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Characterization of a new family of proteins that interact with the C-terminal region of the chromatin-remodeling factor CHD-3[1]

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Abstract The two human proteins Ki-1/57 and CGI-55 have highly similar amino acid sequences but their functions are unknown. We analyzed them by yeast two-hybrid screens and found that they interact with the C-terminal region of the human chromatin-remodeling factor CHD-3 (chromo-helicase-DNA-binding domain protein-3). The interaction of CGI-55 and CHD-3 could be confirmed in vitro and in vivo by co-immunoprecipitations from *Sf*9 insect cells. Mapping showed that CGI-55 interacts with CHD-3 via two regions at its N- and C-terminals. The CGI-55 and Ki-1/57 mRNAs show highest expression in muscle, colon and kidney. A CGI55–GFP fusion protein was localized in the cytoplasm, nucleus and perinuclear regions of HeLa cells. These data suggest the possibility that CGI-55 and Ki-1/57 might be involved in nuclear functions like the remodeling of chromatin.

Key words: Chromatin remodeling; Protein–protein interaction; Two-hybrid; Domain mapping; Cellular localization

1. Introduction

Using the monoclonal antibody Ki-1, the first antibody described that specifically detects the malignant Hodgkin and Sternberg-Reed cells in Hodgkin lymphoma [1], we have previously identified the antigen Ki-1/57, a 57 kDa intracellular phospho-protein [2,3]. In vitro phosphorylation experiments performed with the Ki-1/57 antigen isolated from tumor cells suggested that it is associated with a serine/threonine protein kinase activity [4]. Electron microscopic analysis demonstrated that the Ki-1/57 antigen is not only located in the cytoplasm but also at the nuclear pores and in the nucleus where it is frequently found in association with the nucleolus [5]. Tryptic digestion of the Ki-1/57 antigen resulted in the cloning of a partial cDNA encoding Ki-1/57 [6]. The isolated contig of 1380 bp length, encoded the C-terminal 60% of the Ki-1/57 protein.

By searching for related proteins we discovered a cDNA sequence encoding the protein CGI-55, a possible human paralog of the Ki-1/57 protein of yet unknown function. Its cDNA is complete and encodes a hypothetical protein of 55 kDa. The protein sequence of CGI-55 shows 40.7% identity and 67.4% similarity with Ki-1/57. This suggests that the two human proteins might be paralogs and have similar functions.

In the present study, we wanted to obtain possible clues about the functional context of the proteins CGI-55 and Ki-1/57. We explored the yeast two-hybrid system to identify possible interacting proteins. We found that CGI-55 and Ki-1/57 interact with the C-terminal region of the human protein CHD-3 (chromo-helicase DNA-binding domain protein-3). The CHD proteins are members of the chromodomain family, a class of proteins that are involved in transcriptional regulation and chromatin remodeling [7–16]. Chromatin remodeling is likely to be an important step for the regulation of gene expression next to transcription factor based regulatory mechanisms [17,18]. Proteins that participate in the remodeling of chromatin might affect this process at levels as diverse as the nucleosome packing, the formation of DNA loops, its supercoiling or its attachment to the nuclear matrix [18]. The binding of the proteins CGI-55 and Ki-1/57 to CHD-3 can define them as new family of CHD-3 binding proteins and suggests the possibility that they might be involved in nuclear functions associated with the remodeling of chromatin.

2. Materials and methods

2.1. Plasmid construction

The full-length cDNA (DKFZp564M2423Q3) described in the database report was kindly provided by the Resource Center/Primary Database (Heubnerweg 6, D-14059 Berlin, Germany). This clone had been isolated from a human fetal brain cDNA library (DKFZhfb2) created by Stefan Wiemann (DKFZ, Heidelberg, Germany). Several sets of oligonucleotides were designed to allow sub-cloning of the complete CGI-55 coding region in different expression vectors. Insertion into pGEX-2TK (Amersham Biosciences) allowed to express CGI-55 (1–387) as a C-terminal fusion to GST (GST–CGI55). The cDNAs of CGI-55 and its deletion constructs were also inserted into the yeast two-hybrid expression vector pBTM-116 [19]. In a similar fashion the cDNA fragment encoding the C-terminal 60% of the Ki-1/57 antigen (122–413) was inserted in pBTM116. The cDNAs encoding CGI-55 (1–387), Ki-1/57 (1–413) and the C-terminal of CHD-3 (1839–2000) were inserted into the baculovirus (BV) transfervector pVL1392 vector (Pharmingen). This CHD-3 fusion protein contains N-terminal

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[1] Nucleotide sequence accession numbers: huCGI55: AL080119, AF151813; huKi-1/57: U77327; hu-IHABP: AF241831; CeCGI-55, CGI-55 from *Caenorhabditis elegans*: AF016672.

Abbreviations: BV, baculovirus; CHD-3, chromobox helicase DNA-binding domain protein-3

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HA- and 6×His tags for immunodetection (6×His-HA-CHD3). The complete cDNA of CGI-55 was cloned into vector pEGFP-N1 (Clontech).

2.2. Northern blot analysis

A human 12-lane multiple tissue Northern RNA blot was obtained from Clontech and consisted of poly(A)-enriched RNA from the following tissues: brain, heart, skeletal muscle, colon, thymus, spleen, kidney, liver, small intestine, placenta, lung, and peripheral blood leukocytes. A cDNA fragment of 1161 bp length encompassing the whole coding region of the CGI-55 protein was radiolabeled with α-[^{32}P]dATP using the random prime labeling kit (Roche). Hybridization and exposure of membranes to Kodak films were performed according to manufacturer of membrane (Clontech). The membrane was subsequently striped and reprobed first with a Ki-1/57 cDNA probe of 550 bp length and finally with the 2 kb control actin probe.

2.3. Yeast two-hybrid screening and interaction analysis

The pBTM116-CGI-55 [19] and pBTM116-Ki-1/57 (122–413) vectors were used to express the proteins CGI-55 and Ki-1/57 (C-terminal) linked to the C-terminus of LexA DNA-binding domain peptide in *Saccharomyces cerevisiae* strain L40. A human fetal brain cDNA library (Clontech) expressing GAL4 activation domain (AD) fusion proteins was co-transformed separately with both recombinant pBTM116 vectors. Selection of transformants, β-galactosidase activity test, plasmid DNA extraction and sequencing were performed as described [20].

2.4. Bacterial expression and protein purification

GST and GST–CGI55 proteins were expressed in *Escherichia coli* BL21-CodonPlus-RIL (Stratagene), and purified using glutathione–Sepharose 4B (Amersham) according to manufacturer.

2.5. Expression of 6×His-HA-CHD3, 6×His-CGI55 and Ki-1/57 in Sf9 insect cells

The recombinant transfer vectors pVL1393-HA-CHD3, pVL1392-CGI55 or pVL1392-Ki-1/57 were co-transfected with BV DNA (BaculoGold™, Pharmingen) in *Sf*9 insect cells by lipid transfection (DOTAP, Roche). Recombinant BV were separately amplified three to four times with fresh *Sf*9 cells. Cells were collected and sonicated in PBS, 0,1% Triton X-100 with protease inhibitors. 6×His-HA-CHD3 was purified by Ni-NTA Sepharose affinity chromatography.

2.6. Production of anti-CGI-55 monoclonal antibody

Monoclonal antibodies against CGI-55 were essentially generated as described [6,21]. Briefly, BALB/c mice were immunized four times with intervals of 2–4 weeks intraperitoneally with 100 μg of bacterial GST–CGI55 fusion protein. Spleen cells were fused with X63-Ag8.653 myeloma cells. Hybridoma supernatants were screened by ELISA for the presence of CGI-55 antibodies. The supernatant of re-cloned anti-CGI-55 hybridoma 10.5.6 was used for the experiments.

2.7. In vitro binding assay and Western blot analysis

14 μg of 6×His-HA-CHD3 (1839–2000) fusion protein was coupled to Ni-NTA Sepharose beads. Next 14 μg of GST–CGI55 or GST control protein were incubated for 2 h with the beads and then washed with buffer (20 mM Tris–HCl, pH 7.5, 150 mM NaCl). Proteins bound to the beads were separated by SDS–PAGE, transferred to a PDVF membrane and visualized by immuno-chemiluminescence using a mouse anti-GST antibody and secondary anti-mouse IgG-HRP conjugate.

2.8. In vivo binding assay and Western blot analysis

1.0×10^7 *Sf*9 cells were infected with wild type BV (BV-WT) or recombinant BV (BV-6×His-HA-CHD3(1839–2000), BV-6×His-CGI55, BV-Ki-1/57) at a multiplicity of infection >20. Two days after infection, cells were lysed in 1 ml Tris–HCl (pH 8.5) containing protease inhibitors. Lysate was treated with DNase (Promega) and cleared at 14 000×*g* for 15 min. Next 20 μl protein A Sepharose beads (Pharmacia) were loaded with the indicated antibodies (anti-HA mAb: Clontech), washed (Tris–HCl, pH 8.5) and incubated with the indicated lysates for 2 h at 4°C. After further two washes with the same buffer the beads were resuspended in SDS–PAGE loading buffer, boiled and analyzed by SDS–PAGE and Western blot using different mAb.

Western blots were developed by chemiluminescence as described [20].

2.9. Analysis of CGI55–EGFP (enhanced green fluorescence protein) fusion protein by fluorescence microscopy

HeLa cells were maintained at 37°C in 5% CO_2 in Dulbecco's modified Eagle's medium containing 10% heat inactivated fetal calf serum. Cells were cultured on glass coverslips for 24 h and were transfected with either wild type pEGFP vector (Clontech) or with recombinant vector CGI55–pEGFP-N1 using the lipid transfection method (Lipofectamine, Invitrogen). After 24 h coverslips were washed and mounted in 80% glycerol/10 mM Tris–HCl (pH 7.5) on coverglasses and analyzed with a fluorescence microscope (Eclipse E600, Nikon).

3. Results

3.1. Sequence analysis of CGI-55 and Ki-1/57

An alignment of the deduced amino acid sequences of human CGI-55, human Ki-1/57 and a possible *Caenorhabditis elegans* CGI-55 ortholog (Fig. 1) revealed possible ATP-binding motifs and nuclear localization signals. The central of the two ATP-binding motifs present in the CGI-55 sequence is conserved in the Ki-1/57 sequence and also in an ortholog protein sequence from *C. elegans*, whereas the possible C-terminal ATP-binding motif of CGI-55 is not conserved in the other two proteins. The putative nuclear localization sequence

A

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          * * *           :  :** *:*:*    **::   ** :::: ::        :*:
huKi-1/57 MKGALGSPVAAAGAAMQESFGCVVANRFHQLLDDES---DPFDILREAERRQQQLQRKR  57
huCGI-55  MPGHL-----------QEGFGCVVTNRFDQLFDDES---DPFEVLKAAE------NKKKE  40
CeCGI-55  ----------------MSTEYGCQVTNKFGLPSDDDDEYDDPRELIQKVS----QIAAKKK  41

            :   :     :: : * :: : *:   :    : * :   *      :    :
huKi-1/57 RDEAAAAAGAGPRGGRSPAGASGHRAGAGGRRESQKERKSLPAP---VAHRPD--SPGGG 112
huCGI-55  AGGGGVGGPGAKSAAQAAAQTNSNAAGKQLRKESQKDRKNPLPPSVGVVDKKEETQPPVA 100
CeCGI-55  EEKSVKPAQPVKPAAAPVAATKTDGAAGRGRGGRGRGRGGAGRP---RDGERVSNENGDR  98

            :  : :   **  ::       *      *  :    ** *      :      :*   :
huKi-1/57 LQAPGQKRTPRRGEQQGWNDSRGPEGMLER-AERRSYREYRPYETERQADFTAEKFPDEK 171
huCGI-55  LKKEGIRRVGRRPDQQ----LQGEGKIIDRRPERRPPRERR-FEKPLEEKGEGGEFSVDR 155
CeCGI-55  PQGENRRGGPRRGGER------GAARPAGRGGRGGFTRENR----EGEEPKQEVSFEDGQ 148

                  *: * ***   *   : ***   :    : ::   *:*:* :*:*: :::
huKi-1/57 PGDRFDRDRPLRGRGGPRGGMR-GRGRGGPGNRVFDAFDQRGKREFERYGGNDKIAVRTE 230
huCGI-55  P----IIDRPIRGRGGLGRGRG-GRGRGMGRG---DGFDSRGKREFDRHSGSDRSGLKHE 207
CeCGI-55  D------TRAPRRRGGFTLGGGPSGGRGGGRG----GRG----RQFDRQSGSDRTGVRSF 194

          *: :* *  :**  **    :        :   *: :     *:  :    *       :
huKi-1/57 DNMGGCGVRTWGSGKDT-SDVEPTAPMEEPTVVEESQGTPEEESPAKVPELEVEEETQVQ 289
huCGI-55  DKRGGSGSHNWGTVKDELTDLDQSNVTEETPEGEEHHPVADTEN--KENEVEEVKEEGPK 265
CeCGI-55  DKKDGHGKGNWGDQKDE---LAGETENIAPEGAESTEPEVPREKTAEELAYEAELAVLAK 251

          : **:*:*   :   :*  ** **: : :     * : ::*:       :*:     :
huKi-1/57 EMTLDEWKNLQEQTRPKPEFNIRKPESTVP---SKAVVIHKSKYRDDMVKDDYEDDSHVF 346
huCGI-55  EMTLDEWKAIQNKDRAKVEFNIRKPNEGADGQWKKGFVLHKSKSEEAHAEDSVMD--HHF 323
CeCGI-55  QKTLKEFKAAAKADAPK--FNTRKAGEGAADTFGKLVPIKKEVIP-DREEDEVVVIHKAP 308

          **:: **:  :: :  :  * :*: *    * **      ::             :*   :
huKi-1/57 RKPANDITSQLEINFGNLPRPGRGARGGTRGGRGRIRRAENNYPRAEVVMQDV-APNPDD 405
huCGI-55  RKPANDITSQLEINFGDLGRPGRGGRGG-RGGRGRGGRPNRGSRTDKSSAS---APDVDD 379
CeCGI-55  RKQVLDISITFRNDRPERERNDRSERPQRGGPRGGGRGGGRGGQRQGGHGGRNNTPFNAS 368

           : ****:
huKi-1/57 PEDFPALS--   413
huCGI-55  PEAFPALA--   387
CeCGI-55  DDAFPALGAK   378
```

B

	huCGI-55	huKi-1/57	CeCGI-55
huCGI-55	-	-	-
huKi-1/57	40,7* / 67,4:	-	-
CeCGI-55	28,8 / 55,3	23,9 / 53,2	-

Fig. 1. Protein sequence alignment of human Ki-1/57, human CGI-55 and *C. elegans* CGI-55. A: Comparison of huKi-1/57 (U77327 and AF241831), huCGI-55 (AL080119 and AF151813) and CeCGI-55 (CGI-55 from *C. elegans*: AF016672). Asterisks (*) indicate identical whereas colons (:) mark similar residues. Predicted ATP-binding motifs are boxed. Basic motif: G-X-G-X-X-G-$(X)_{13-22}$-K; [26,27] with white highlighting. Putative NLSs were predicted by the program PSORT II and are indicated by boxes and gray highlighting. B: Table comparing the identity (*) and similarity (:) values between the analyzed protein sequences.

(NLS) of CGI-55 begins at proline 132, whereas that of Ki-1/57 is located closer to the N-terminus at arginine 55. The amino acid sequence similarity between Ki-1/57 and CGI-55 is high: 40.7% of the residues are identical and 16.7% are similar. These data suggest that both human proteins might be paralogs with similar or overlapping functions. In the *C. elegans* amino acid sequence only the N-terminal of the ATP-binding motifs is conserved. Its NLS is found approximately 20 amino acids downstream of that found in the human CGI-55 (proline 153).

3.2. Comparison of the tissue expression of CGI-55 and Ki-1/57

To investigate the human CGI-55 and Ki-1/57 expression pattern in different human tissues we conducted a Northern blot analysis. As shown in Fig. 2 two major transcripts of human CGI-55, of ~5.5 kb and ~4 kb as well as a minor transcript of ~2.2 kb, were observed. The first two transcripts might be unprocessed pre-mRNA, whereas the transcript of ~2.2 kb is the mature CGI-55 transcript. The signal intensity decreases in the order: heart, skeletal muscle, kidney, placenta, liver and brain. The other tissues showed only very faint bands of the two larger CGI-55 transcripts, indicating low levels of CGI-55 expression in these tissues. In contrast, two transcripts of human Ki-1/57 of ~2.8 kb and ~2 kb were observed in the order of decreasing signal intensity in brain, kidney, heart, and skeletal muscle. All other tissues showed weak expression of predominantly the ~2 kb transcript. A control hybridization with the β-actin probe confirmed the uniform loading of the lanes with poly(A)$^+$ RNA.

3.3. Yeast two-hybrid screens

To gain functional insights via the identification of interacting proteins of CGI-55 and Ki-1/57, the yeast two-hybrid system [19,20,22,23] was employed, utilizing a human fetal brain cDNA library. For CGI-55 a screen of 0.6×10^6 co-transformants yielded 125 clones positive for both His3 and

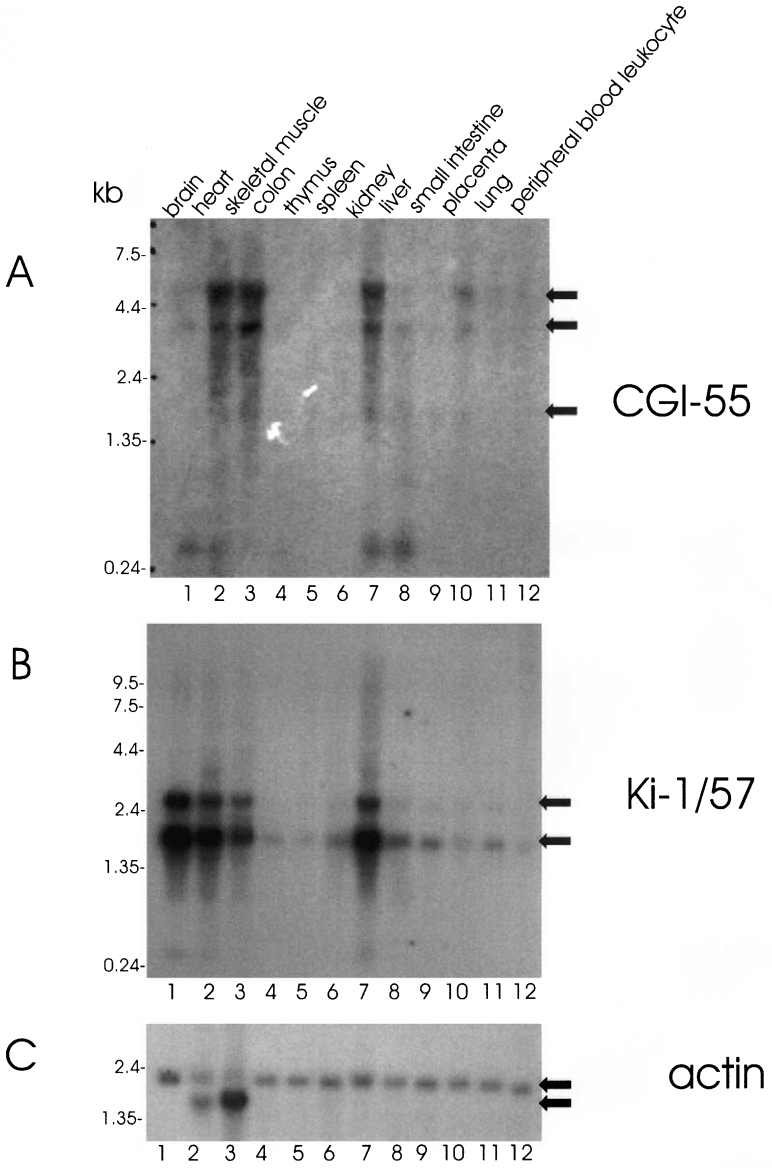

Fig. 2. Differential expression of CGI-55 and Ki-1/57 mRNAs in human tissues. A Northern blot of poly(A)$^+$ RNA isolated from several human tissues (Clonetech) was hybridized with human CGI-55 (A), Ki-1/57 (B) and β-actin (C) cDNA probes.

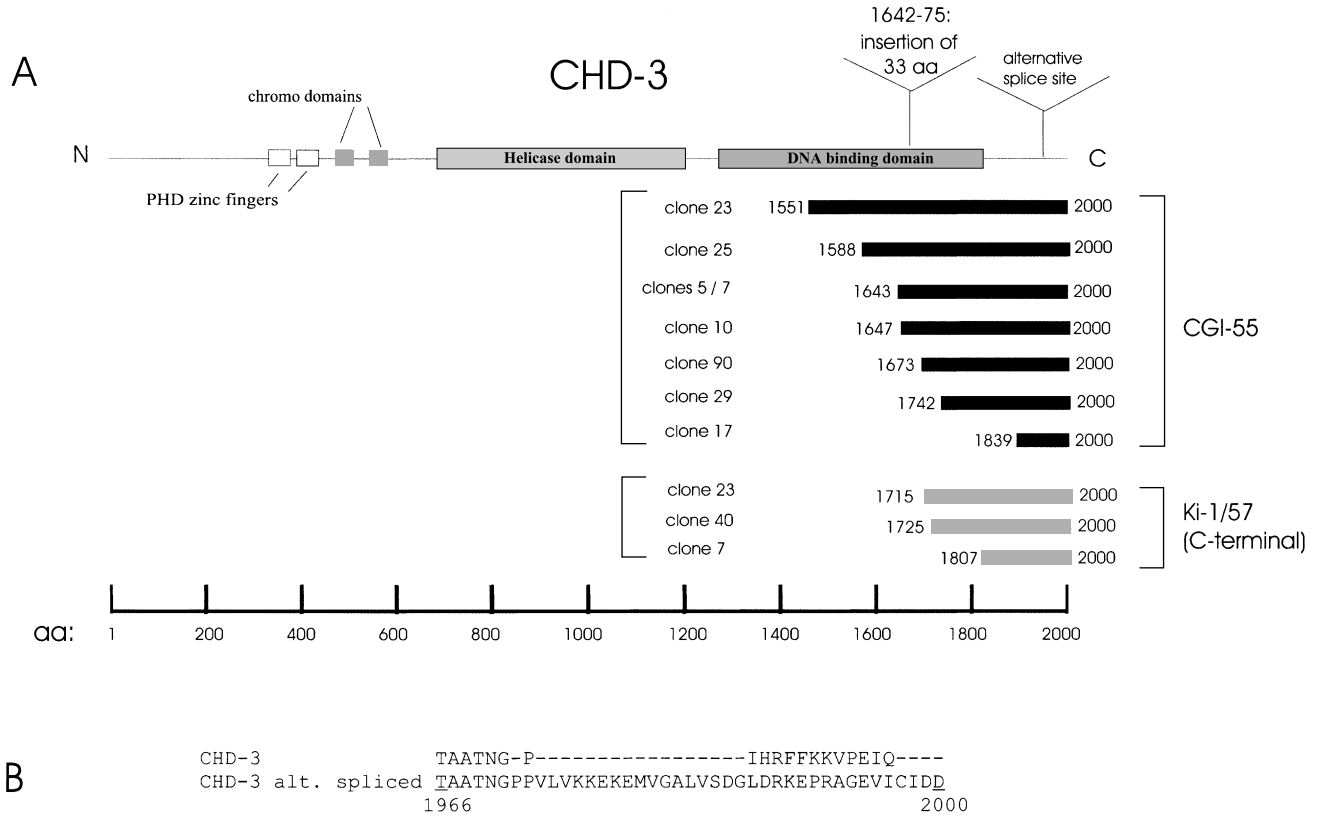

Fig. 3. CGI-55 and Ki-1/57 interact with the C-terminal region of CHD-3. A: Schematic representation of the domain organization of the human protein CHD-3. The position and of the identified CGI-55 interacting clones is shown along the full-length CHD-3 protein. The ruler indicates the length of CHD-3 in amino acids. B: Sequence alignment of the classical [13] and alternatively spliced C-terminal of CHD-3 [11].

LacZ reporter constructs. Library plasmids of 19 clones were sequenced. 42% of the sequenced clones encoded the C-terminal region of human chromatin remodeling factor CHD-3 (amino acid residues 1551–2000) (Fig. 3) [13]. All clones represent an alternative version of the CHD-3 protein that probably rises through alternative splicing and had been termed SNF2-like zinc-finger helicase [11,13] (Fig. 3B). For Ki-1/57 we performed a two-hybrid screen of the same cDNA library using the construct pBTM116-Ki-1/57(122–413). We isolated three independent alternatively spliced CHD-3 clones (Fig. 3).

3.4. Mapping the interaction site of CGI-55 with CHD-3

Next, we mapped the CGI-55 region required for the interaction CHD-3 using the yeast 2-hybrid method (Fig. 4). N- and C-terminal deletion constructs of the CGI-55 protein were fused to the lexA DNA-binding domain and tested for their ability to bind CHD-3. Only the construct 4 of CGI-55 (128–259), that contains the central region of CGI-55, failed to bind to CHD-3. The co-transformation of pBTM116-CHD3(1893–2000) with an unrelated 'bait' construction (pBTM116-AUF1) [24] showed no interaction. Our data suggest that CGI-55 interacts with CHD-3 via two independent binding sites that are located in its N- and C-terminal regions.

3.5. In vitro confirmation of the CGI55–CHD-3 interaction with purified proteins

We carried out pull down assay with purified recombinant proteins that had been expressed in *E. coli* (GST, GST–CGI55) or in the BV system (6×His-HA-CHD3) to confirm the interaction between CGI-55 and CHD-3 in vitro. As shown in Fig. 5B GST–CGI55 bound specifically to the C-terminal of CHD-3, while the control protein GST did not. The input controls identify the corresponding proteins in the blot. In Fig. 5A we controlled the equal loading of the Ni-NTA Sepharose beads with 6×His-HA-CHD3(1893–2000) by developing the Western blot with an anti-4×His mAb.

3.6. Co-immunoprecipitation from BV infected Sf9 insect cells

In order to test if CGI-55 (or Ki-1/57) and CHD-3 can form a stable complex when they are co-expressed in animal cells we employed the BV expression system for co-infection and co-immunoprecipitation studies. When *Sf*9 cells were co-infected with the two recombinant BVs BV-HA-CHD3(1893–2000) and BV-CGI55 and the recombinant protein HA-CHD3(1893–2000) was immunoprecipitated with an anti-HA tag mAb, the protein CGI-55 co-precipitated (Fig. 5C, lane 3) and could be detected by an immunoblot with anti-CGI55 mAb. This immunoprecipitation was specific, since no CGI-55 protein was detected when the *Sf*9 cells were infected with BV-WT or with the recombinant BV-HA-CHD3 alone (Fig. 5C, lanes 1 and 2). The lysate of the *Sf*9 cells infected with BV-CGI55 alone was used to identify the recombinant CGI55 protein in the anti-CGI55 immunoblot (Input lane; Fig. 5C, lane 4). In a similar fashion when CGI-55 was immunoprecipitated (using ant-CGI55 mAb 10.5.6) the protein HA-CHD3(1893–2000) co-precipitated only from the lysate of *Sf*9 cells that had been co-infected by both recombinant BVs: BV-HA-CHD3 and BV-CGI55 (Fig. 5D, lane 3). The immunoprecipitation of lysates of *Sf*9 cells that had been infected with BV-WT or BV-CGI55 alone, did not result in the

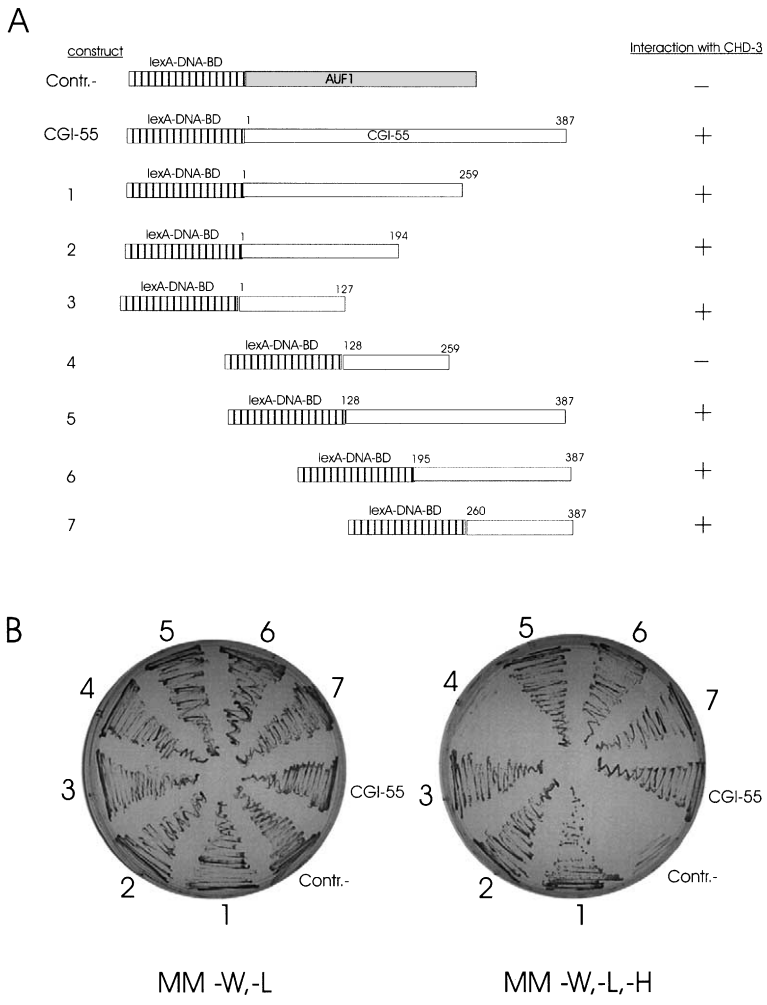

Fig. 4. Mapping of the regions of CGI-55 that interact with CHD-3. A: Various N- and C-terminal truncations of human CGI-55 were fused in frame to the DNA-binding domain of lexA in plasmid pACT2 and transformed into yeast L40 together with the fusion CHD3-Gal4-AD cloned in pBTM116. B: Interaction was determined by the ability of the co-transformant cells to grow on minimal medium (MM),-W,-L,-H (right). Presence of 'bait' and 'prey' plasmids in the co-transformed cells was controlled by growth on MM-W,-L (left).

detection of HA-CHD3 protein (Fig. 5D, lanes 1 and 2). The lysate of *Sf*9 cells infected only with BV-HA-CHD3 identifies the HA-CHD3 protein in the anti-HA immunoblot (Fig. 5D, lane 4, Input). In a parallel approach we were able to demonstrate that also Ki-1/57 specifically co-precipitates with HA-CHD3 protein (Fig. 5E).

3.7. Subcellular localization of CGI-55

Human HeLa cells were transfected with a vector containing GFP alone or with a vector that contains the cDNA encoding CGI-55 fused to the N-terminal of GFP (Fig. 6). In the control an even distribution of GFP was observed in the HeLa cells. In the case of the CGI55–GFP fusion construct we observed a less intense overall staining that appeared in a punctuated pattern throughout the cell. The speckled pattern was found in both the cytoplasm and to a lesser extend also in the nucleus. In addition we observed a marked perinuclear accumulation of the fluorescence in the transfected cells.

4. Discussion

Very few functional data exist about the proteins CGI-55 and Ki-1/57 [6,25,28]. The data that are available in the literature so far suggest, that CGI-55 (also named PAI-RBP1 for plasminogen activator inhibitor mRNA–RNA-binding protein 1) is a mRNA-binding protein [28]. These researchers studied proteins that bind to the 3′-terminal most 134 nt of the PAI-1 (plasminogen activator inhibitor 1) mRNA, that might be involved in the control of this mRNA's stability. They suggested that CGI-55 might be a protein that is involved in the regulation of the stability of the PAI-1 mRNA.

The only functional studies concerning Ki-1/57 come from our group [2–6] and from one other group [25]. The latter had described Ki-1/57 as a novel hyaluronan-binding protein and re-named it IHABP4 (for intracellular hyaluronan binding protein 4). They also found that IHABP4 (= Ki-1/57) binds to other negatively charged glycosaminoglycans like chondroitin sulfate, heparane sulfate, and also RNA, although with lower affinity. The binding of IHABP4/Ki-1/57 to a series of negatively charged macromolecules might be due to its relatively high content of positively charged amino acids, in particular Arg (12.8%). The biological meaning of the interaction of a protein localized in the cytoplasma and nucleus [2–5,25] with glycosaminoglycans, which are mainly found outside the cell [25], remains open.

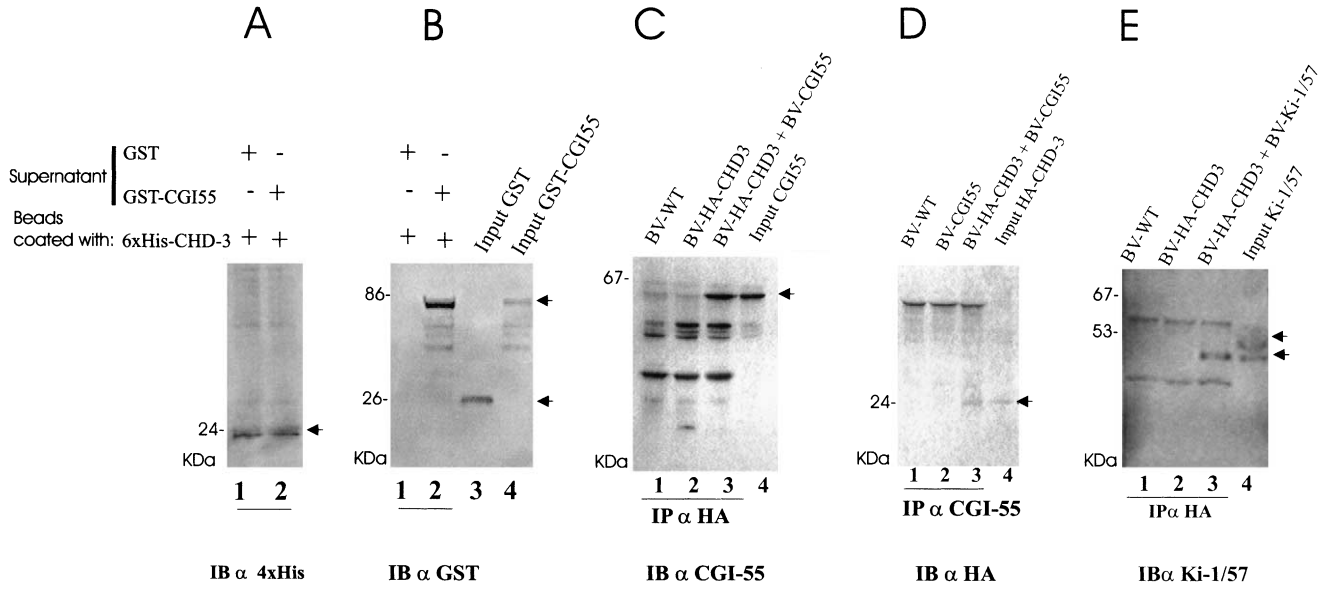

Fig. 5. In vitro binding assay and co-immunoprecipitation from *Sf*9 insect cells infected with different combinations of recombinant BV. A,B: In vitro binding assay. Ni-NTA Sepharose beads were loaded with purified 6×His-HA-CHD3(1983–2000) protein. Beads were then incubated with purified GST or GST–CGI55, washed three times, separated by SDS–PAGE, transferred to PVDF membrane and probed with anti GST antibody (B, lanes 1 and 2) or anti-4×His mAb (A, lanes 1 and 2). Purified GST and GST–CGI55 proteins were used as input controls (B, lanes 3 and 4). C,E: Co-immunoprecipitation of CGI-55 and Ki-1/57 with HA-CHD3. *Sf*9 cells were infected with the BV indicated on top of the panel. C: HA-CHD3(1983–2000) was immunoprecipitated (IP) with anti-HA antibody. The immunoprecipitates were probed (IB, immunoblot) with anti-CGI-55 antibody 10.5.6. Input: expression and identity of CGI-55 were controlled by applying lysate of *Sf*9 cells infected with BV-CGI55 (C, lane 4). D: CGI-55 was immunoprecipitated with antibody 10.5.6. Immunoprecipitates were probed with anti-HA antibody. Input: Lysate of *Sf*9 cells infected with BV-HA-CHD3 (D, lane 4). E: HA-CHD3(1983–2000) was immunoprecipitated with anti-HA antibody. The immunoprecipitates were probed with anti-Ki-1/57 antibody Ki-1. Input: Lysate of *Sf*9 cells infected with BV-Ki-1/57. Molecular mass markers are shown in kDa on left side of the panels. Arrows on the right indicate specific proteins identified by immunoblot.

In order to identify a functional context for the protein paralogs Ki-1/57 and CGI-55 we set out to perform yeast two-hybrid screens to identify possible interacting protein partners. Screens of a human fetal brain cDNA library with both CGI-55 and Ki-/57 identified an alternative spliced version of the DNA remodeling factor CHD-3. The longest CHD-3 clone encodes its C-terminal 412 amino acids and includes part of the predicted DNA-binding domain. The shortest of the interacting clones however, includes only the C-terminal 161 amino acids of the CHD-3 protein, suggesting that the interaction occurs in a very defined region at the C-terminal region of CHD-3. Ki-1/57 also interacted with clones that represent the C-terminal region (1807–2000) of CHD-3.

Our analysis of the sub-cellular localization of the EGFP–CGI55 fusion protein in human HeLa cells suggests that CGI-55 might have both cytoplasmic and nuclear functions and it is tempting to speculate that the distribution of CGI-55 between these two compartments might be regulated like that of other proteins shuttling between the nucleus and the cytoplasma.

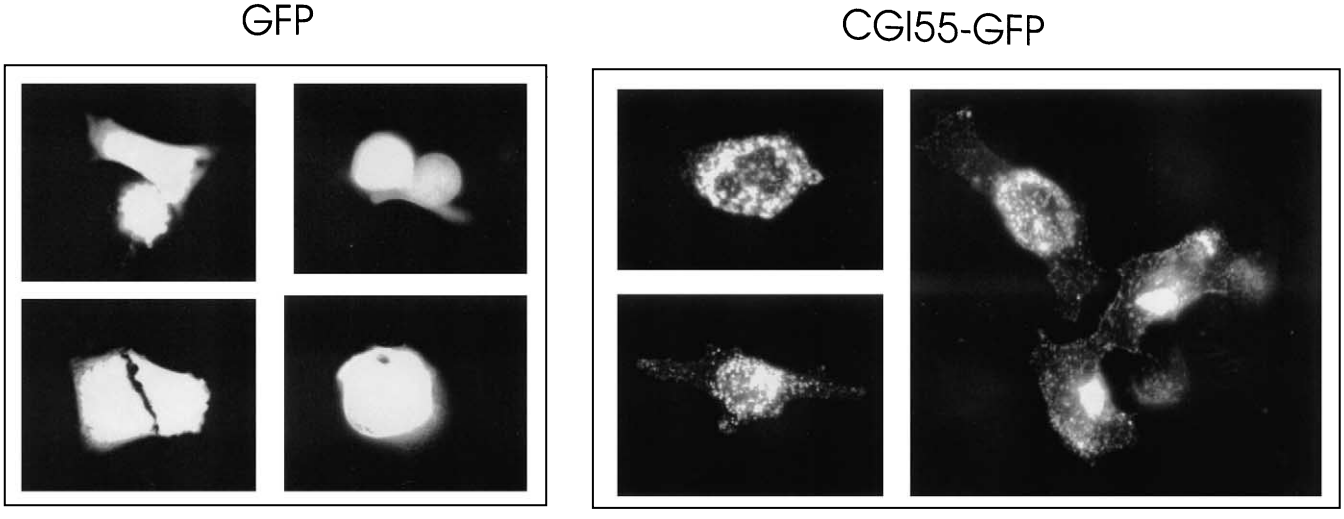

Fig. 6. Cellular Localization of CGI55–EGFP. HeLa cells were transiently transfected with expression vector pEGFP or recombinant expression vector CGI55–pEGFP-N1. After 24 h transfected cells were examined with a Nikon microscope.

In summary, the new functional data on the homologous proteins Ki-1/57 and CGI-55 that we described here, demonstrate that these proteins interact with the nuclear protein CHD-3, which is involved in chromatin-remodeling and transcriptional regulation. To our knowledge, this is the first report that describes a specific protein–protein interaction for CGI-55 and Ki-1/57 and might define them as a new family of proteins. It is tempting to speculate that these interactions might be relevant for the regulation of the CHD-3 mediated chromatin-remodeling.

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