Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 308 (2003) 170–176
BBRC
www.elsevier.com/locate/ybbrc

CHD1 associates with NCoR and histone deacetylase as well as with RNA splicing proteins

Helen H. Tai,[a,b,*] Margit Geisterfer,[a] John C. Bell,[a,c] Mariko Moniwa,[d] James R. Davie,[d] Lorrie Boucher,[a] and Michael W. McBurney[a,c]

[a] *Ottawa Regional Cancer Centre, Ottawa, Ont., Canada*
[b] *Department of Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa, Ont., Canada*
[c] *Department of Medicine, University of Ottawa, Ottawa, Ont., Canada*
[d] *Manitoba Institute of Cell Biology, Winnipeg, MB, Canada*

Received 19 May 2003

Abstract

CHD1 is one of a family of nuclear proteins containing two chromodomains, a SWI/SNF-like helicase/ATPase domain and a DNA binding domain. We found that CHD1 co-immunoprecipitates with histone deacetylase (HDAC) activity and that CHD1 also associates with NCoR, a transcriptional corepressor, in yeast two-hybrid and in vitro pull-down assays. NCoR is known to associate with HDACs to effect its repressive activity, suggesting that the predicted chromatin remodeling activity of CHD1 plays a role in this repression. Yeast two-hybrid assays also showed that CHD1 interacts with splicing proteins mKIAA0164, Srp20, and SAF-B. Splicing assays show that CHD1 overexpression can affect alternative splicing. These results suggest that CHD1 may function in both chromatin mediated transcriptional repression and RNA splicing.

Modulating chromatin structure has emerged as an important step in the control of gene expression. Chromatin remodeling refers to changes in the positioning of nucleosomes and requires ATP as an energy source [1]. In addition to ATP-driven chromatin remodeling, modification of histone proteins by acetylation, methylation, ubiquitination, or phosphorylation can change chromatin structure and activity [2]. Several transcriptional activators have intrinsic histone acetylase activity linking histone acetylation to transcriptional activation [3]. Histone deacetylases (HDACs), on the other hand, are thought to mediate transcriptional repression as part of multiprotein complexes [3] such as the mammalian Sin3 complex which consists of Sin3, SAP18, SAP30, HDAC1, HDAC2, RbAp46, and RbAp48 [4–6]. The Sin3 complex is recruited to specific chromatin sites by transcriptional corepressor proteins, such as SMRT, NCoR, Mad, and Ume6 [7–11].

NuRD is another multiprotein complex in mammalian cells that consists of HDAC1, HDAC2, RbAp46, and RbAp48 along with CHD3, CHD4, MTA1, MTA2, and MBD3 [12–15]. The NuRD complex links proteins capable of histone deacetylation and chromatin remodeling and is associated with transcriptional repression [16]. CHD3 and CHD4, members of the CHD family of proteins, are the components of the NuRD complex responsible for its chromatin remodeling activity [17]. CHD3 and CHD4, like several other chromatin remodeling proteins, have a SWI/SNF2-like helicase/ATPase domain. All members of the CHD family contain two chromodomains near the N-terminus of the protein, a SWI/SNF2-like helicase/ATPase domain and a DNA binding domain [18]. The chromodomain is associated with repressive chromatin effects because it is present in proteins involved in gene silencing [19] and it functions as an interaction domain [20]. In addition, CHD proteins also have a C-terminal DNA binding domain. The CHD1 DNA binding

* Corresponding author. Present address: Canadian Forest Service, Natural Resources Canada, P.O. Box 4000, Fredericton, NB, Canada E3B 5P7. Fax: +506-452-3525.
E-mail address: htai@nrcan.gc.ca (H.H. Tai).

0006-291X/$ - see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/S0006-291X(03)01354-8

domain binds to AT-rich DNA [21], but little else is known about DNA binding properties for CHD proteins.

We are interested in CHD1, a mouse member of the CHD family. Studies have shown that CHD1 can associate with SSRP1, a protein involved in transcription regulation [22] and the *Drosophila* homolog, dCHD1, is localized to puff and interband regions of polytene chromosomes associated with transcriptional activity [23]. This information suggests that CHD1, unlike CHD3 and CHD4, may function in transcriptional activation rather than repression. Recent studies have shown that the CHD1 homologs in yeast, *Saccharomyces cerevisiae* Hrp1, and *Saccharomyces pombe* Chp1p are involved in transcription elongation [24,25] and termination [26].

In this study we present evidence that mouse CHD1 is associated with HDAC activity and with NCoR, a corepressor of nuclear hormone receptor transcription. This suggests a function for CHD1 in repression of gene expression. Interestingly, we also found that CHD1 interacts with splicing proteins and can affect splice site selection of a test reporter transcript. Together these studies suggest that CHD1 is involved in a broad range of functions in the nucleus.

Materials and methods

Co-immunoprecipitation. 293T human kidney epithelial cells were transfected with Fugene (Boehringer–Mannheim) according to manufacturer's instructions. P19 embryonal carcinoma cells [27] and transfected 293T cells were lysed with 10 mM Tris, pH 7.5, 150 mM NaCl, 5 mM EDTA, and 1% Triton X-100 with protease inhibitors (200 μg/ml PMSF, 2 μg/ml aprotinin, and 5 μg/ml leupeptin). One hundred micrograms of protein lysate was precleared with 50 μl of a 50% slurry of IgG beads (gammabind, Pharmacia) for 30 min at 4 °C. Anti-CHD-1 polyclonal antisera [21] (1/1000), anti-myc 9E10 monoclonal supernatant (1/200), or anti-HDAC1 polyclonal antibody (Santa Cruz, final dilution 1/1000) was added to the precleared protein lysate and incubated overnight with rotation at 4 °C. The next day 50 μl of a 50% slurry of IgG beads (gammabind, Pharmacia) equilibrated in lysis buffer was added to the protein lysate antibody mixture and incubated at 4 °C with rotation for 30 min. The beads were pelleted with a brief centrifugation and washed four times with lysis buffer containing protease inhibitors. Precipitated proteins were analyzed for the presence of HDAC activity or eluted from beads, run on 10% SDS–PAGE [28], immunoblotted, and probed with anti-myc 9E10.

Myc-tagging CHD1 peptides. CHD1 a.a. 81–1711 (myc-CHD), a.a. 195–401 (myc-C1), and a.a. 81–532 (myc-C1C2) were fused in-frame with the 6-myc tag in the mouse Pgk-1 based expression vector [29]. All CHD-1 sequences were myc epitope tagged on the 5′ end.

HDAC assays. Immunoprecipitated complexes were washed five times with LS buffer (phosphate-buffered saline and 0.5% Nonidet P-40) containing 1 mM phenylmethylsulfonyl fluoride and protease inhibitors and resuspended in HD buffer (20 mM Tris (pH 8.0), 150 mM NaCl, and 10% glycerol). HDAC assays were performed as described in Hendzel et al. [30]. Briefly, immunoprecipitated complexes were incubated for 30 min at 37 °C with 180 μg (150,000 dpm) of acid-soluble histones isolated from [^{3}H]acetate chicken erythrocytes. Released [^{3}H]acetate was extracted using ethyl acetate and quantified by scintillation counting.

Yeast two-hybrid analysis. The CHD1 C1 bait containing a.a. 195–401 of the CHD1 protein was fused to the GAL-4 DNA binding domain in the plasmid pAS2. The C1 bait was co-transformed with the mouse 17-day embryo Matchmaker two-hybrid library (Clontech, Palo Alto, CA) into the Y190 strain of *S. cerevisiae*. All yeast growth and two-hybrid screening protocols were carried out according to manufacturer's specifications (Clontech). β-Galactosidase expression assays with the X-gal substrate were used to confirm bait and prey interactions. The QIAprep Spin miniprep kit (Qiagen) was used to isolate plasmid from yeast with the following modifications of the manufacturer's instructions—yeast grown in liquid media were pelleted and resuspended in yeast lysis solution (2% Triton X-100, 1% SDS, 100 mM, 10 mM Tris–Cl, pH 8.0, and 1 mM EDTA) with 10 μg/ml RNase A instead of P1. Yeast two-hybrid clones were sequenced using automated dye terminator cycle sequencing (ABI). The BLAST algorithm [31] was used to search for sequence homology in GenBank [32].

Additional baits C1C2 and ΔC1C2 were constructed by fusing a.a. 81–532 of CHD1 and 81–299, respectively, with the GAL4 DNA binding domain of pAS2. The CLK1 bait is the full length cDNA fused with the GAL4 DNA binding domain [33].

Specific bait and prey were tested for interaction using co-transformation of the Y190 cell line followed by testing yeast colonies for β-galactosidase activity.

His-tagging NCoR. The 5′ end of NCoR was his-tagged using RT-PCR. The sequence of the oligonucleotide used to prime cDNA synthesis from C57/Bl mouse brain total RNA is CGATCGACA CGATCC (NCoR 569A). CCGCGTCCATACCATGAGAGGATC GCATCACCATCACCATCACATGTCAAGTTCAGGTTATCCTC CCAACCAGGGGGCG (hisNCoRATG) and GCTCTGTATCAG CTCTT (NCoR 552A) were used to PCR amplify a 534 bp sequence encoding the N-terminal end of NCoR. It was noted that the NCoR clone obtained from the C57/Bl mouse T-cell lymphoma yeast two-hybrid library [34] was identical in sequence with the cDNA clone obtained with RT-PCR but only 97% identical to the published sequence at the amino acid level. Therefore, the clone we obtained is likely C57/Bl mouse allele of NCoR. The his-NCoR RT-PCR product encodes the 6-his tag plus the MRGS epitope for antibody recognition [35] fused to the N-terminus of the protein. To create the his-NCoR expression plasmid the RT-PCR product was ligated into the mouse Pgk-1 based expression vector [29].

In vitro pull-down assay. 293T cells grown on 60 mm tissue culture dishes were transfected with the his-NCoR, his-NCoR plus myc-C1, or myc-C1 alone. The cells were lysed in 100 μl his-tag lysis buffer (HLB) (50 mM Tris–Cl, pH 8.0, 150 mM NaCl, and 1% NP-40) with protease inhibitors (200 μg/ml PMSF, 2 μg/ml aprotinin, and 5 μg/ml leupeptin). One hundred milligrams of protein from the lysate was added to 50 μl of a 50% slurry of Ni–NTA cross-linked agarose beads (Ni beads). The Ni beads and protein lysate were incubated overnight at 4 °C with rotation. The next day the Ni beads were pelleted with a brief spin and washed four times with HLB containing proteases and 10 mM imidazole. Proteins were eluted in 100 μl of his tag elution buffer (HEB) (50 mM sodium phosphate buffer, pH 6.0, 100 mM KCl, 2% glycerol, 0.2% NP-40, and 250 mM imidazole) with rotation for 1 min at 23 °C. Eluted proteins were run on 10% SDS–PAGE [28], immunoblotted [36], and probed with the 1/1000 anti-his antibody (Qiagen), 1/200 anti-myc 9E10 monoclonal supernatant, or 1/50 anti-tubulin antisera for analysis of protein interaction.

Splicing assays. Splicing assays were done according to Duncan et al. [37] using the *CR-1 Clk1* minigene splice reporter. CMV *Clk1* [37], myc-CHD1, myc-C1, or myc-C1C2 was co-transfected with the *Clk1* minigene splice reporter. RT-PCR was used to amplify alternative spliced transcripts expressed from the *Clk1* minigene.

Results

CHD1 associates with HDAC

Since the CHD family members, CHD3 and CHD4, are associated with HDAC1 and 2, we set out to determine if CHD1 is also associated with HDAC activity. Anti-CHD1 polyclonal antisera [21] were used to immunoprecipitate the endogenous CHD1 protein from P19 cell lysates. HDAC activity was detected in the immunoprecipitates indicating an association between CHD1 and HDAC (Fig. 1A). Anti-HDAC1 and non-immune serum immunoprecipitates were used as positive and negative controls for this experiment, respectively.

To confirm this interaction between CHD1 and HDAC, myc-tagged CHD1 constructs, myc-CHD and myc-C1 (Fig. 1B), were transfected into 293T cells and the 9E10 anti-myc monoclonal antibody was used to immunoprecipitate these myc-tagged proteins. Western blots and immunofluorescence experiments confirmed that the myc-tagged CHD1 and C1 constructs encoded proteins of the expected size and nuclear location (data not shown). HDAC activity was co-immunoprecipitated with both transfected proteins (Fig. 1B). The association of myc-C1 with HDAC indicates that the chromodomain may be the site responsible for HDAC binding. In contrast, the association between CHD4 and HDAC1 is mediated by the PHD fingers not the chromodomains in CHD4 [12].

CHD1 interacts with NCoR and splicing proteins

To further study the proteins associated with CHD1, a yeast two-hybrid screen was carried out in which the C1 peptide of CHD1 fused with the GAL4 DNA binding domain was used as bait (Fig. 2A). The yeast two-hybrid screen identified a mouse homolog of KIAA0164 [38] (mKIAA0164) as a protein that bound CHD1 (Fig. 2B). In an independent yeast two-hybrid screen, mKIAA0164 was also found to bind CLK1, a dual-specificity protein kinase that is known to phosphorylate SR splicing proteins and regulate alternative splicing, [33,34,37]. mKIAA0164 also contains an SR domain and recent studies showed that mKIAA0164 can affect splice site selection in an alternative splicing assay (unpublished data). The mKIAA0164 cDNA clones isolated from the yeast two-hybrid screens baited with CHD1 and CLK1 are labeled A and B, respectively (Fig. 2B). mKIAA0164-B is missing the SR-domain and interacts with both CHD1 and CLK1. mKIAA0164-A is smaller and located within B but interacts only with CHD1. Thus CLK1 and CHD1 may bind to different regions of the mKIAA0164 protein.

A number of other clones isolated from the CLK1 yeast two-hybrid screen were tested for interaction with

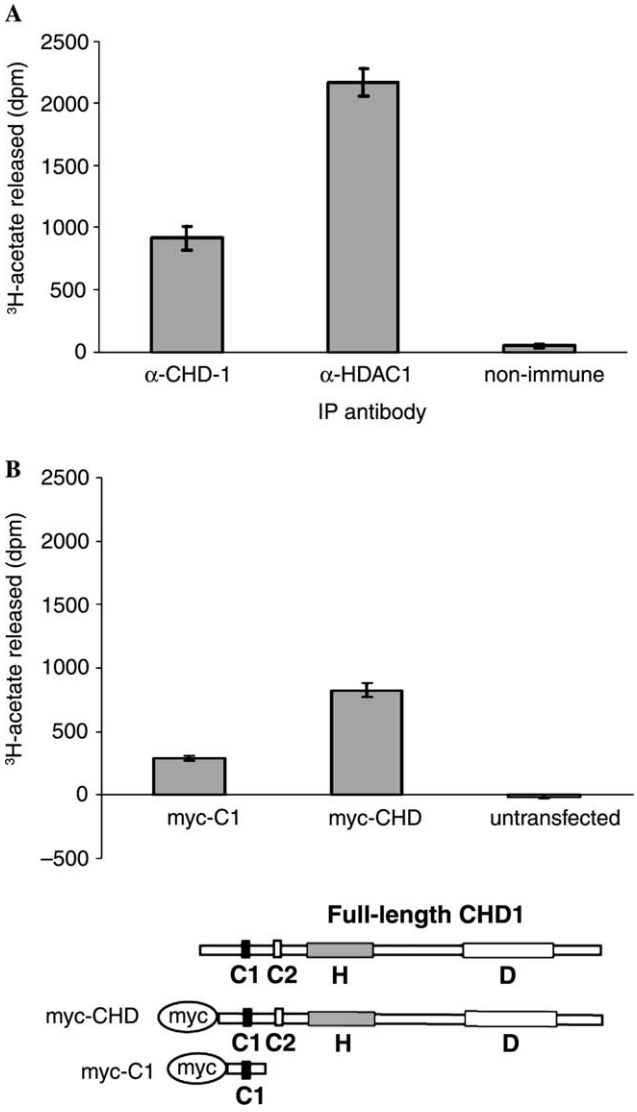

Fig. 1. CHD1 is associated with HDAC. (A) Anti-CHD1, anti-HDAC1, and non-immune serum were used to immunoprecipitate proteins from P19 cell lysates. The *y*-axis shows the amount of histone deacetylase activity present in protein immunoprecipitated with the indicated IP antibodies on the *x*-axis. Histone deacetylase activity is measured by the amount of [^{3}H]acetate released from [^{3}H]acetylated histones in the presence of immunoprecipitated proteins. (B) CHD1 a.a. 81–1711 (myc-CHD) and a.a. 195–401 (myc-C1) were myc_6 tagged and expressed in 293T cells. The anti-myc 9E10 monoclonal antibody was used to immunoprecipitate myc-tagged protein and the immunoprecipitates were tested for the presence of co-precipitated HDAC activity. Untransfected cell lysates served as the negative control. The bottom panel shows schematics of the CHD1 protein along with those encoded by the myc-tagged constructs. C1 and C2 refer to the two chromodomains, H is the helicase/ATPase domain and D is the DNA binding domain.

CHD1 using the yeast two-hybrid assay (Fig. 2B). Remarkably, three other proteins tested appeared to interact with both CHD1 and CLK1—NCoR, Srp20, and SAF-B. NCoR is a corepressor of nuclear hormone receptor transcription [39] and Srp20 and SAF-B are, like KIAA0164, involved in RNA splicing [40,41].

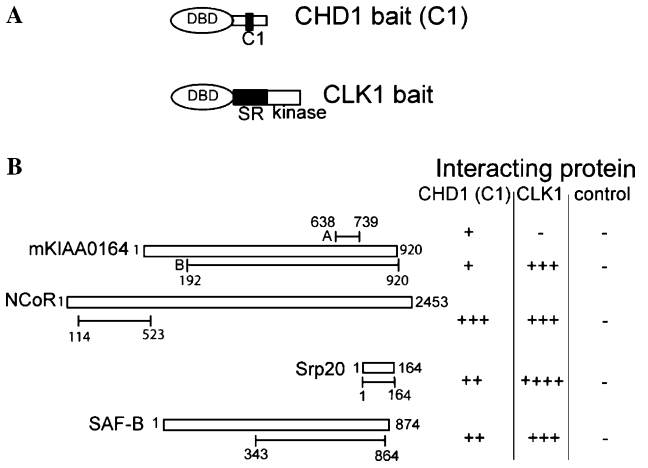

Fig. 2. CHD1 and CLK1 interact with similar proteins in the yeast two-hybrid system. (A) CHD1 a.a. 195–401 (C1) and full-length CLK1 were fused with the GAL DNA binding domain to create bait proteins for testing protein interactions in yeast two-hybrid assays. (B) The test constructs were co-transformed with the CHD1 bait (C1) or the CLK1 bait. Co-transformation with empty pAS vector served as a negative control. Transformed yeast were grown on leu-, trp, and his-selection media and tested for expression of β-galactosidase using an X-gal substrate. The bars above and below the rectangles indicate the location of the yeast two-hybrid clone. The amino acid numbers are also indicated. The SAF-B amino acid numbers are from the rat sequence while the rest are all from mouse sequences. The adjacent table indicates the strength of interaction as measured by the intensity of X-gal substrate conversion. The negative control was the empty pAS vector. "++++" indicates blue color product formation by 5 min, which is the strongest interaction, "+++" 15 min, "++" 30 min, "+" 45 min, "+/−" >1 h, and "−" no product formation.

No interaction was detected between CLK1 and the C1 peptide of CHD1 (data not shown).

In vitro pull-down assay confirms interaction between CHD1 and NCoR

To confirm the interaction between CHD1 and NCoR an in vitro pull-down assay was carried out with mammalian cell lysates. An NCoR cDNA encoding the N-terminal end (a.a. 1–523, his-NCoR) of the protein was tagged with the 6-histidine epitope and expressed in 293T cells along with myc-C1 Fig. 3), which contains the first chromodomain of CHD1. Ni–NTA agarose beads were used to pull down his-NCoR and associated proteins. The results show that myc-C1 was pulled down in the presence of his-NCoR, confirming that CHD1 and NCoR associated in mammalian cells (Fig. 3). Tubulin, a cytoplasmic protein, served as a negative control in this experiment. This experiment also shows that the chromodomain of CHD1 may be sufficient for association with NCoR.

NCoR is known to be associated with HDACs [42] so the interaction found between CHD1 and NCoR and the association of CHD1 with HDAC activity suggest that CHD1 may be present in a multiprotein

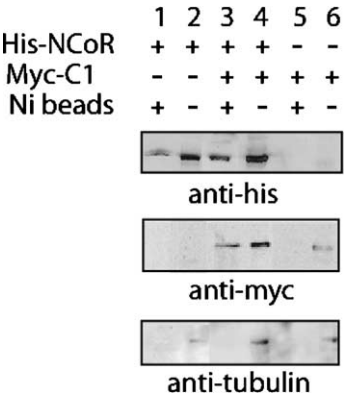

Fig. 3. In vitro pull-down assays confirm that NCoR interacts with CHD1. 293T cells were transfected with his-NCoR (lanes 1 and 2), Myc-C1 (lanes 5 and 6), or both (lanes 3 and 4) and proteins were either loaded directly onto 10% SDS–PAGE (lanes 2, 4, and 6) or Ni–NTA agarose beads were used to pull down his-NCoR and associated proteins (lanes 1, 3, and 5). Anti-his (top box), anti-myc (9E10) (middle box), and anti-tubulin (bottom box) were used to probe the same immunoblot.

transcriptional repressor complex along with NCoR and HDAC.

CHD1 and CLK1 interact with different regions of NCoR

To further define the region of NCoR that is responsible for association with CHD1 and CLK1, deletions of the NCoR cDNA were made and co-transformed with the CHD1 and CLK1 baits (Fig. 4). The association of NCoR with CHD1 did not require the SANT domain [50] and interaction was not lost until repressor domain I [39] was deleted. The interaction with NCoR was slightly decreased with the presence of the second chromodomain. However, there was no interaction with NCoR in the absence of both chromodomains (Fig. 4). Hence the interaction between CHD1 and NCoR requires the presence of at least one chromodomain of CHD1 and repressor domain I of NCoR.

CLK1 interaction with NCoR, on the other hand, depended on the presence of the SANT domain (Fig. 4). Deletion of the SANT domain resulted in a complete loss of interaction between NCoR and CLK1.

CHD1 and splicing

The interaction between CHD1 and splicing proteins implies that CHD1 may also influence pre-mRNA splicing. To test this idea, we co-transfected the *Clk1* minigene splice reporter (Fig. 5A) with either myc-CHD, myc-C1, or myc-C1C2 in 293T cells. Overexpression of CLK1 induced a shift towards exclusion of the alternatively spliced exon, as previously reported [37]. The ratio of transcripts with the alternate exon spliced in to spliced out is reduced by 50% in the

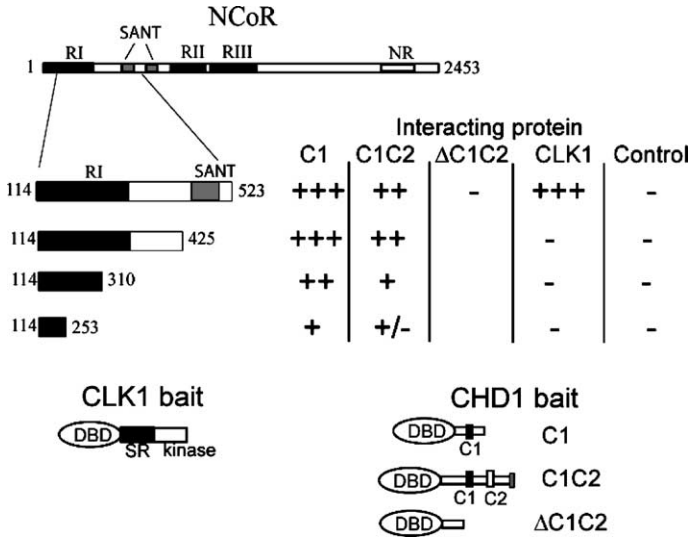

Fig. 4. CHD1 and CLK1 interaction with NCoR. Full-length NCoR protein sequence is shown at the top of the figure. Locations of the SANT domains, nuclear receptor interaction (NR) domain, and Repressor domains I, II, and II, (RI, RII, and RIII, respectively) are indicated. The table shows the results of the yeast two-hybrid assay testing for interactions between deleted NCoR proteins (shown on the left) and the CLK1 and CHD1 baits (C1, C1C2, and ΔC1C2) on the bottom. The pAS vector was the negative control. Relative β-galactosidase activity levels are shown as in Fig. 2.

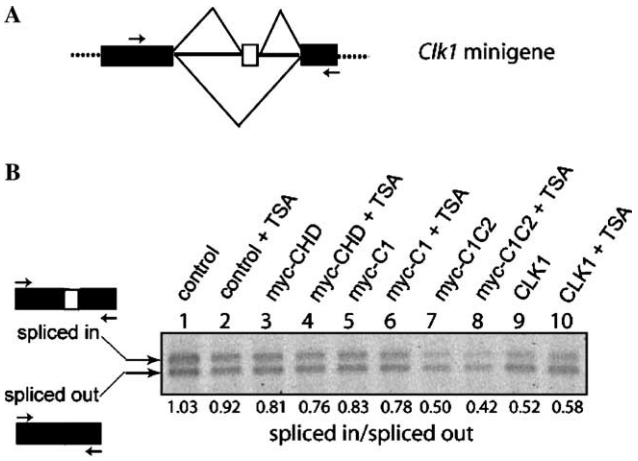

Fig. 5. Effect of CHD1 on splicing of the Clk1 minigene. (A) Schematic of the *Clk1* minigene splice reporter showing the alternatively spliced exon as an open box. Primers used for amplification by RT-PCR are indicated with arrows. (B) RT-PCR of Clk1 minigene alternatively spliced transcripts. Locations for the spliced in and spliced out transcripts are indicated. Lane 1 is the control transfection with the empty vector. Lane 9 shows the alternative splicing pattern with CLK1 transfection, lanes 3, 5, and 7 show the alternative splicing pattern when cells are transfected with the indicated CHD1 peptides (see Figs. 1 and 4). Lanes 2, 4, 6, 8, and 10 show the pattern of alternative splicing in the presence of TSA. The ratio of intensities of the spliced in/spliced out bands was calculated from the densitometric scan.

presence of CLK1 (Fig. 5B). Myc-CHD and myc-C1 induced a 20% shift towards exclusion of the alternatively spliced exon whereas the effect of myc-C1C2 on splicing was about 50%, the same as CLK1. These results suggest that CHD1 associates with splicing proteins in vivo. Since the CHD1 protein is associated with HDAC, the effect of a HDAC inhibitor, trichostatin A, on splicing was also tested. Trichostatin A had little effect on the splicing reaction in the presence or absence of CHD1 or CLK1 (Fig. 5B), indicating that HDAC is not involved in pre-mRNA splicing.

Discussion

One of the major conclusions of this work is that CHD1 is a component of a multiprotein complex that also contains NCoR and HDAC activity. The region of CHD1 surrounding and containing the first chromodomain appears to be the binding site required for its association with this complex, probably through interaction with the repressor domain I of the NCoR protein. NCoR is known to associate with a number of HDACs including HDAC3, 4, and 5 [43–49]. Xue et al. [15] found that CHD1 did not co-immunoprecipitate with HDAC1, suggesting that the CHD1/NCoR complex may contain only a subset of HDAC proteins. Another member of the CHD protein family, CHD4, associates with HDAC1 through its PHD finger consistent with the idea that different CHD family members are components of distinct multiprotein complexes.

The association of CHD1 with NCoR and HDAC suggests a role for this complex in transcriptional repression. Others have evidence to suggest that CHD1 has a role in transcriptional activation [22,23] while the yeast CHD1 homolog appear to play roles in transcription termination and elongation [24–26]. Thus, CHD1 appears to play a role in various aspects of gene expression. One explanation is that CHD1 is a general chromatin remodeling protein, which is recruited where necessary.

CHD1 and CLK1 bind to different regions of the NCoR protein. CHD1 appeared to bind through the repressor domain I while the SANT domain [50] is responsible for association with CLK1. Repressor domain I of NCoR is the region that associates with the Sin3 complex [8]. The SANT domain of NCoR associates with HDAC3 [43,51] and is also required for association with PRP4K, another protein kinase involved with splicing [52]. PRP4K is phosphorylated by CLK1 [53], suggesting that these proteins form a complex that associates with NCoR.

A second unexpected inference from our data is that CHD1 may be involved in RNA splicing. We found three RNA splicing proteins, mKIAA0164, Srp20, and SAF-B, associated with CHD1 in our yeast two hybrid assays. Another SR splicing protein, p54, interacted with CHD1 in a yeast two hybrid screen done by Kelley et al. [22]. Splicing assays using a splice reporter show that CHD1 modulated splice site selection, suggesting that CHD1 may be involved in pre-mRNA splicing. This suggests that the CHD1/NCoR complex has a role in both chromatin modification and RNA splicing. The association between NCoR and CLK1/PRP4K [52] is additional evidence that RNA splicing and chromatin structure are linked. There is also indirect evidence to suggest that RNA splicing and chromatin structure are linked. Transgenes carrying introns and exons are able to escape inactivation following integration into the genomes of transfected stem cells while similar transgenes with no introns are readily inactivated under similar conditions [54,55].

Acknowledgments

We are grateful to Robert Perry and David Stokes of the Fox Chase Cancer Centre in Philadelphia, USA, for their gift of the anti-CHD1 antisera used in our study and to Tony Pawson and Karen Colwill of the Samuel Lunenfeld Institute at Mount Sinai Hospital in Toronto, Canada, for the gift of the yeast two-hybrid clones interacting with CLK1. This work was supported by a grant from the Medical Research Council of Canada. H.H.T. was also supported by a fellowship from the Medical Research Council of Canada.

References

[1] A. Flaus, T. Owen-Hughes, Mechanisms for ATP-dependent chromatin remodelling, Curr. Opin. Genet. Dev. 11 (2001) 148–154.
[2] S.L. Berger, Histone modifications in transcriptional regulation, Curr. Opin. Genet. Dev. 12 (2002) 142–148.
[3] K. Struhl, Histone acetylation and transcriptional regulatory mechanisms, Genes Dev. 12 (1998) 599–606.
[4] C.A. Hassig, T.C. Fleischer, A.N. Billin, S.L. Schreiber, D.E. Ayer, Histone deacetylase activity is required for full transcriptional repression by mSin3A, Cell 89 (1997) 341–347.
[5] Y. Zhang, R. Iratni, H. Erdjument-Bromage, P. Tempst, D. Reinberg, Histone deacetylases and SAP18, a novel polypeptide, are components of a human Sin3 complex, Cell 89 (1997) 357–364.
[6] C.D. Laherty, W.M. Yang, J.M. Sun, J.R. Davie, E. Seto, R.N. Eisenman, Histone deacetylases associated with the mSin3 corepressor mediate mad transcriptional repression, Cell 89 (1997) 349–356.
[7] L. Nagy, H.Y. Kao, D. Chakravarti, R.J. Lin, C.A. Hassig, D.E. Ayer, S.L. Schreiber, R.M. Evans, Nuclear receptor repression mediated by a complex containing SMRT, mSin3A, and histone deacetylase, Cell 89 (1997) 373–380.
[8] T. Heinzel, R.M. Lavinsky, T.M. Mullen, M. Soderstrom, C.D. Laherty, J. Torchia, W.M. Yang, G. Brard, S.D. Ngo, J.R. Davie, E. Seto, R.N. Eisenman, D.W. Rose, C.K. Glass, M.G. Rosenfeld, A complex containing N-CoR, mSin3 and histone deacetylase mediates transcriptional repression, Nature 387 (1997) 43–48.
[9] L. Alland, R. Muhle, H. Hou Jr., J. Potes, L. Chin, N. Schreiber-Agus, R.A. DePinho, Role for N-CoR and histone deacetylase in Sin3-mediated transcriptional repression, Nature 387 (1997) 49–55.
[10] D. Kadosh, K. Struhl, Repression by Ume6 involves recruitment of a complex containing Sin3 corepressor and Rpd3 histone deacetylase to target promoters, Cell 89 (1997) 365–371.
[11] S.E. Rundlett, A.A. Carmen, N. Suka, B.M. Turner, M. Grunstein, Transcriptional repression by UME6 involves deacetylation of lysine 5 of histone H4 by RPD3, Nature 392 (1998) 831–835.
[12] Y. Zhang, G. LeRoy, H.P. Seelig, W.S. Lane, D. Reinberg, The dermatomyositis-specific autoantigen Mi2 is a component of a complex containing histone deacetylase and nucleosome remodeling activities, Cell 95 (1998) 279–289.
[13] Y. Zhang, H.H. Ng, H. Erdjument-Bromage, P. Tempst, A. Bird, D. Reinberg, Analysis of the NuRD subunits reveals a histone deacetylase core complex and a connection with DNA methylation, Genes Dev. 13 (1999) 1924–1935.
[14] J.K. Tong, C.A. Hassig, G.R. Schnitzler, R.E. Kingston, S.L. Schreiber, Chromatin deacetylation by an ATP-dependent nucleosome remodelling complex, Nature 395 (1998) 917–921.
[15] Y. Xue, J. Wong, G.T. Moreno, M.K. Young, J. Cote, W. Wang, NURD, a novel complex with both ATP-dependent chromatin-remodeling and histone deacetylase activities, Mol. Cell 2 (1998) 851–861.
[16] J. Ahringer, NuRD and SIN3; histone deacetylase complexes in development, Trends Genet. 16 (2000) 351–356.
[17] H.B. Wang, Y. Zhang, Mi2, an auto-antigen for dermatomyositis, is an ATP-dependent nucleosome remodeling factor, Nucleic Acids Res. 29 (2001) 2517–2521.
[18] T. Woodage, M.A. Basrai, A.D. Baxevanis, P. Hieter, F.S. Collins, Characterization of the CHD family of proteins, Proc. Natl. Acad. Sci. USA 94 (1997) 11472–11477.
[19] R. Paro, D. Hogness, The polycomb protein shares a homologous domain with a heterochromatin-associated protein of *Drosophila*, PNAS 88 (1991) 263–267.
[20] G. Cavalli, R. Paro, Chromo-domain proteins: linking chromatin structure to epigenetic regulation, Curr. Opin. Cell Biol. 10 (1998) 354–360.
[21] D.G. Stokes, R.P. Perry, DNA-binding and chromatin localization properties of CHD1, Mol. Cell. Biol. 15 (1995) 2745–2753.
[22] D.E. Kelley, D.G. Stokes, R.P. Perry, CHD1 interacts with SSRP1 and depends on both its chromodomain and its ATPase/helicase-like domain for proper association with chromatin, Chromosoma 108 (1999) 10–25.
[23] D.G. Stokes, K.D. Tartof, R.P. Perry, CHD1 is concentrated in interbands and puffed regions of *Drosophila* polytene chromosomes, Proc. Natl. Acad. Sci. USA 93 (1996) 7137–7142.
[24] R. Simic, D.L. Lindstrom, H.G. Tran, K.L. Roinick, P.J. Costa, A.D. Johnson, G.A. Hartzog, K.M. Arndt, Chromatin remodeling protein Chd1 interacts with transcription elongation factors and localizes to transcribed genes, EMBO J. 22 (2003) 1846–1856.

[25] N.J. Krogan, M. Kim, S.H. Ahn, G. Zhong, M.S. Kobor, G. Cagney, A. Emili, A. Shilatifard, S. Buratowski, J.F. Greenblatt, RNA polymerase II elongation factors of *Saccharomyces cerevisiae*: a targeted proteomics approach, Mol. Cell. Biol. 22 (2002) 6979–6992.
[26] C. Alen, N.A. Kent, H.S. Jones, J. O'Sullivan, A. Aranda, N.J. Proudfoot, A role for chromatin remodeling in transcriptional termination by RNA polymerase II, Mol. Cell 10 (2002) 1441–1452.
[27] M.W. McBurney, B.J. Rogers, Isolation of male embryonal carcinoma cells and their chromosome replication patterns, Dev. Biol. 89 (1982) 503–508.
[28] U.K. Laemmli, Cleavage of structural proteins during the assembly of the head of bacteriophage T4, Nature 227 (1970) 680–685.
[29] M.W. McBurney, L.C. Sutherland, C.N. Adra, B. Leclair, M.A. Rudnicki, K. Jardine, The mouse Pgk-1 gene promoter contains an upstream activator sequence, Nucleic Acids Res. 19 (1991) 5755–5761.
[30] M. Hendzel, G. Delcuve, J. Davie, Histone deacetylase is a component of the internal nuclear matrix, J. Biol. Chem. 266 (1991) 21936–21942.
[31] S.F. Altschul, W. Gish, W. Miller, E.W. Meyers, D.J. Lipman, Basic local alignment search tool, J. Mol. Biol. 215 (1990) 403–410.
[32] D.A. Benson, I. Karsch-Mizrachi, D.J. Lipman, J. Ostell, B.A. Rapp, D.L. Wheeler, GenBank, Nucleic Acids Res. 30 (2002) 17–20.
[33] K. Colwill, T. Pawson, B. Andrews, J. Prasad, J.L. Manley, J.C. Bell, P.I. Duncan, The Clk/Sty protein kinase phosphorylates SR splicing factors and regulates their intranuclear distribution, EMBO J. 15 (1996) 265–275.
[34] K. Colwill, in: Medical Genetics, University of Toronto, Toronto, 1997, p. 169.
[35] T. Lu, M. Vandyke, M. Sawadogo, Protein–protein interaction studies using immobilized oligohistidine fusion proteins, Anal. Biochem. 213 (1993) 318–322.
[36] H. Towbin, T. Staehelin, J. Gordon, Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications, Proc. Natl. Acad. Sci. USA 76 (1979) 4350–4354.
[37] P. Duncan, D. Stojdl, R. Marius, J. Bell, In vivo regulation of alternative pre-mRNA splicing by the Clk1 protein kinase, Mol. Cell. Biol. 17 (1997) 5996–6001.
[38] T. Nagase, N. Seki, K. Ishikawa, A. Tanaka, N. Nomura, Prediction of the coding sequences of unidentified human genes. V. The coding sequences of 40 new genes (KIAA0161–KIAA0200) deduced by analysis of cDNA clones from human cell line KG-1, DNA Res. 3 (1996) 17–24.
[39] A.J. Horlein, A.M. Naar, T. Heinzel, J. Torchia, B. Gloss, R. Kurokawa, A. Ryan, Y. Kamei, M. Soderstrom, C.K. Glass, et al., Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor, Nature 377 (1995) 397–404.
[40] H. Jumaa, J.L. Guenet, P.J. Nielsen, Regulated expression and RNA processing of transcripts from the Srp20 splicing factor gene during the cell cycle, Mol. Cell. Biol. 17 (1997) 3116–3124.
[41] O. Nayler, W. Stratling, J.P. Bourquin, I. Stagljar, L. Lindemann, H. Jasper, A.M. Hartmann, F.O. Fackelmayer, A. Ullrich, S. Stamm, SAF-B protein couples transcription and pre-mRNA splicing to SAR/MAR elements, Nucleic Acids Res. 26 (1998) 3542–3549.
[42] P.L. Jones, Y.B. Shi, N-CoR–HDAC corepressor complexes: roles in transcriptional regulation by nuclear hormone receptors, Curr. Top Microbiol. Immunol. 274 (2003) 237–268.
[43] M.G. Guenther, O. Barak, M.A. Lazar, The SMRT and N-CoR corepressors are activating cofactors for histone deacetylase 3, Mol. Cell. Biol. 21 (2001) 6091–6101.
[44] J. Li, J. Wang, Z. Nawaz, J.M. Liu, J. Qin, J. Wong, Both corepressor proteins SMRT and N-CoR exist in large protein complexes containing HDAC3, EMBO J. 19 (2000) 4342–4350.
[45] H.-Y. Kao, M. Downes, P. Ordentlich, R.M. Evans, Isolation of a novel histone deacetylase reveals that class I and class II deacetylases promote SMRT-mediated repression, Genes Dev. 14 (2000) 55–66.
[46] E.Y. Huang, J. Zhang, E.A. Miska, M.G. Guenther, T. Kouzarides, M.A. Lazar, Nuclear receptor corepressors partner with class II histone deacetylases in a Sin3-independent repression pathway, Genes Dev. 14 (2000) 45–54.
[47] Y.-D. Wen, V. Perissi, L.M. Staszewski, W.-M. Yang, A. Krones, C.K. Glass, M.G. Rosenfeld, E. Seto, The histone deacetylase-3 complex contains nuclear receptor corepressors, PNAS 97 (2000) 7202–7207.
[48] C. Underhill, M.S. Qutob, S.-P. Yee, J. Torchia, A novel nuclear receptor corepressor complex, N-CoR, contains components of the mammalian SWI/SNF complex and the corepressor KAP-1, J. Biol. Chem. 275 (2000) 40463–40470.
[49] P.L. Jones, L.M. Sachs, N. Rouse, P.A. Wade, Y.-B. Shi, Multiple N-CoR complexes contain distinct histone deacetylases, J. Biol. Chem. 276 (2001) 8807–8811.
[50] R. Aasland, A.F. Stewart, T. Gibson, The SANT domain: a putative DNA-binding domain in the SWI-SNF and ADA complexes, the transcriptional co-repressor N-CoR and TFIIIB, Trends Biochem. Sci. 21 (1996) 87–88.
[51] J. Zhang, M. Kalkum, B.T. Chait, R.G. Roeder, The N-CoR–HDAC3 nuclear receptor corepressor complex inhibits the JNK pathway through the integral subunit GPS2, Mol. Cell 9 (2002) 611–623.
[52] G. Dellaire, E.M. Makarov, J.J. Cowger, D. Longman, H.G. Sutherland, R. Luhrmann, J. Torchia, W.A. Bickmore, Mammalian PRP4 kinase copurifies and interacts with components of both the U5 snRNP and the N-CoR deacetylase complexes, Mol. Cell. Biol. 22 (2002) 5141–5156.
[53] T. Kojima, T. Zama, K. Wada, H. Onogi, M. Hagiwara, Cloning of human PRP4 reveals interaction with Clk1, J. Biol. Chem. 276 (2001) 32247–32256.
[54] M.W. McBurney, X. Yang, K. Jardine, M. Cormier, A role for RNA processing in regulating expression from transfected genes, Somat. Cell Mol. Genet. 24 (1998) 203–215.
[55] M.W. McBurney, S. Fournier, K. Jardine, L. Sutherland, Intragenic regions of the murine Pgk-1 locus enhance integration of transfected DNAs into genomes of embryonal carcinoma cells, Somat. Cell Mol. Genet. 20 (1994) 515–528.