* embryos obtained from overnight egg lays by the method of Franke *et al.* (18) or the method of Kamakaka and Kadonaga (19). The nuclei were extracted for 30 min at 4°C in either buffer B (18) containing 0.6 M NaCl or buffer HEMG (19) containing 0.6 M KCl. The extracts were centrifuged at 12000 × *g* for 20 min (18) or 100,000 × *g* for 1 hr (19), and the supernatants were retained as the nuclear extract. Whole 0- to 24-hr embryo extracts were obtained by boiling dechorionated embryos in SDS/PAGE loading buffer. SDS/PAGE and immunoblotting were carried out as described (13) with 30–50 μg of nuclear extract protein and an indeterminate amount of whole embryo protein. Primary antiserum was used at a 1:1000 dilution.

Polytene chromosome spreads were made according to the protocol of Zink *et al.* (20) from Oregon-R wild-type wandering third instar larvae. Squashes were stored in PBS/50% glycerol at −20°C overnight. Heat-shock spreads were obtained from larvae that had been placed in an Eppendorf tube and immersed in a 37°C water bath for 30 min. The immunostaining was carried out as described (13) except that the biotinylated secondary antibody was revealed with fluorescein isothiocyanate (Pierce), and the DNA was stained by including 25 ng of propidium iodide per ml (Sigma) in one of the final washes. Coverslips were mounted in Gel/Mount (Biomeda, Foster City, CA). and viewed with a Bio-Rad MRC-600 laser scanning confocal microscope equipped with a Nikon 60 × Plan Apo objective (oil immersion). Images were captured to a Silicon Graphics workstation (Mountain View, CA) and processed using Voxel View software (Vital Images, Fairfield, IA). The chromosome spreads were also visualized with phase-contrast microscopy to help identify the various puff loci.

RESULTS

Characterization of the *D. melanogaster* CHD1 homologue. Sequencing of a set of overlapping cDNA clones revealed an open reading frame of 5649 nt, which would encode a protein of 1883 aa (Fig. 1). A putative translational initiation codon, with stop codons preceding it in all three frames, was found at position 110 of the cDNA. The initiation sequence conforms to the Kozak consensus at key positions, most importantly at position −3, where it has an A residue (21, 22). Overall, the *D. melanogaster* protein sequence is 50% identical and 68% similar to the mouse CHD1 sequence, the *Drosophila* protein being slightly larger [1883 aa (211 kDa) versus 1711 aa (196 kDa)]. The spacing of the three signature domains and the sequences of the domains themselves are highly conserved

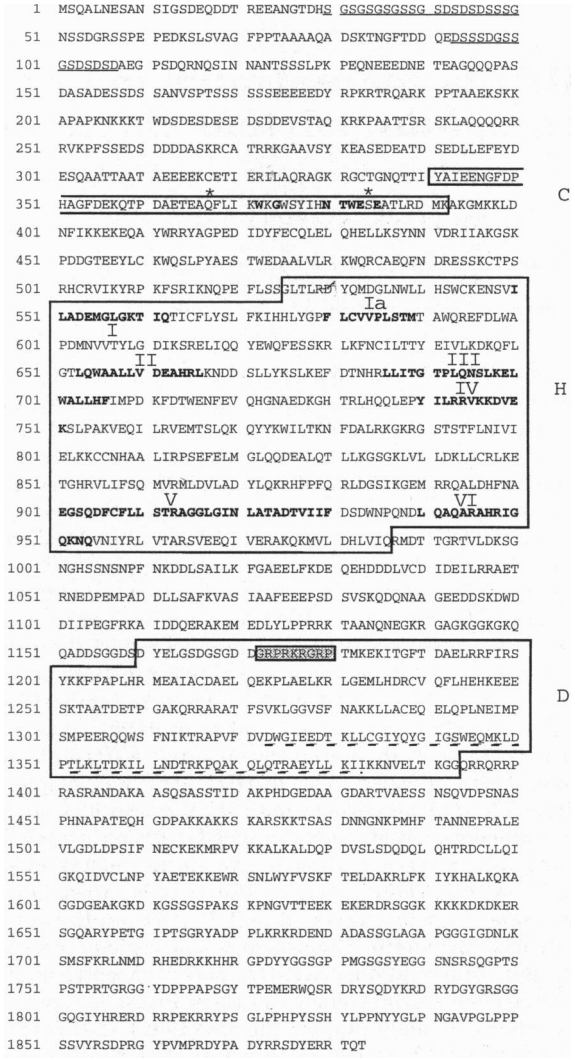

FIG. 1. Amino acid sequence of dCHD1 deduced from cDNAs. Numbers at the left refer to adjacent amino acids. The boxed sequences indicate the C domain, the H domain, and the D domain. Invariant residues within the C domain are in boldface, and asterisks denote residues diagnostic of the CHD1 C domains. Bold residues with adjacent roman numerals in the H domain indicate the seven highly conserved motifs that are found in this family. The shaded box in the D domain indicates a sequence with similarity to motifs that have been implicated in minor groove DNA-binding activity. The dashed underline sequence in the D domain denotes a highly conserved sequence in CHD1 homologues that is essential for the DNA-binding activity of mCHD1 (see ref. 13). The complete cDNA sequence of 6248 bp has been deposited in GenBank (accession no. L77907).

(Fig. 2*A*). A dot-plot comparison of the mouse and *Drosophila* proteins shows that there are significant stretches of sequence similarity even outside of the three signature domains (Fig. 2*B*). Based on these striking similarities, we consider the proteins to be homologues and have designated them as dCHD1 and mCHD1. Complete sequences of a CHD1 homologue in chicken (R. Griffiths, personal communication) and a putative homologue in yeast [designated as open reading frame 4 of chromosome V (GenBank accession no. L10718)] are also known. In addition, C and H domain sequences of probable homologues in other species are available in sequence databases.

An *in situ* hybridization analysis of *D. melanogaster* polytene chromosomes with a cDNA probe indicated that the gene encoding dCHD1 is located in region 23C–24A of chromosome 2L (data not shown). A Northern blot analysis of late embryonic, adult, and Schneider cell RNAs revealed a single

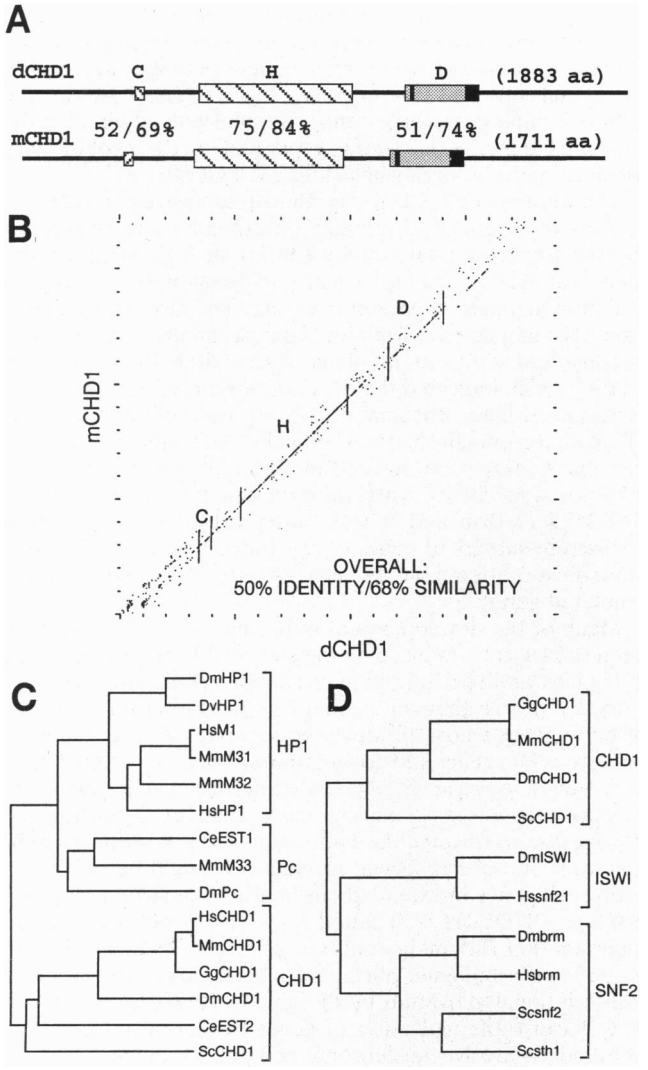

FIG. 2. Comparison of dCHD1 with homologues and members of related families. (*A*) Schematic representation of the primary structures of dCHD1 and mCHD1. The positions of the three signature domains are indicated by rectangles. The percent identity and similarity of the amino acid sequences of each domain is shown between the two drawings. The two black boxes within the D domain indicate the locations of the two conserved sequences highlighted in Fig. 1. (*B*) Dot-plot comparison of dCHD1 (abcissa) and mCHD1 (ordinate). The scales are marked at 100-aa intervals. The positions of the C, H, and D domains are indicated. The plot was obtained with the COMPARE program (Wisconsin Sequence Analysis Package) set for a word size of two and suppression of random off-diagonal points. (*C*) Dendogram of an amino acid sequence comparison of the C domains of CHD1 homologues, other C domain-containing proteins, and expressed sequence tags encoding C domains. (*D*) Dendogram of an amino acid sequence comparison of the H domains of CHD1 homologues and members of related families. The dendograms were generated by the PILEUP program (Wisconsin Sequence Analysis Package). Except for Gg CHD1, all of the sequences are accessible on the GenBank data base. Hs, *Homo sapiens*; Mm, *Mus musculus*; Gg, *Gallus gallus*; Dm, *D. melanogaster*; Dv, *Drosophila virilis*; Ce, *Caenorhabditis elegans*; and Sc, *Saccharomyces cerevisiae*.

major mRNA component of ≈7 kb (data not shown). The multiplicity of CHD1 mRNA species observed in various mouse cells (five discrete components; refs. 1 and 13) was not evident in *Drosophila*.

Distinctive Features of CHD1 Proteins. In the amino-terminal region of dCHD1, there are stretches that are especially rich in serine, glycine, and aspartic acid (Fig. 1, underlined). Similar stretches are also present in the amino terminal portion of mCHD1. Within highly conserved portions of the C domain (23–25), the various CHD1 homologues exhibit similar variations at certain positions. One noteworthy example is in the sequence NTWEXE (Fig. 1, positions 380 to 385). In virtually all other C domain-containing proteins, there is a proline at position X (indicated in boldface type), whereas in the CHD1 homologues there is a threonine or serine at this position. Another example is the glutamine at position 367, which is replaced by glutamic acid in the C domains of other proteins. Such distinctions may serve as diagnostic features of CHD1 C domains. This inference was reinforced by the dendogram shown in Fig. 2*C*. In this analysis, we compared the C domain sequences of members of the CHD1, Polycomb, and HP1 families by progressive, pairwise alignments and looked for clustering relationships. Indeed, we found that the CHD1 C domain appears to constitute a distinct subgroup that diverged early in evolution from the HP1 and Pc subgroups, classified by Pearce and colleagues (25).

The H domains of dCHD1 and mCHD1 are strikingly similar, both at the sequence level (Fig. 2*A*) and at the level of the spacing of the seven highly conserved motifs (Fig. 1, I–VI) that define this domain. Notably, motif II in the CHD1 homologues contains the sequence DEAH, as do various members of the SNF2 family of proteins (26, 27). Nevertheless, when a sequence comparison analysis was made on the H domains of various CHD1 homologues and SNF2-related proteins, the CHD1 proteins segregated as a distinct subgroup (Fig. 2*D*). The apparent novelty of the CHD1 H domain was also noted in a broad survey of H domain-containing proteins (28).

The D domain previously characterized in mCHD1 is also present at a similar location in dCHD1 (Fig. 2*A*). The two sequence motifs, D1 and D2 (Fig. 1, shaded box and dashed underline; Fig 2*A*, small black boxes), which were shown to be important for the sequence-selective DNA-binding characteristics of mCHD1 (13), are conserved at the sequence level, and their positions within the domain are also conserved.

Antibodies to dCHD1 Recognize a ≈200-kDa Protein from *D. melanogaster* Nuclear Extracts and Localize to Interbands and Puffs in Polytene Chromosomes. Polytene chromosomes are thought to be structurally and biochemically representative of the chromatin of interphase nuclei and can therefore provide valuable clues about the function of chromatin-associated proteins (29). To visualize the distribution of dCHD1 on polytene chromosomes, polyclonal antibodies were raised against a GST fusion protein that contained a C terminal fragment (residues 1419–1644) of dCHD1. This fragment was selected as an antigen to avoid crossreactivity with proteins containing related signature domains. The specificity of these antibodies was tested by an immunoblot analysis of embryonic nuclear and whole cell extracts (Fig. 3, lanes 1 and 2). In both extracts the antibodies recognized a protein of ≈200–210 kDa, which is the size predicted for dCHD1 (211,629 Da). These bands were not seen when the antiserum was applied to cytoplasmic extracts, nor were they seen when preimmune serum was substituted for the antiserum. To eliminate antibodies with incidental crossreactivity to other *Drosophila* proteins, the anti-dCHD1 antibodies were affinity-purified by elution from a column containing the GST fusion protein.

The affinity-purified antibodies, which specifically recognized the 200- to 210-kDa protein in nuclear extracts from embryos and cultured Schneider cells (Fig. 3, lanes 3 and 4), were used to stain polytene chromosomes from third instar larval salivary glands. To visualize the band/interband and puffing patterns of the polytene chromosomes, the chromosomes were counterstained with propidium iodide, which paints bands bright red, while leaving interbands and puffs as unstained or lightly stained regions. This pattern is reflective of the DNA density, with the compacted DNA within the bands staining more intensely than the less compacted (extended) interband and puffed regions (30, 31). dCHD1 local-

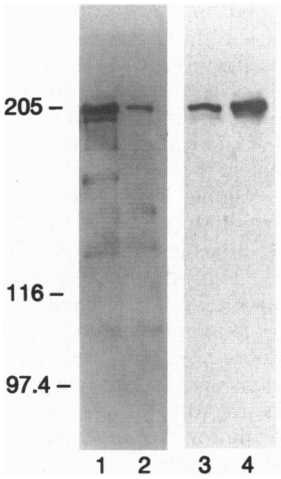

FIG. 3. Immunoblot analysis of *Drosophila* extracts with anti-dCHD1 antibodies. Proteins from *Drosophila* embryonic nuclear extracts (lanes 1 and 3), a whole-embryo extract (lane 2), and a Schneider cell nuclear extract (lane 4) were subjected to SDS/PAGE and immunoblotting with anti-dCHD1 polyclonal antiserum (lanes 1 and 2) or affinity-purified antibodies (lanes 3 and 4). The sizes of marker proteins in kDa are indicated at the left. A minor band of slightly greater mobility was observed in certain nuclear extracts but was not evident in the whole-embryo extract, even after lengthy exposure of the autoradiograph. This band may represent a degradation product.

ization was revealed with the green fluorescent dye, fluorescein isothiocyanate. In superimposed images, a green signal is obtained where the protein is located on extended chromatin, and a yellow signal is obtained where the protein overlaps with compacted DNA. As can be seen in Fig. 4, the dCHD1 staining was almost exclusively restricted to interband regions and certain puffs, where the chromatin is in the least compacted state. The distribution of CHD1 was locus-restricted in that not all of the interband/puff regions were immunostained (compare regions marked by open and closed arrowheads Fig. 4 *C* and *D*) The chromocenter, which represents the heterochromatic satellite-centrosomal DNA, did not show any dCHD1 signal.

We were able to identify a number of the puffs that stained with anti-dCHD1 antibodies. These include developmental puffs (Fig. 4 *C–E*, labeled arrows) and heat shock-induced puffs (Fig. 4*E*). Prominent among the developmental puffs brightly stained by CHD1 antibody are those that occur in response to pulses of the hormone ecdysone during the late larval and prepupal periods—e.g., puffs 75B, 74EF, 72D, 71DE, and 2B5. These puffs are correlates of the increased transcriptional activity of genes located within these loci (32, 33). At each locus, the CHD1 stain appears to be evenly dispersed over the entire puff region. In polytene chromosomes from larvae that had been heat-shocked for 30 min, CHD1 was located at several heat-shock puff loci—e.g. 63C, 64F, 93D, and 95D. Interestingly, the localization of CHD1 on the prominent developmental puffs was not detectably altered by the heat-shock treatment (compare puffs 75B and 74EF in Fig. 4 *C* and *D*).

DISCUSSION

Based on a sequence analysis of 6242 bp of cDNA encoding the *D. melanogaster* CHD1 homologue, we have determined that dCHD1 is a protein of 1883 aa, which is 50% identical and 68% similar to the mouse CHD1 sequence. The three signature domains, C, H, and D, are well conserved both in sequence and in their relative position within the protein. When the sequences of the C and H domains in various CHD1 homologues were compared with the sequences of similar domains in other proteins, certain distinctive features of the CHD1 C and H domains were revealed. These features have been conserved over a long period of evolution; whether or not they have subtle functional consequences remains to be established. Among the CHD1 homologues, the extent of sequence divergence in the C and H domains is strikingly parallel to the evolutionary distance between organisms (Fig. 2 *C* and *D*).

The finding that CHD1 is almost exclusively located in regions of extended chromatin (interbands) and regions of intense transcriptional activity (puffs) strongly supports the idea that CHD1 facilitates gene expression by helping to maintain an open chromatin structure. The absence of CHD1 from the compacted chromatin of bands and the chromocenter is consistent with our previous observations of mammalian CHD1, which indicated that it is not present in the condensed centromeric heterochromatin of interphase cells and that it is off-loaded from chromatin when cells enter mitosis (13). The fact that CHD1 is not located in all interbands indicates that it has locus specificity. This is also the case for related proteins, SNF2/SWI2, Brm, and Pc, which are involved in the regulation of discrete subsets of genes (3, 7). Indeed, Brm and Pc exert antagonistic effects on the same subset of developmentally regulated genes.

Many of the developmental puffs that harbor CHD1 have been shown to be induced by the steroidal hormone ecdysone in the late larval period (reviewed in ref. 34). Ecdysone, bound to its receptor, is thought to stimulate the transcription of a set of early genes whose products up-regulate the expression of numerous late genes and down-regulate the expression of their own genes. Several of the ecdysone-responsive genes are exceptionally large, requiring more than an hour for the polymerase to traverse the entire gene (35). It seems reasonable to suppose that special mechanisms might be needed to keep such genes in extended chromatin. The structural properties of CHD1 are well-suited for such a function. Interestingly, another H domain-containing protein, SNF2/SWI2, and its human homologue, hbrm, have been shown to influence transcriptional activation by the glucocorticoid receptor (36, 37). Because the activation of genes by steroid hormones is believed to involve nucleosome reorganization, it has been theorized that the SNF2/SWI2-like proteins may be at least partly responsible for these alterations. We hypothesize that CHD1 implements the activation of ecdysone responsive genes by helping to disrupt higher-order chromatin structure.

A comparison of the CHD1 interband/puff staining pattern with the patterns of other proteins that have been immunolocalized by similar protocols revealed some interesting similarities and differences. Like CHD1, RNA polymerase II and topoisomerase I are located mostly in interbands and puffs and are dispersed over the entire puff region (38, 39). However, in contrast to CHD1, these proteins are depleted from developmental puffs under heat-shock conditions. The fact that CHD1 remains in developmental puffs after a 30-min heat-shock treatment would seem to indicate that the association of CHD1 with interphase chromatin is relatively stable and that its presence at a particular locus does not require concomitant transcriptional activity. The CHD1 staining is clearly different from that of the boundary element protein, BEAF 32 (31), the heat-shock transcription factor, HSF (30), and the B52 splicing factor (40), all of which are located in discrete zones within or at the borders of puffs. Our previous studies of the DNA-binding activity of mCHD1 showed that it preferentially interacts with (A+T)-rich sequences and that multiple binding sites are present in a matrix/scaffold attachment region fragment from a mouse immunoglobulin gene (13). Whether or not the localization pattern of dCHD1 on polytene chromosomes involves interactions with matrix/scaffold attachment region elements remains to be determined.

Proteins related to CHD1 via similarities in the H domain have been shown to exist in large multiprotein complexes.

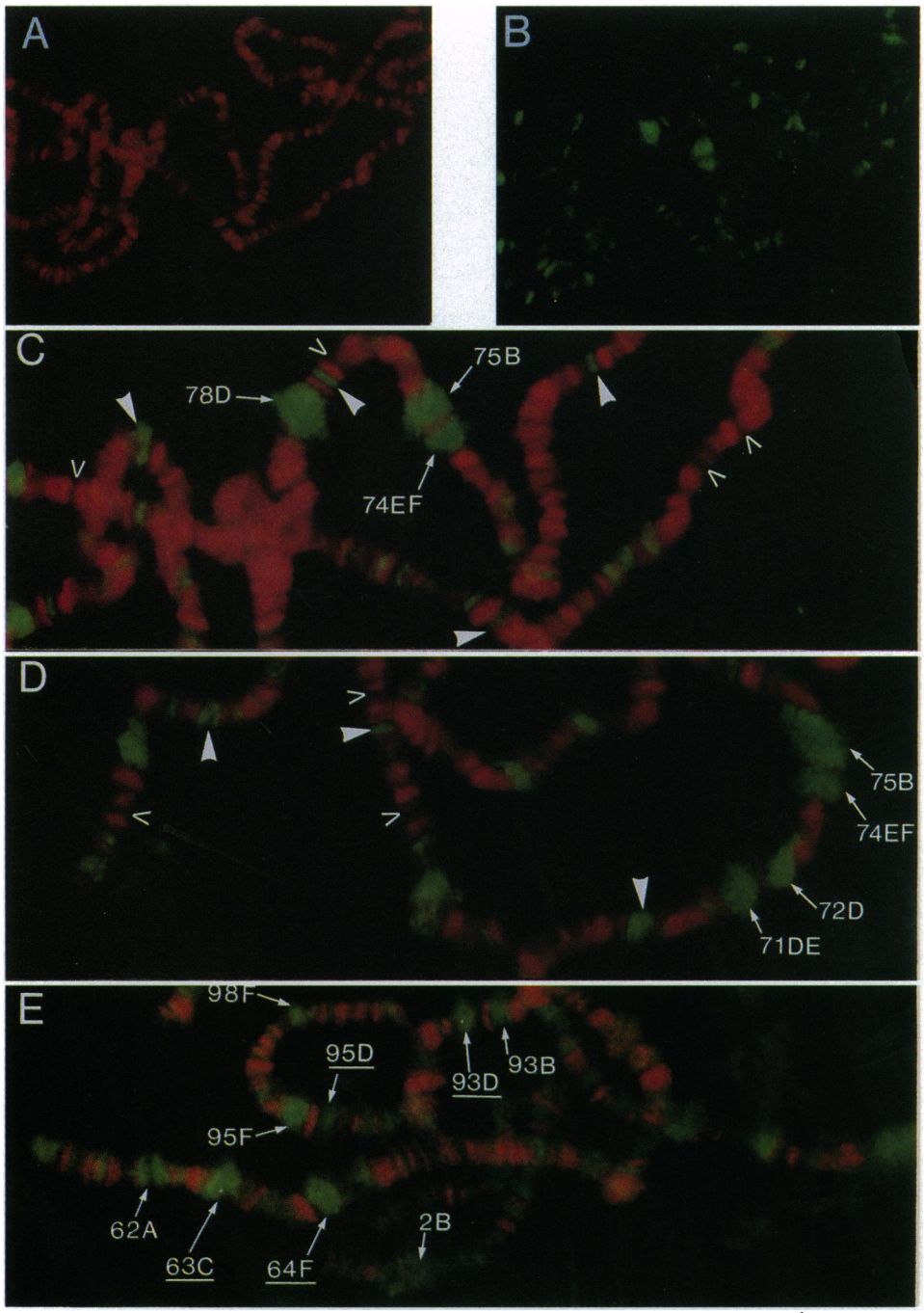

FIG. 4. dCHD1 immunolocalizes to interbands and puff regions of polytene chromosomes. Polytene chromosome spreads from normal (*A–C*) and heat-shocked (*D* and *E*) third instar larvae. (*A*) Chromosomes stained with propidium iodide (red) to visualize the DNA banding pattern and puff regions. (*B*) The staining pattern of anti-CHD1 antibody (green) on the same chromosomes. (*C–E*) Enlarged merged images of the propidium iodide and anti-dCHD1 staining, which vividly illustrate the localization pattern of dCHD1 with respect to the bands, interbands, and puffs. *C* is from the same spread as *A* and *B*. Arrows indicate puffs that stain with dCHD1 antibodies, labeled according to their locus designation. The heat-shock puffs are underlined. Closed arrowheads indicate interband regions that stain with the antibodies, and open arrowheads show interband regions that do not stain.

SNF2/SWI2/Brm proteins have been isolated from yeast, *Drosophila*, and mammalian cells as components of 2-MDa complexes (8, 9, 11, 41), which in some cases may be associated with the RNA polymerase II holoenzyme (42). Another H-domain protein, ISWI, has been identified as a component of a ≈500 kDa complex termed NURF, which is believed to participate in ATP-dependent chromatin remodeling at *Drosophila* heat-shock loci (43). What about CHD1? Preliminary biochemical studies of mammalian CHD1 indicate that it is a component of a ≈650-kDa complex (T. A. Owen-Hughes, J. L. Workman, and D.G.S., unpublished observations). Presumably, a similar complex exists in *Drosophila*. NURF contains an as yet unidentified component of ≈215 kDa, which conceivably might be CHD1. Alternatively, CHD1 could be part of a distinct complex with different locus selectivity and/or different functional attributes.

In conclusion, the results presented here are very suggestive of a role for CHD1 in facilitating the transcription of certain loci, presumably by functioning to remodel chromatin architecture. The identification of a CHD1 homologue in an organism such as *Drosophila*, which is ideal for exploring genetic interrelationships, should help us greatly in our efforts to understand the action of this interesting protein.

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