Identification and Analysis of Chromodomain-Containing Proteins Encoded in the Mouse Transcriptome

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The chromodomain is 40–50 amino acids in length and is conserved in a wide range of chromatic and regulatory proteins involved in chromatin remodeling. Chromodomain-containing proteins can be classified into families based on their broader characteristics, in particular the presence of other types of domains, and which correlate with different subclasses of the chromodomains themselves. Hidden Markov model (HMM)-generated profiles of different subclasses of chromodomains were used here to identify sequences encoding chromodomain-containing proteins in the mouse transcriptome and genome. A total of 36 different loci encoding proteins containing chromodomains, including 17 novel loci, were identified. Six of these loci (including three apparent pseudogenes, a novel HP1 ortholog, and two novel Msl-3 transcription factor-like proteins) are not present in the human genome, whereas the human genome contains four loci (two CDY orthologs and two apparent CDY pseudogenes) that are not present in mouse. A number of these loci exhibit alternative splicing to produce different isoforms, including 43 novel variants, some of which lack the chromodomain. The likely functions of these proteins are discussed in relation to the known functions of other chromodomain-containing proteins within the same family.

[Supplemental material is available online at www.genome.org.]

The chromodomain (CD) is a domain of 40–50 amino acids long contained in various proteins involved in chromatin remodeling and the regulation of gene expression in eukaryotes during development (Cavalli and Paro 1998). Examples of such proteins include the *Drosophila melanogaster* proteins Heterochromatin Protein 1 (HP1), the histone acetyltransferase MOF, Polycomb, and Suppressor of variegation (3–9), and their homologs in other organisms. The CD has been variously reported to be a protein interaction module (Cowell and Austin 1997; Strutt and Paro 1997; Lachner et al. 2001), an RNA-binding module (Akhtar et al. 2000), and most recently a DNA-binding module (Bouazoune et al. 2002). These functions may in fact be interdependent, since the CD confers binding specificity to chromatin (Platero et al. 1995; Kelley et al. 1999). In the case of HP1, the CD has been shown to recognize histone H3 methylated at lysine 9 (Lachner et al. 2001), whereas the CD of polycomb recognizes histone H3 methylated at lysine 27 (C.D. Allis, pers. comm.). However, HP1 has also been reported to recognize chromatin-RNA complexes (Maison et al. 2002; Muchardt et al. 2002), and there is good general evidence that epigenetic modification is RNA-directed (Mattick 2001). CD-containing proteins may therefore be recognizing various types of histone codes in defined regions of chromatin which themselves are marked as a consequence of trans-acting RNA signals at specific DNA/protein targets, followed by the recruitment of other proteins into larger complexes (Hashimoto et al. 1998).

We clustered known CD sequences from all organisms were clustered into discrete subclasses using the Protein Distance Method (Felsenstein 1989; BioManager, http://bn2.angis.org.au/). Hidden Markov model (HMM) profiles (consensus primary structure models with position-specific residue scores and insertion/deletion penalties; Eddy 1996, 1998), were then generated for each subclass using HMMER (see Methods; K. Tajul-Arifin, R. Teasdale, and J.S. Mattick, in prep.). This analysis revealed 26 distinct subclasses of CDs, termed A–Z, some of which overlap with what has been previously referred to as the "chromo shadow domain." These profiles were then used to identify CD-containing proteins in various databases.

We identified 13 families of CD-containing proteins that are present in the genomically well studied eukaryotes *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Drosophila melanogaster*, *Arabidopsis thaliana, Caenorhabditis elegans*, human, and mouse (K. Tajul-Arifin, R. Teasdale, and J.S. Mattick, in prep.). These families include the chromodomain-helicase-DNA-binding (CHD) family, the histone methyl transferase family, the HP1 family, the Polycomb family, the Msl-3 ho-

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molog family, the histone acetyltransferase (HAT) family, the retinoblastoma-binding protein 1 (RBBP1) family, the enoyl-CoA hydratase family, the SWI3 family, and the plant-specific chromomethylase family (Table 1). We also found that particular subclasses of CDs are associated with particular families of CD-containing proteins, indicating that the function and/or specificity of the CD are subtly different in the different types of CD proteins.

In the present study we used the HMM profiles of the various subclasses of CDs (see Supplementary information) to identify all CD-containing proteins and their alternatively spliced products in mouse. The HMM profiles were used to query the Variant-based Proteome Set (VPS) of RIKEN's FANTOM2 Representative Transcripts and Protein Sets (RTPS; The FANTOM Consortium and the RIKEN Genome Exploration Research Group Phase I and II Team, 2002). The VPS data set consists of alternative transcription products within FANTOM2 clusters that have CDS sequences different from the cluster representative. In addition, VPS also contains known mouse proteins from GenPept and SWISS-PROT. The HMM profiles were also used to query the Ensembl mouse protein data set for CD-containing proteins, and the results from the two analyses were integrated. These protein sequences were then analyzed using SMART and Pfam (see Methods) to identify other domains. These data were then compared to the human genome to determine the extent of similarity between human and mouse regarding their repertoires of CD-containing proteins. Our analysis confirms a number of gene predictions and also provides the first detailed insight into the extent and functional impact of alternative splicing in CD-containing proteins in mammals.

RESULTS

Table 2 lists the source of the identified CD-containing proteins in the RIKEN VPS database, with cross-reference information where relevant. The RIKEN VPS database contains most but not all mouse CD-containing proteins. Table 3 shows all of the CD-containing loci identified in the mouse genome, as well as in the human genome, derived from analysis of both the RIKEN VPS and Ensembl databases. A total of 36 loci encoding CD-containing proteins, including 17 novel loci, were identified in mouse, which included representatives of 10 of the 13 eukaryotic CD-containing protein families. Our analyses also show that particular subclasses of chromodomains are associated with particular CD-containing protein families (Table 4), which presumably reflects different substrate specificity of different subclasses of CDs and their associated protein families (K. Tajul-Arifin, R. Teasdale, and J.S. Mattick, in prep.). A large number of alternative splice variants, including novel variants, were also identified, and these are discussed in more detail below in terms of the different CD-containing protein families (Table 4). A number of other transcripts from these loci were also found in the RIKEN and Ensembl databases, but were not included in this analysis unless they exhibited a different exon structure consistent with alternative splicing, as opposed to 3′- or 5′-truncated reverse transcripts.

Chromodomain-Helicase-DNA-Binding (CHD) Family

CHD proteins are typically characterized by two CDs near the N-terminus and an ATP-dependent helicase domain. This family can be further subdivided into three subfamilies, according to the presence of additional functional domains and the types of CDs. The CHD-1/2 subfamily contains CDs of subclasses O and H; the CHD-3/4 subfamily contains CDs of subclasses P and J, with PHD and/or RING zinc-finger domains near the N-terminus; and the CHD-5 subfamily contains CD subclasses Q and I with BRK and/or SANT DNA-binding domains in the C-terminal region. Mouse proteins belonging to all three subfamilies were identified in this study.

CHD-1/2 Subfamily

CHD-1/2 proteins are chromatin-binding proteins and appear to be both positive and negative regulators of gene transcription, based on mutational and functional studies (Delmas et al. 1993; Stokes and Perry 1995; Stokes et al. 1996; Jin et al. 1998; Kelley et al. 1999; Yoo et al. 2000). They are dispersed

Table 1. CD Families Identified and Their Description With CD Subclass Assignments

CD family	Description	CD subclass(es)
Chromodomain-Helicase-DNA-binding domain (CHD)		
Subfamily 1: CHD-1/2	General description: 2 CDs near N-terminal and ATP-department helicase region	O and H
Subfamily 2: CHD-3/4	Additional RING and/or PHD DNA-binding domains near N-terminal	P and J
Subfamily 3: CHD-5	Additional SANT and/or BRK domains near C-terminal	Q and I
Histone methyltransferase	Contain SET flanked by PreSET and PostSET domains	X
HP1	Containing 2 CDs	S and T
Polycomb (Pc)	Homologs of *D. melanogaster* Polycomb protein	B
Msl-3 homolog	Homologs of Msl-3 protein of *D. melanogaster*	E
Histone acetyltransferases	Contain a putative acetyltransferase domain near C-terminal	F
Retinoblastoma-binding protien 1 (RBBP1)	Proteins containing TUDOR and BRIGHT domains, binds to Retinoblastoma (Rb) protein	E
Enoyl-CoA hydratase	Contain EnoylCo A domain	L
SWI3	SWI/SNF-related protein with SANT DNA-binding domain	G
Ankyrin family	A CD near the N-terminus and 3 ankyrin repeats near the C-terminus of protein	L
Chromomethyltransferase	DNA methylases containing CD	M
Integrases	Plant CD-containing proteins with reverse transcriptase and intergrase domains	W
AAAs	ATPases proteins of yeasts	A

Identification of CD families was done previously (K. Tajul-Arifin, R. Teasdale, and J.S. Mattick, in prep.). All except three CD families, chromomethyltransferase, integrases, and AAAs families, are represented in mouse genome.

Table 2. A List of FANTOM2 VPS ID of Proteins Within the Same Cluster as Proteins Identified to Contain CD

No.	Locus	VPS ID	Database	Database ID	Reference
1a	CHD-1	PA1318.0	SWISS-PROT	P40201	Delmas et al. 1993
1c		PC1318.2	FANTOM2	4930428D05	
3a	CHD-3/4–Mi2a	PC71098.0	FANTOM2	B230201M16	
4a	CHD-3/4–Mi2b	PC50515.0	FANTOM2	9430004K15	
6a	CHD-5a	PC14728.3	FANTOM2	B430211I15	
7a	CHD-5b	PC26677.0	FANTOM2	A330063D19	
7b		PC26677.1	FANTOM2	A530057H05	
10a	Suv39h1	PB5441.0	GenPept	NP_035644	Aagaard et al. 1999; Bultman and Magnuson 2000; Carninci et al. 2000; Czvitkovich et al. 2001
		PC5441.1	FANTOM2	E430014P09	
11a	Suv39h2	PB8025.0	GenPept	NP_073561	Carninci et al. 2000; Shibata et al. 2000
		PC8025.2	FANTOM2	D030020B18	
11b		PC8025.1	FANTOM2	B130047D07	
12	HP1α	PB1167.0	GenPept	NP_031652	Le Douarin et al. 1996; Carninci et al. 2000; Shibata et al. 2000
		PC1167.1	FANTOM2	6620401G22	
		PC70076.0	GenPept	AAF80993	Li et al. 2001
13a	HP1β	PA1163.0	SWISS-PROT	P23197	Singh et al. 1991; Ball et al. 1997; Kawai et al. 2001
13b		PC1163.1	FANTOM2	5730433G16	
14	HP1γ	PA1165.0	SWISS-PROT	P23198	Singh et al. 1991, Horsley et al. 1996; Le Douarin et al. 1996
18a	Cbx6	PA14827.0	SWISS-PROT	Q9DBY5	Kawai et al. 2001
		PC14827.1	FANTOM2	9530005K03	
18b		PC14827.2	FANTOM2	G630026N14	
19	Cbx2 (M33)	PA1164.0	SWISS-PROT	P30658	Pearce et al. 1992
20	Cbx4 (MPc2)	PA1166.0	SWISS-PROT	O55187	Alkema et al. 1997
21	Cbx8 (MPc3)	PA6755	SWISS-PROT	Q9QXV1	Hemenway et al. 2000
22a	Cbx7	PC11218.0	FANTOM2	B230307D15	
22b		PC11218.1	GenPept	AAH21398	R. Strausberg 2002, GenBank submission
23	Msl-3	PB3758.0	GenPept	NP_034962	Prakash et al. 1999
24	Tex189/MRG15	PB5593.0	GenPept	NP_077751	Lopez-Fernandez and del Mazo 1996; Carninci et al. 2000; Shibata et al. 2000; Bertram and Pereira-Smith 2001
28a	Myst1	PC9534.0	FANTOM2	1110001P03	
28b		PC9534.1	FANTOM2	4833401K19	
32a	EnoylCoA protein	PC16290.0	FANTOM2	4930453I21	
33	Cdyl	PB1292.0	GenPept	NP_03401I	Lahn and Page 1999; Carninci et al. 2000
38	SRG3	PB5215.0	GenPept	NP_033237	Jeon et al. 1997
40a	MPP8	PB15875.0	GenPept	NP_076262	Carninci et al. 2000; Shibata et al. 2000

Proteins listed in FANTOM2 VPS obtained from either GenPept or SWISS-PROT are indicated with their references cited. Left-hand column; Numbering used to refer to the gene in Table 3.

evenly in the nucleus and not exclusively localized to heterochromatic regions.

Two genes encoding CHD-1 and CHD-2 homologs were identified in mouse. The CHD-1 gene is located on chromosome 17 band A2, with the full-length protein encoded by 35 exons. This protein had already been identified in the public database as well as in the Ensembl mouse database (protein No. 1a).

A protein from FANTOM2 was identified as a possible alternative splicing product for this locus (No. 1b). The transcript contains 23 exons, whereby the first exon is located about 1.6 kb upstream from the first exon of protein 1a transcript. This strongly supports the use of an alternative promoter in transcribing the mRNA for this protein. The translated protein contains all of the recognized structural domains of the full-length protein, but lacks the C-terminal region, due to an in-frame stop codon encoded by a 3′ extension of exon 23.

The second CHD protein identified in this subfamily is CHD-2. Described as a novel mouse protein in Ensembl, with a homolog in human, the mouse gene is located on chromosome 7 band D1. Four alternatively spliced transcripts were identified, each of which contains one or more exons not present in one or more of the other transcripts (Table 4). Each transcript codes for isoforms of CHD-2 protein that contain only one CD of subclass H (Table 4), whereas the human CHD-2 homolog has two CD domains (O and H), which suggests that the mouse CHD-2 protein recorded transcripts are incomplete. Indeed, analysis of the genomic region surrounding this locus in mouse revealed sequences located between the recorded exons 8 and 9 capable of encoding a second CD, suggesting that these sequences represent a cryptic exon, which presumably is included in other splice variants. This suggests that different isoforms of this protein may include one or both CD domains, with potentially important functional consequences.

CHD–3/4 Subfamily

Analysis of the Ensembl mouse database revealed three loci that encode for CHD-3/4 proteins. All three are novel mouse genes with corresponding human homologs (Table 3), and are characterized by CDs of subclasses P and J (Table 4). Homologs of these proteins in various organisms have been reported to induce ATP-dependent nucleosome remodeling during development, and negatively control gene transcription (Kehle et al. 1998; Brehm et al. 2000; Solari and Ahringer 2000; von Zelewsky et al. 2000). In addition, the human

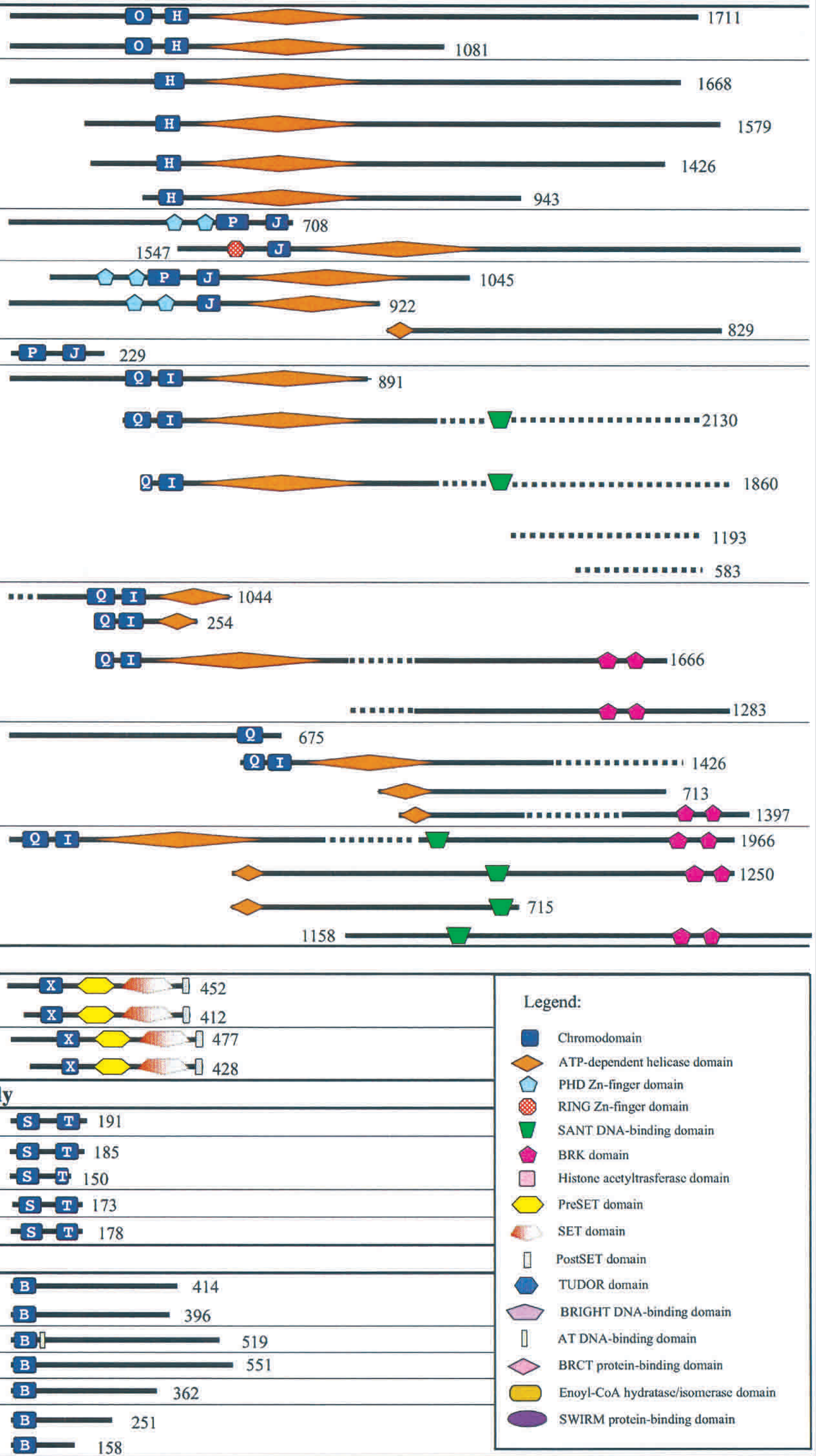
Table 3. Summary of Results of Loci Encoding Proteins Containing CD in Mouse

No	Family	Protein	Ensemble Gene ID	VPS ID	Chrom.	Band	Human homolog	Ensemble Gene ID	Chrom.	Band
1	**CHD**	Chd1	ENSMUSG00000023852	PA1318.0 PC1318.2	17	A2	Chd1	ENSG00000153922	5	q22.2
2		Chd2 (novel)	ENSMUSG00000025788	**Not Available**	7	D1	Chd2	ENSG00000165893	15	q26.1
3		Chd3/4-Mi2a (novel)	ENSMUSG00000006551	PC71098.0	11	B4	CHD3 (Mi2α)	ENSG00000170004	17	p12
4		Chd3/4-Mi2b (novel)	ENSMUSG00000038307	PC50515.0	6	F2	CHD4 (Mi2β)	ENSG00000111642	12	p13.31
5		Chd3/4-Mi2c (novel)	ENSMUSG00000039622	**Not Available**	4	E2	CHD3/4	ENSG00000116254	1	p36.31
6		Chd5a (novel)	ENSMUSG00000035795	PC14728.3	2	H3	Chd5	ENSG00000124177	20	q12
7		Chd5b (novel)	ENSMUSG00000033329	PC26677.0	8	C5	Chd5	ENSG00000087255	16	q12.2
8		Chd5c (novel)	ENSMUSG00000035714	**Not Available**	14	C1	Chd5	ENSG00000100888	14	q11.12
9		Chd5d (novel)	ENSMUSG00000041235	**Not Available**	4	A1	Chd5	ENSG00000171316	8	q12.2
10	**Histone methyltransferase**	Suv39h1	ENSMUSG00000039231	PB5441.0 PC5441.0	X	A1.1	SUV39H1	ENSG00000101945	X	p11.23
11		Suv39h2(Riken)	ENSMUSG00000026646	PB8025.0 PC8025.2 PC8025.1	2	A1	SUV39H2	ENSG00000152455	10	p13
12	**HP1**	HP1α	ENSMUSG00000009575	PB1167.0 PC1167.1 PC70076.0	15	F3	HP1α	ENSG0000094916	12	q13.13
13		HP1β	ENSMUSG00000018666	PA1163.0 PC1163.1	11	D	HP1β	ENSG00000108468	17	q21.32
14		HP1γ	ENSMUSG00000029836	PA1165.0	6	B3	HP1γ	ENSG00000122565	7	p15.2
15		HP1γ (pseudogene)	ENSMUSG00000004979		14	D2	*No human homolog*			
16		HP1γ (pseudogene)	ENSMUSG00000038158		1	C2	*No human homolog*			
17		HP1γ homolog (novel)	ENSMUSG00000038108	**Not Available**	4	C3	*No human homolog*			
18	**Polycomb**	Cbx6	ENSMUSG00000022424	PA14827.0 PC14827.1 PC14827.2	15	E2	Cbx6 homolog	ENSG00000100261	22	q13.1
19		Cbx2 (M33)	ENSMUSG00000025577	PA1164.0	11	E2	Cbx2 homolog (novel)	ENSG00000141584	17	q25.3
20		Cbx4 (MPc2)	ENSMUSG00000039989	PA1166.0	11	E2	Cbx4 homolog	ENSG00000141582	17	q25.3
21		Cbx8 (MPC3)	ENSMUSG00000025578	PA6755.0	11	E2	Cbx8 homolog	ENSG00000141570	17	q25.3
22		Pc homolog Cbx7 (novel)	ENSMUSG00000000488	PC11218.0 PC11218.1	15	E2	Cbx7	ENSG00000100307	22	q13.1
23	**Msl-3 homologs**	Msl-3 homolog	ENSMUSG00000031358	PB3758.0	X	F5	Msl3-like 1	ENSG00000005302	X	p22.22
24		Tex189/MRG15	ENSMUSG00000032361	PB5593.0	9	E3.2	MRG15	ENSG00000140393	15	q25.1
25		Tex189/MRG15(pseudogene)	ENSMUSG00000036311		19	D1	*No human homolog*			
26		Transcription factor-like (novel)	ENSMUSG00000040827	**Not Available**	18	A2	*No human homolog*			
27		Transcription factor-like (novel)	ENSMUSG00000034236	**Not Available**	18	C	*No human homolog*			
28	**Histone acetyltransferase**	MYST1	ENSMUSG00000030801	PC9534.0 PC9534.1	7	F4	MYST1	ENSG00000103510	16	p11.2
29		Tip60 homolog (novel)	ENSMUSG00000024926		19	A	Tip60	ENSG00000172977	11	q13.1
30	**Retinoblastoma binding protein-1 (RBBP1)**	RBBP1 (novel)	ENSMUSG00000034629		12	C3	RBBP1	ENSG00000032219	14	q23.1
31		RBBP1-related (novel)	ENSMUSG00000039219		13	A2	RBBP1-related protein	ENSG00000054267	1	q42.3
32	**Enoyl-CoA Hydratase**	EnoylCoA protein (ECH) (novel)	ENSMUSG00000031758	PC16290.0	8	E1	EnoylCoA (novel)	ENSG00000166446	16	q23.1
33		Cdyl	ENSMUSG00000009589	PB1292.0	13	A5	CDYL	ENSG00000153046	6	p25.1
34		*No mouse homolog*					CDY1	ENSG00000172288	Y	q11.223
35		*No mouse homolog*					CDY1	ENSG00000172352	Y	q11.223
36		*No mouse homolog*					CDY2 (pseudogene)	ENSG00000129871	Y	q11.221
37		*No mouse homolog*					CDY2(pseudogene)	ENSG00000129873	Y	q11.221
38	**SWI3**	SRG3 (SMARCC1)	ENSMUSG00000032481	PB5215.0	9	F2	SMARCC1 homolog	ENSG00000160819	3	p21.31
39		SMARCC2 homolog (novel)	ENSMUSG00000025369	**Not Available**	10	D3	SMARCC2	ENSG00000139613	12	q13.2
40	**Ankyrin Family**	ANK protein (MPP8)	ENSMUSG00000021934	PB15875.0 PC15875.1	14	C2	MPP8	ENSG00000083771	13	q12.11

Respective human homolog for each loci is also listed. Novel proteins are indicated as such in parentheses. Each loci is assigned a number that is used as a reference to the proteins produced from that loci throughout this article, as listed in left-most column.

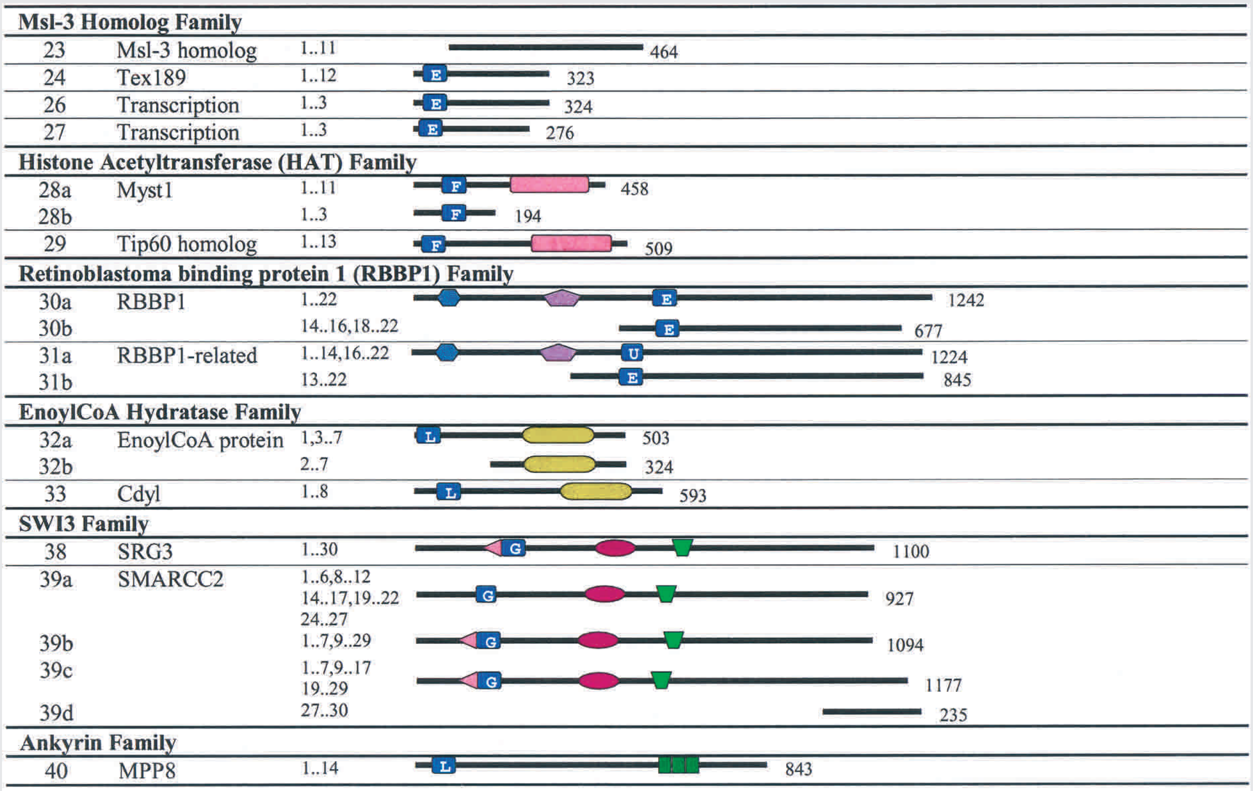
Table 4. Clusters of CD-Containing Proteins and Their Predicted Alternative Splicing Products Grouped According to CD Family

No.	Protein	Exons	Protein Domain Structure
Chromodomain-Helicase-DNA-binding (CHD) Family			
1a	CHD-1	2..36	1711
1b		1..23	1081
2a	CHD-2	1..8,10..31,34 37..39,41..42	1668
2b		6..8,10,12..34 37..40,42..43	1579
2c		6..8,10,12..30 35..40,42..43	1426
2d		9..10,12..30	943
3a	CHD-3/4 / Mi-2a	1..14	708
3b		6,7,9,12..39	1547
4a	CHD-3/4 / Mi-2b	4..20	1045
4b		1..8,10..19	922
4c		20..37	829
5	CHD-3/4 / Mi-2c	1..4	229
6a	CHD-5a	1..16	891
6b		5..25,27..29 31,32,34..36 40..42	2130
6c		7..26,29,31 32,34..36,39 41,42	1860
6d		22..25,29..36 39,42	1193
6e		35..38,40..42	583
7a	CHD-5b	1,3..16	1044
7b		2..13	254
7c		7..10,12..29 32,33,35,36 38..42	1666
7d		25..43	1283
8a	CHD-5c	1..10	675
8b		8..27,29..33	1426
8c		16..26,29..30	713
8d		16..38	1397
9a	CHD-5d	1..23,25..35	1966
9b		12..23,26..33 35	1250
9c		12..22,26..27	715
9d		19..24,26..35	1158
Histone Methyltransferase Family			
10a	Suv39h1	2..8	452
10b		1,4..8	412
11a	Suv39h2	1..6	477
11b		1,3..6	428
Heterochromatin Protein 1 (HP1) Family			
12	HP1α	1..5	191
13a	HP1β	1..6	185
13b		1..4,6	150
14	HP1γ	1..6	173
17	HP1γ homolog	1..4	178
Polycomb (Pc) Family			
18a	Cbx6	1..5	414
18b		1..5	396
19	Cbx2 (M33)	1..5	519
20	Cbx4 (MPc2)	1..5	551
21	Cbx8 (MPc3)	1..5	362
22a	Cbx7	1..7,7a,8	251
22b		1..7,8	158


Legend:
Chromodomain
ATP-dependent helicase domain
PHD Zn-finger domain
RING Zn-finger domain
SANT DNA-binding domain
BRK domain
Histone acetyltrasferase domain
PreSET domain
SET domain
PostSET domain
TUDOR domain
BRIGHT DNA-binding domain
AT DNA-binding domain
BRCT protein-binding domain
Enoyl-CoA hydratase/isomerase domain
SWIRM protein-binding domain

(continued)

Table 4. *Continued*



Msl-3 Homolog Family				
23	Msl-3 homolog	1..11		464
24	Tex189	1..12	E	323
26	Transcription	1..3	E	324
27	Transcription	1..3	E	276
Histone Acetyltransferase (HAT) Family				
28a	Myst1	1..11	F	458
28b		1..3	F	194
29	Tip60 homolog	1..13	F	509
Retinoblastoma binding protein 1 (RBBP1) Family				
30a	RBBP1	1..22	E	1242
30b		14..16,18..22	E	677
31a	RBBP1-related	1..14,16..22	U	1224
31b		13..22	E	845
EnoylCoA Hydratase Family				
32a	EnoylCoA protein	1,3..7	L	503
32b		2..7		324
33	Cdyl	1..8	L	593
SWI3 Family				
38	SRG3	1..30	G	1100
39a	SMARCC2	1..6,8..12 14..17,19..22 24..27	G	927
39b		1..7,9..29	G	1094
39c		1..7,9..17 19..29	G	1177
39d		27..30		235
Ankyrin Family				
40	MPP8	1..14	L	843

▪▪▪ represents sequence that is shortened and not to scale to the rest of the sequence represented by ▬ . Numbers at the end of each protein denote the length in amino acids.

Number at the end of each figure denotes the number of amino acids. Letters contained in CD denote the CD subclass determined previously (K. Tajul-Arifin, R. Teasdale, and J.S. Mattick, in prep.; see Suppl. information). Each protein is referred to by its designated number used as reference throughout this paper, as listed in the left-most column. Pseudogenes are not presented.
▪▪▪ represents sequence that is shortened and not to scale to the rest of the sequence represented by ▬.

CHD-4 (Mi-2b) has been reported to be an autoantigen for dermatomyositis (Seelig et al. 1996; Wang and Zhang 2001).

The first of three CHD-3/4 proteins, also known as Mi-2a, is encoded by a gene located on chromosome 11 band B4. The protein in Ensembl is encoded by 31 exons, and domain analyses identified a RING-PHD Zn-finger domain overlap near the N-terminal, one CD of subclass J, and an ATP-dependent helicase region, which is typical of this subfamily. The FANTOM2 protein, encoded by 14 exons, contains two PHD domains with two CDs of subclass P and J. It seems that exons 10 and 11 encode for the CD of subclass P, whereas exon 8 may encode for part of the PHD domain sequence, whereby splicing the exon creates a sequence that constructs a RING-PHD domain overlap (Table 4).

The second CHD-3/4 protein, Mi-2b, is encoded on chromosome 6 band F2, and is represented in the FANTOM2 and Ensembl databases by several different transcripts (Table 4), none of which may be full-length. Indeed, two of these transcripts (4b and 4c) are presented as originating from separate adjacent loci in Ensembl, but probably arise from a single locus, in comparison with other known Mi-2b homologs. This conclusion is also supported by examination of the FANTOM2 transcript (4a), which spans both 4b and 4c. Proteins 4a and 4b contain two PHD domains and an ATP helicase domain and either one or two CDs, via alternative splicing, similar to that observed in CHD-2.

A third gene encodes another member of this subfamily (which we have termed Mi-2c), and is located on mouse chromosome 4 band E2. The Ensembl transcript includes four exons and encodes a short protein of 229 amino acids that contains just two CDs. However, the transcript is not full-length, and comparison with the human homolog indicates that the full-length protein would contain two PHD domains, two CDs, and an ATP-helicase domain. No transcripts from this locus were present in FANTOM2.

CHD–5 Subfamily

There are four genes encoding CHD-5 proteins in mouse, all of them novel. All of these proteins contain CDs of profiles Q and/or I. The first gene (which we have designated CHD-5a) is located on chromosome 2 band H3, from which there are a total of five possible protein isoforms predicted from alternatively spliced transcripts (with unique exon combinations; Table 4). These transcripts contain two CDs of profiles Q and

I, an ATP-dependent helicase domain, and a SANT DNA-binding domain.

The second novel CHD-5 locus is designated CHD-5b (No. 7). Located on chromosome 8 at band C5, four alternative spliced products were identified for this locus, two from FANTOM2 and two from Ensembl. The longest protein (No. 7c) is encoded by 30 exons (1666 aa), and contains two CDs, an ATP-dependent helicase domain, and two BRK domains within an extended C-terminal region. The function of BRK domains is unknown but is described by SMART as a domain found in transcriptional and CD helicase proteins. The second isoform identified in Ensembl (No. 7d) is encoded by 18 exons (1283 aa) and contains just two BRK domains. The proteins identified in FANTOM2 are probable alternative splicing products which include two CDs and the ATP-dependent helicase domain (Table 4).

The third and fourth CHD-5 genes in mouse (CHD-5c and CHD-5d, Nos. 8 and 9, respectively) are not represented in FANTOM2. The CHD-5c gene is located on chromosome 14 band C1, with four alternatively spliced transcripts identified in Ensembl encoding various isoforms of the protein, none of which may be full-length (Table 4). The CHD-5d locus is on chromosome 4 band A1, with four alternatively spliced transcripts identified in Ensembl. The possible full-length protein (No. 9a) is coded by 34 exons, and contains two CDs of subclasses Q and I near the N-terminal, an ATP-dependent helicase region, a SANT DNA-binding domain, and two BRK domains near the C-terminal (Table 4).

The functions of CHD-5 proteins in mammals have not been elucidated, but by analogy with homologs such as kismet in *D. melanogaster* (Daubresse et al. 1999), they are predicted to regulate genes involved in development. Interestingly, only one copy of the CHD-5 gene has been identified in *D. melanogaster* and *C. elegans* (Table 5) and none in yeast. Here we describe four CHD-5 genes in mouse, each one with a corresponding homolog in human (Table 3; Schuster and Stoger 2002). This suggests that the number of CHD-5 proteins correlates closely with developmental complexity.

Histone Methyltransferase Family

Two genes encoding histone methyltransferase CD family proteins were identified, Suppressor of variegation (3–9) homolog 1 (Suv39h1) and Suppressor of variegation (3–9) homolog 2 (Suv39h2). The Suv39h1 gene is located on the X chromosome band A1.1, and the Suv39h2 gene is located on chromosome 2 band A1.

The histone methyltransferase CD family contains a single CD of subclass X together with PreSET, SET, and PostSET domains. The SET domain confers the catalytic activity of the histone methyltransferase proteins. The name itself comes from the name of proteins from which the domain was first identified; the strongest PEV suppressor gene *Su(var)3-9* (Tschiersch et al. 1994), the Pc-G gene *Enhancer of zeste* (*E[z]*) (Jones and Gelbart 1993), and the activating trithorax group (trx-G) gene *trithorax* (Jenuwein et al. 1998). The SET domain is considered a 'promiscuous' domain as it is found in both chromatin repressors and activators. It has been suggested that the SET domain represents a surface for the assembly of either activating or repressing chromatin complexes, dependent on interactions with accessory Trx-G or Pc-G proteins, respectively (Jenuwein et al. 1998).

Two Suv39h1 proteins were identified in FANTOM2 VPS (Table 2) and two from Ensembl. As the two proteins in FANTOM2 VPS share 99% identity, differing only at aa position 364, they are considered to be synonymous (No. 10a). The Ensembl proteins are encoded by seven and six exons, producing proteins of 452 aa and 412 aa in length, respectively. Analysis shows that the FANTOM2 proteins correspond to the smaller-sized Ensembl protein (Table 4).

The Suv39h2 locus is represented by four proteins, three from the FANTOM2 VPS and one from Ensembl. Three of the proteins are similar (No. 11a) except for one or two mismatches in the aa sequence, whereas the fourth (No. 11b) appears to be a novel alternative splicing product, deduced from the transcript from FANTOM2. The longer proteins are encoded by six exons, and the shorter protein is encoded by five exons, with exon 2 spliced out. Exon 2 is 146 bases long, and partially codes for the CD, the removal of which shortens the CD region by 14 aa.

Suv39h1 and Suv39h2 genes in mouse are homologs of the suppressor of variegation (3–9) gene in *D. melanogaster* (Su[var]3–9; Table 5). Mutation of this gene in *Drosophila* suppresses the position variegation effect, whereby an active gene is silenced when it is physically translocated near a repressive region of the chromosome (Tschiersch et al. 1994). Su(var)3–9 controls heterochromatin-dependent gene silencing by histone H3-Lys9 methylation (Schotta et al. 2002).

The functions of the mouse and human Suv39h2 genes are not known, but mouse Suv39h2 transcripts are specifically expressed in adult testis (O'Carroll et al. 2000). The identification of alternative splicing products of both Suv39h1 and Suv39h2 genes raises questions as to how these proteins and their alternative spliced products may function. The alteration of the CD sequence as a result of alternative splicing in Suv39h2 is interesting, as it may change the target specificity or the nature and range of interactions of this protein.

Heterochromatin Protein 1 (HP1) Family

HP1 was one of the first CD-containing proteins discovered. HP1 proteins and their homologs are short proteins that contain just two CDs of subclasses S and T. The C-terminal CD is also known as the chromo shadow domain (CSD; Aasland and Stewart 1995). However, our sequence analysis has shown that the various CSD sequences are not significantly different from a range of other CD sequences, and in fact overlap with several CD subclasses (K. Tajul-Arifin, R. Teasdale, and J.S. Mattick, in prep.). Three HP1 loci are known in mouse and human (HP1α, β, and γ), and all were identified in the FANTOM2 VPS.

The HP1α gene is located on chromosome 15 band F3. Three HP1α proteins were identified from the FANTOM2 VPS and one was identified in Ensembl. Taking sequencing errors and strain polymorphisms into consideration, all of these proteins appear to be synonymous, and no alternative splicing protein products have been identified (No. 12).

The HP1β gene is located on chromosome 11 band D, and two proteins have been identified from both FANTOM2 VPS and Ensembl. The longest protein is 185 aa in length (186 aa in Ensembl) and appears to be the full-length protein, encoded by six exons (No. 13a). An alternatively spliced product has also been identified in both FANTOM2 and Ensembl, and is encoded by five exons whereby exon 5 is spliced out in the smaller protein (No. 13b). Exon 5 partially codes for the C-terminal CD, and its removal shortens the CD sequence by 22 aa.

The HP1γ locus is located on chromosome 6 band B3. Only one predicted protein from FANTOM2 VPS and Ensembl was identified for this gene. Mapping of HP1γ in Ensembl

Table 5. Homologs of Mouse CD-Containing Proteins in Human, Fly, and Worm

No.	Family	Protein	Human homolog	*D. melanogaster* homologs	*C. elegans* homologs
1	CHD	Chd1	Chd1	Chd1 (Stokes et al. 1996)	Chd-1 (Accession no. NP_491994)
2		Chd2 (novel)	Chd2 (novel)	*No fly homolog*	*No worm homolog*
3		Chd3/4-Mi2a (novel)	CHD3 (Mi2α)	2 Chd3/4 proteins (Accession nos. NP_649154 and NP_649111)	Chd3, Chd4 (Let-418) (Solari and Ahringer 2000; von Zelewsky et al. 2000)
4		Chd3/4-Mi2b (novel)	CHD4 (Mi2β)		
5		Chd3/4-Mi2c (novel)	CHD3/4		
6		Chd5a (novel)	Chd5	Kismet (Daubresse et al. 1999)	Chd-5 (Accession no. NP_491426)
7		Chd5b (novel)	Chd5		
8		Chd5c (novel)	Chd5		
9		Chd5d (novel)	Chd5		
10	Histone methyltransferase	Suv39h1	SUV39H1	Su(var)3-9 (Tschiersch et al. 1994)	*No worm homolog*
11		Suv39h2(Riken)	SUV39H2		
12	HP1	HP1α	HP1α	HP1a	Hpl-1, Hpl-2 (Accession nos. NP_510199 [Hpl-1] and NP_499373 [Hpl-2])
13		HP1β	HP1β	HP1b	
14		HP1γ	HP1γ	HP1c	
15		HP1γ (pseudogene)	HP1γ	(Platero et al. 1995; Singh et al. 1991)	
16		HP1γ (pseudogene)	*No human homolog*		
17		HP1γ homolog (novel)	*No human homolog*		
18	Polycomb	Cbx6	Cbx6 homolog	Polycomb (Pc) (Messmer et al. 1992)	*No worm homolog*
19		Cbx2 (M33)	Cbx2 homolog (novel)		
20		Cbx4 (MPc2)	Cbx4 homolog		
21		Cbx8 (MPC3)	Cbx8 homolog		
22		Pc homolog Cbx7 (novel)	Cbx7		
23	Msl-3 homologs	Msl-3 homolog	Msl3-like 1 isoform d	Msl-3 (Gorman et al. 1995)	Ce-1/MRG-1 (Fujita et al. 2002)
24		Tex189/MRG15	MRG15	MRG15 (Bertram and Pereira-Smith 2001)	
25		Tex189/MRG15(pseudogene)	*No human homolog*		
26		Transcription factor-like (novel)	*No human homolog*	*No fly homolog*	
27		Transcription factor-like (novel)	*No human homolog*	*No fly homolog*	
28	Histone acetyltransferase	MYST1	MYST1	Mof (Hilfiker et al. 1997)	VC5.4, K03D10.3 (Accession nos. NP_504796 [VC5.4] and NP_493467 [K03D10.3])
29		Tip60 homolog	Tip60		
30	Retinoblastoma binding protein-1	RBBP1	RBBP1	*No fly homolog*	*No worm homolog*
31		RBPB1-related (novel)	RBP1-related protein		
32	Enoyl-CoA hydratase	EnoylCoA protein (ECH)	EnoylCoA (novel)	*No fly homolog*	*No worm homolog*
33		Cdyl	CDYL		
34		*No mouse homolog*	CDY1		
35		*No mouse homolog*	CDY1		
36		*No mouse homolog*	CDY2 (pseudogene)		
37		*No mouse homolog*	CDY2 (pseudogene)		
38	SWI3	SRG3 (SMARCC1)	SMARCC1 homolog (novel)	Moira (Crosby et al. 1999)	*No worm homolog*
39		SMARCC2 homolog (novel)	SMARCC2		
40	Ankyrin family	ANK protein (MPP8)	MPP8	*No fly homolog*	*No worm homolog*

References of fly and worm proteins are given where available. Numbers in the first colum refer to mouse genes assigned throughout this article.

shows that the protein is coded by four exons. No alternatively spliced product has been identified. There are also two HP1γ pseudogenes, on chromosome 14 band D2 and chromosome 1 band C2. There are two other HP1γ loci, on chromosome 14 band D2 and chromosome 1 band C2, which code for identical proteins but are likely to represent pseudogenes, as they are composed of a single exon.

Another HP1γ locus (No. 17) is also identified in Ensembl on chromosome 1 band C3, but not represented in FANTOM2. Encoded by four exons, the protein contains two CDs (Table 4), with the sequence showing significant overall similarity to the known mouse HP1γ protein sequence. We therefore termed this gene a novel HP1γ homolog (Table 3). The CDs subclasses (S and T) are the same as other HP1 proteins described, confirming the protein's identity. However, unlike other HP1 genes discussed, there is no homolog found in human.

HP1 proteins in mouse have not been extensively studied. In *D. melanogaster*, HP1α is heterochromatin-specific, HP1β localizes to both heterochromatin and euchromatin, and HP1γ is euchromatin-specific (James and Elgin 1986; Smothers and Henikoff 2001; Volpe et al. 2001). HP1 proteins appear to function as transcriptional repressors (Jones et al. 2000; Vassallo and Tanese 2002). The difference in localization is due to targeting by the C-terminal CD and to some extent the hinge region between the N- and C-terminal CDs (Smothers and Henikoff 2001). In this context the alternative splicing of HP1β is interesting, as it shortens the C-terminal CD, and may therefore alter the target specificity of HP1β or interfere with the function of the normal HP1β.

Polycomb (Pc) Family

Five different Pc-like genes were identified in the mouse. Pc proteins contain one CD of subclass B with no other (as yet) recognized domains, and appear to act as part of a large multiprotein complex to maintain epigenetic gene repression in euchromatic regions of the chromosome (Pearce et al. 1992; Alkema et al. 1997; Satijn et al. 1997; Bardos et al. 2000; Hemenway et al. 2000). The CD of Pc proteins appears to recognize histone H3 methylated at lysine 27 (C.D. Allis, pers. comm.) and plays an important role in maintaining the complex at target sites, whereby mutations in the CD sequence not only result in a loss of binding of Pc at its target sites, but also lead to an apparent disintegration of the entire Pc group complex (Strutt and Paro 1997). Five Pc genes were identified, including one novel gene.

Chromobox Protein Homolog 6 (Cbx6)

There are four Cbx6 proteins identified in this study, three from FANTOM2 VPS and one from Ensembl, all derived from the same locus on chromosome 15 band E2. Two of the identified proteins from FANTOM2 VPS (No. 18a) are synonymous with the Cbx6 protein identified in Ensembl and are encoded by five exons. The third Cbx6 protein identified in FANTOM2 (No. 18b) is similar, but the transcript sequence lacks the first 54 bases of exon 5 that encodes for 18 aa, presumably as a result of the usage of an alternative splice site, which does not affect the CD sequence.

Cbx2 (M33)

The Cbx2 gene is located on chromosome 11 band E2. Cbx2 is different from other identified Pc proteins as it contains an AT-hook domain, which is a small DNA-binding domain. The Cbx2 protein identified in the FANTOM2 VPS is a protein from the public database (No. 19), and a synonymous protein was also identified in Ensembl. The protein is encoded by five exons, and no alternative splicing product was identified.

Cbx4 (MPc2)

The gene that encodes for Cbx4 protein, also known as MPc2, is also located on chromosome 11 band E2, and consists of five exons. The protein identified in Ensembl is 551 aa in length, identical to that identified in FANTOM2 (No. 20). No alternative splicing product was found.

Cbx8 (MPc3)

The gene encoding Cbx8, also known as MPc3, is also located on chromosome 11 band E2. The proteins identified from FANTOM2 VPS and Ensembl are identical, 362 aa in length, and are encoded by five exons (No. 21). No alternative splicing product was identified for this protein.

Cbx7

The gene encoding Cbx7 is located on chromosome 15 band E2. Two proteins were identified from the FANTOM2 VPS (Nos. 22a and 22b). The shorter protein is synonymous with the protein identified in Ensembl and is encoded by seven exons. The longer protein in FANTOM2 is encoded by a transcript that also contains seven exons (No. 22b), but with an additional exon between exons 4 and 5 (280 bases) of the Ensembl EST sequence, which extends the translated protein sequence to 251 aa. Other interesting features of the FANTOM2 transcript include a cytosine insertion at position 716 (within exon 6), and the last exon comprises exons 7 and 8 and the intronic sequence in between, exon 7a (Table 4).

Msl-3 Homologs

Five Msl-3 homologs in mouse were identified, with two being novel. Msl-3 in *Drosophila* is a component of multisubunit histone acetyltransferase complexes (Marin and Baker 2000), which suggests that Msl-3 and its homologs are involved in transcriptional regulation via histone acetylation and chromatin modification. Msl-3 and its homologs are characterized by a CD of subclass E near the N-terminus, with no other as yet recognized domains, although there are uncharacterized conserved regions within the C-terminal region among these proteins.

A mouse Msl-3 protein which is similar to the *Drosophila* Msl-3 protein was identified in Ensembl and the FANTOM2 VPS set, but does not contain a CD, unlike other known Msl-3 homologs. The gene is located on chromosome X band F5, and the corresponding cDNA is encoded by 11 exons. We presume that the cDNA is not full-length but an alternatively spliced variant that lacks a CD, because its human homolog contains a CD. Potential CD encoding sequences are present at this locus in the mouse and rat genomes.

Another Msl-3 homolog was identified, the MORF-related gene 15 (MRG15), also known as Tex-189. It is 323 aa in length, and is encoded by 12 exons. The gene is located on chromosome 9 band E3.2. The two proteins identified in FANTOM2 VPS and Ensembl are synonymous. A likely pseudogene for Tex-189 was also identified on chromosome 19 band D1. It codes for the full-length protein but consists of just one exon. Studies have shown that mouse MRG15/Tex-189 is localized in dendrites as well as in the nuclei of Purkinje cells (Matsuoka et al. 2002), and may function as a chromatin structure regulator. It may be involved in the gene expression for synaptic plasticity and may link synaptic activity to gene expression.

In addition to the two proteins described above, two novel Msl-3 homologs were identified in Ensembl, both encoded by three exons separately on chromosome 18 bands A2 and C. Neither gene has a homolog in human, which represents one of the relatively rare cases of such an occurrence. These two proteins were not identified in FANTOM2.

Histone Acetyltransferase (HAT) Family

HAT proteins are postulated to activate genes by altering chromosome architecture (Bannister and Miska 2000). CD-containing HATs, which are localized in the nucleus, show distinct specificity in histone acetylation (H4 Lys5, H3 Lys14, and H2A Lys4), which is different from other known HATs which do not contain CD (Lucchesi 1998; Ding et al. 2000). Two CD-containing HAT genes were identified in our analysis. Members of this family contain a CD of subclass F and a C-terminal HAT domain.

The first protein identified is the Myst1 (Kawai et al. 2001), the gene for which is located on chromosome 7 band F4. Two FANTOM2 transcripts were identified for this gene. The longer transcript, which encodes for the full-length protein, is comprised of 11 exons (No 28a). This protein is identical to the one identified in Ensembl. The additional transcript identified encodes a smaller protein of 194 aa in length and contains only the CD. The cDNA transcript of this protein is comprised of exons 1–3 of the full-length transcript, with an in-frame stop codon within the intron sequence following exon 3.

The second CD-containing HAT gene identified is the mouse homolog of the human protein Tip60 (Table 3). The mouse homolog gene is located on chromosome 19 band A and is comprised of 13 exons. This gene is not represented in FANTOM2. Mouse Tip60 has been suggested to have a developmental function in early embryogenesis and organ development (McAllister et al. 2002).

Retinoblastoma-Binding Protein 1 (RBBP1) Family

Two mouse genes encoding CD-containing proteins of the RBBP1 family were identified in this study. RBBP1 proteins are characterized as containing a CD of subclass E, together with TUDOR and BRIGHT (also known as ARID) DNA-binding domains. Studies of human RBBP1 protein indicates that it plays an important role in repressing E2F-dependent promoters via interactions with the retinoblastoma protein (Lai et al. 1999).

The first mouse RBBP1 gene is located on chromosome 12 band C3. Two alternatively spliced transcripts were identified in Ensembl for this gene, coding for the full-length protein containing all three CD, TUDOR, and BRIGHT domains (No. 30a) and a shorter transcript that codes for a protein which contains just the CD (No. 30b; Table 4).

The gene for RBBP1-related protein is located on chromosome 13 band A2. In Ensembl, two isoforms of the protein were identified, with the long isoform containing a CD, as well as TUDOR and BRIGHT domains, and the shorter isoform containing just the CD. Interestingly, because of the splicing out of exon 15 in the long isoform, which encodes for part of the CD sequence, the CD subclass is transformed into subclass U. Perhaps the longer isoform protein is targeted to a region that is different from that of the shorter isoform.

Enoyl-CoA Hydratase (EnoylCoAH) Family

Two loci encoding EnoylCoAH family proteins were identified, encoding proteins containing one CD of subclass L, and an EnoylCoAH domain. The EnoylCoAH domain is found in many CoA-dependent acylation enzymes, including napthoate synthase, carnitate racemase, 3-hydoxybutyryl-CoA dehydratase and dodecanoyl-CoA δ-isomerase.

The first locus encodes a protein that is identified in FANTOM2 VPS with two corresponding proteins identified in Ensembl. The gene is located on chromosome 8 band E1, with both protein isoforms encoded by six exons. The full-length proteins identified in both FANTOM2 and Ensembl are synonymous. They contain a CD near the N-terminal and an EnoylCoAH domain (No. 32a). The shorter transcript encodes for a protein that lacks the CD as a result of the use of an alternative first exon. Presumably this indicates the use of an alternative promoter for the transcription of mRNA for both proteins. The function of this protein in mouse is not yet known, and thus far this family has only been identified in mammals (Table 5).

The second gene identified that encodes for CD-containing EnoylCoAH protein is located on chromosome 13 band A5. The gene encodes for the Cdyl protein, which plays a major role in spermatogenesis in mouse and shares 93% identity with its human homolog, CDYL. Cdyl is deduced to activate genes in spermatogenesis by acetylating histone H4 (Lahn et al. 2002). The two proteins identified in both FANTOM2 VPS and Ensembl are identical, and no alternative splicing product was observed for this protein (No. 33).

SWI3 Family

Two genes were identified that encode for proteins that match the characteristics of the CD-containing SWI3 family, the archetypal member of which (SWI3) was first characterized in yeast, but which in fact does not contain a CD. CD-containing SWI3 proteins are characterized by one CD of subclass G, which may overlap with a BRCT protein-binding domain, a SWIRM protein-binding domain, and a SANT DNA-binding domain in the C-terminal region (Table 4).

The first SWI3 family member in mouse is SRG3, also known as SMARCC1. Mouse SRG3 is essential for early embryogenesis and plays an important role in mice brain development (Kim et al. 2001), as well as being a regulatory component of T-cell development in the thymus (Jeon et al. 1997). The gene is located on chromosome 9 band F2, and the protein is encoded by 30 exons (No. 38). Synonymous proteins were identified in both FANTOM2 VPS and Ensembl, and no alternative splicing product was recorded.

The second gene of the mouse SWI3 family is on chromosome 10 band D3, the mouse homolog of the human SMARCC2 gene (No. 39; Table 3). Four alternatively spliced transcripts were identified at the locus in Ensembl, three of them coding for very similar proteins, and the fourth transcript of four exons coding for a short peptide (No. 39d). Proteins 39a, 39b, and 39c are very similar to each other except for their protein lengths. Alternative splicing of the exons does not cause any major differences in proteins 39b and 39c, but the splicing of exon 7 in 39a causes the protein to lose the BRCT protein-binding domain and shortens that CD region slightly (Table 3). Exon 7 therefore presumably encodes for part of the BRCT domain and CD sequence. Transcripts for this gene were not identified in FANTOM2.

Ankyrin Family

There is only one protein identified in mouse (MPP8) that fits the characteristic of this family. MPP8 contains one CD of subclass L near the N-terminal, with three copies of ankyrin repeats near the C-terminal (No. 40). The gene is located on chromosome 14 band C2 with 14 exons, and the proteins identified in both FANTOM2 and Ensembl are synonymous. The function of this protein is not yet known.

CD Families Not Detected in the Mouse Transcriptome

Three of 13 CD families which have been identified (K. Tajul-Arifin, R. Teasdale, and J.S. Mattick, in prep.) were not present in the mouse, nor in human, and presumably are absent from mammalian genomes in general. These are the chromomethylase (CMT) family, the integrase family, and the AAA (ATPases associated with diverse cellular activities) family.

CMT family proteins contain a CD of subclass M embedded within an extended DNA methylase domain, with a BAH (bromo adjacent homology) domain near the N-terminal of the protein. These proteins are unique to plants and have not been identified in animals (Genger et al. 1999). They are also the only eukaryotic DNA methylases which contain CDs (Henikoff and Comai 1998). The BAH domain is frequently associated in proteins with other modules which are implicated in epigenetic mechanisms of gene regulation such as bromo and SET domains, as well as PHD fingers, and the BAH domain appears to be tightly associated with replication events (Callebaut et al. 1999). CMT proteins are postulated to methylate DNA specifically at CNG motifs in gene silencing (Lindroth et al. 2001).

The integrase family members also appear to be plant-specific and have not thus far been identified in animals (K. Tajul-Arifin, R. Teasdale, and J.S. Mattick, in prep.). These proteins contain reverse transcriptase and integrase core domains, with a CD of subclass W located at the C-terminal end of the protein. The function of these proteins is unknown. The integrases form a novel group within Ty3/Gypsy retrotransposons, which have been proposed to be named the 'Chromovirus' (Marin and Llorens 2000).

The last CD family which has not been identified in either mouse or human is the AAA family. The proteins have been identified in *S. cerevisiae* and *S. pombe,* but not in plants or animals (K. Tajul-Arifin, R. Teasdale, and J.S. Mattick, in prep.). The proteins are characterized by two AAA domains, with a CD of subclass A embedded within the C-terminal AAA domain. Yef3 (yeast elongation factor 3) of *S. cerevisiae*, a component of yeast protein elongation machinery, is proposed to play a role in the ribosomal optimization of the accuracy of fungal protein synthesis by altering the conformation and activity of a ribosomal 'accuracy center' (Sandbaken et al. 1990; Belfield et al. 1995; Chakraburtty and Triana-Alonso 1998). The function of CD in these proteins is not known.

CD-Containing Proteins in Human

In this analysis, we also identified 34 genes that code for CD-containing proteins in human. Of the 34 genes identified, four do not have homologs in mouse. These are all CDY genes, including two probable pseudogenes. On the other hand, six of the mouse genes are not found in human. These are the two HP1γ pseudogenes, the Tex189/MRG15 pseudogene, a novel HP1γ gene, and two novel Msl-3 homologs. Besides the genes mentioned, there is good conservation of the CD-related genes between human and mouse. There is also conservation of alternative splicing patterns, at least in some cases (data not shown). Conservation of CD-containing proteins between mouse and human also extends to the conservation of the domain structure of the CD-containing proteins and the type of subclass of the CD within the CD protein family.

DISCUSSION

By combining data from FANTOM2 and Ensembl, we identified 36 loci encoding CD-containing proteins in mouse, including 17 novel loci. In total, 65 CD-related alternatively spliced proteins were identified, with 43 being novel. All of the mouse genes except four are conserved in human, whereas there are six human genes encoding CD-containing proteins that are not conserved in mouse.

CD-containing proteins are generally localized in the nucleus as part of large complexes and are involved in either activating or repressing genes or regions of the chromosome by altering chromatin architecture. Evidence suggests that the CD determines the target specificity of the protein/complex, as domain swapping of CD regions of the Pc and HP1 proteins of *D. melanogaster*, which normally localize to the Polycomb and heterochromatin region, respectively, targeted the Pc proteins/complexes to the heterochromatin region, and vice versa (Platero et al. 1995). In this context it is interesting to note that some of the alternative-splicing products of the mouse CD-proteins have lost part or all of their CDs, and in one case at least, change the subclass of the CD, which may indicate that these proteins have altered specificity or act in opposition to the function of CD-containing isoform.

The absence of three CD families in mouse and human indicates that some CD-containing proteins have evolved to carry out specific function(s) in a particular class of organism. Consequently, three of the CD families described here are only found in mammals (RBBP, EnoylCoAH, and Ankyrin families; Table 5), and mammals lack the three CD families which are found in plants or fungi. Evolutionary and domain accretion studies will provide further insight into the evolution and function of the different CD families.

METHODS

Creating CD Hidden Markov Models (HMM) Profiles

CD-containing protein sequences were obtained from publicly available databases. Proteins were filtered to avoid multiple representation of a protein from the same organism, except for alternatively spliced products. The proteins were analyzed with SMART and Pfam to obtain the CD sequence for each protein. CD sequences were then clustered using the Protein Distance Method in BioManager (http://bn2.angis.org.au), using default settings. An HMM 'profile' for each CD cluster or subclass was built and calibrated using the HMMER package (Version 2.2 August 2001; http://hmmer.wustl.edu/). The CD profiles were then used to query data sets for CD-containing protein. Designation of a particular subclass to a CD in a protein is based on the least E-value given to the alignment between a CD sequence and the profile sequence of a particular subclass. The cut-off point for the E-value is set at 10^{-4} for a sequence to be positive for CD.

Identification and Mapping of Mouse and Human CD-Containing Proteins

CD profiles created were used to identify CD-containing proteins in FANTOM2 VPS from RTPS6.3 with HMMER2.2 (see Supplementary information). Proteins belonging to the same cluster as the identified CD-containing protein were grouped for alternative splicing analysis. cDNA sequences of mouse CD-containing proteins in FANTOM2 were mapped to the mouse genome using the Mouse Genome Server (MGS, version 9.3a.1, last updated December 2, 2002) and the SSAHA server (version 2.0) in Ensembl (www.ensembl.org; Hubbard et al. 2002). Ensembl-predicted proteins from those genes that were initially not identified in FANTOM2 were used to back-query the FANTOM2 VPS using local BLAST (Altschul et al. 1997) to identify any proteins from these loci that lacked the CD. Human homologs for each mouse gene were identified using Ensembl. Proteins identified in both FANTOM2 and Ensembl were combined in relation to listing alternatively spliced products of CD-related genes.

Alternative Splicing Analysis

The nucleotide sequence of each protein identified in VPS was analyzed to determine the exon structure by comparison with Ensembl, and by reference to MouSDB (http://genomes.rockefeller.edu/splice/; Zavolan et al. 2002). A transcript was considered to be an alternative spliced product only if it exhibited an exon structure different from that of other transcripts from the locus, that is, it was clearly not a possible truncated or incomplete reverse transcript (of which many examples occur in these databases).

Domain Analysis

Each protein sequence was analyzed with SMART (Simple Modular Architecture Research Tool, http://smart.embl-heidelberg.de, version 3.4, 641 HMMs, 29 August 2002; Schultz et al. 1998, Letunic et al. 2002) and Pfam (http://pfam.wustl.edu, version 7.5, 4176 models, August 2002; Bateman et al. 2000) for possible functional domains. The results of the two analyses were combined to represent the complete domain structure of each protein.

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http://smart.embl-heidelberg.de; SMART—Simple Modular Architecture Research Tool.

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