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Complete sequence of human vinculin and assignment of the gene to chromosome 10

(cell–matrix junctions/actin cytoskeleton/talin)

P. A. WELLER*, E. P. OGRYZKO†, E. B. CORBEN*, N. I. ZHIDKOVA†, B. PATEL*, G. J. PRICE*‡, N. K. SPURR§, V. E. KOTELIANSKY†, AND D. R. CRITCHLEY*¶

*Department of Biochemistry, University of Leicester, Leicester LE1 7RH, United Kingdom; †Institute of Experimental Cardiology, Cardiology Research Centre of the USSR, Academy of Medical Sciences, 3rd Cherepkovskaya Street 15A, Moscow 121552, USSR; and §Department of Human Genetic Resources, Imperial Cancer Research Fund, Clare Hall Laboratories, Potters Bar, Herts EN6 3LD, United Kingdom

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ABSTRACT **We have determined the complete sequence of human vinculin, a cytoskeletal protein associated with cell–cell and cell–matrix junctions. Comparison of human and chicken embryo vinculin sequences shows that both proteins contain 1066 amino acids and exhibit a high level of sequence identity (>95%). The region of greatest divergence falls within three 112-amino acid repeats spanning residues 259–589. Interestingly, nematode vinculin lacks one of these central repeats. The regions of human vinculin that are N- and C-terminal to the repeats show 54% and 61% sequence identity, respectively, to nematode vinculin. Southern blots of human genomic DNA hybridized with short vinculin cDNA fragments indicate that there is a single vinculin gene. By using a panel of human–rodent somatic cell hybrids, the human vinculin gene was mapped to chromosome 10q11.2–qter.**

Vinculin is a cytoskeletal protein associated with the cytoplasmic face of both cell–cell and cell–extracellular matrix adherens-type junctions (1, 2), where it is thought to function as one of several interacting proteins involved in anchoring F-actin to the membrane (reviewed in ref. 3). In cell–matrix junctions, these proteins include α-actinin (4) and talin (5), although talin is absent from cell–cell junctions (6). Evidence in support of such a role for vinculin comes from *in vitro* biochemical studies that show that vinculin binds to α-actinin (7, 8), an F-actin bundling protein (reviewed in ref. 9), and to talin (10). Talin in turn has been reported to bind to the cytoplasmic domain of the β1 subunit of the fibronectin receptor (11, 12), thus completing the link between the actin cytoskeleton and the extracellular matrix. Interaction between matrix proteins and their receptors is thought to provide the trigger for assembly of these specialized cellular junctions (13), and protein phosphorylation is likely to be involved in the regulation of their stability (reviewed in ref. 3). To obtain a more complete understanding of the role of vinculin in adherens junctions, the sequence of chicken embryo vinculin has been determined (14–16). The sequence of nematode *Caenorhabditis elegans* vinculin also has been published (17). Here we report the complete sequence of human vinculin‖ together with evidence that there is a single human vinculin gene, which we have localized to chromosome 10.

MATERIALS AND METHODS

A partial human vinculin cDNA (HV1) was isolated from a human phage λgt10 fibroblast cDNA library (provided by the late A. R. Macleod of Ludwig Institute, Cambridge, U.K.) by using a 2.89-kilobase (kb) chicken embryo vinculin cDNA probe (14). The HV1 cDNA was used to screen an oligo(dT)-primed human endothelial cell λgt11 library (provided by S. Orkin of Children's Hospital, Boston) (18), and HV2, HV6, and HV7 were isolated. Vh1F was isolated from a human fibroblast λgt11 library (Clontech) by screening with an antibody to human vinculin following standard procedures. Vh2E and Vh3E were isolated from a human endothelial λgt11 library (a gift from M. Chao of Cornell Medical Center, New York) by using Vh1F as a probe. Vh6F was isolated from a human lung fibroblast λgt11 library (Clontech) by using the 0.27-kb 5′ *Eco*RI–*Bam*HI fragment from HV1 as a probe. DNA probes were labeled with [α-^{32}P]dCTP by the random priming method (19). Plaque screening by DNA·DNA hybridization was by standard procedures (20), with a final wash in 0.45 M NaCl/0.045 M sodium citrate, pH 7, at 65°C. cDNAs were subcloned into either M13mp18 or the plasmid vectors Bluescript (Stratagene) or pTZ19 and were sequenced on both strands by the dideoxy chain-termination method (21).

All of the hybrids used in this work have been described (22). Cells were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum at 37°C in 5% CO_2/95% air. To retain human chromosomes X or 17, media were supplemented where appropriate with 0.1 mM hypoxanthine, 10 μM methotrexate, and 16 μM thymidine. All hybrids were checked for the presence of human chromosomes by isoenzyme and karyoptic analysis (22) at the same passage as that used to prepare high molecular weight genomic DNA.

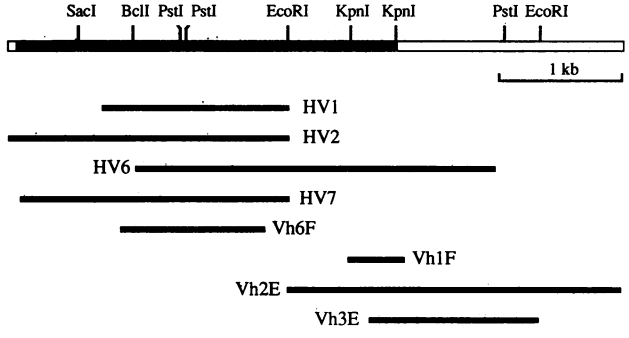

FIG. 1. Restriction map of overlapping human vinculin cDNAs. ■, Coding sequence; □, untranslated sequence. Clones HV1, HV2, Vh1F, Vh6F, Vh2E, and Vh3E were completely sequenced on both strands. The sequence across the *Eco*RI site within the coding region was confirmed by using clone HV6. There were no differences between the sequences of these cDNAs in the regions of overlap. The origins of the cDNA clones are described in *Materials and Methods*.

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‡Present address: Roche Products, Welwyn Garden City, Herts, U.K.
¶To whom reprint requests should be addressed.
‖The sequence reported in this paper has been deposited in the GenBank data base (accession no. M33308).

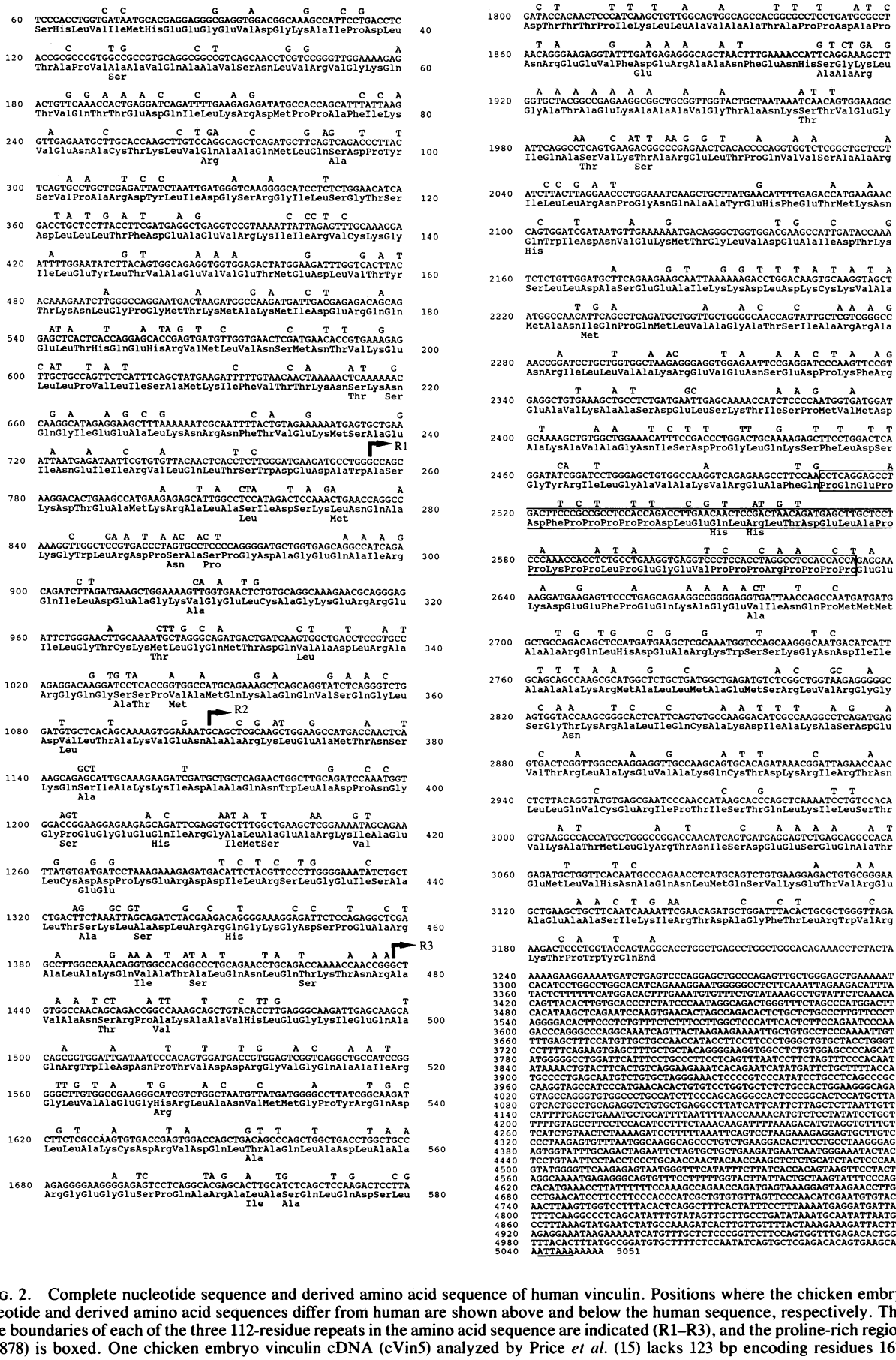

FIG. 2. Complete nucleotide sequence and derived amino acid sequence of human vinculin. Positions where the chicken embryo vinculin nucleotide and derived amino acid sequences differ from human are shown above and below the human sequence, respectively. The positions of the boundaries of each of the three 112-residue repeats in the amino acid sequence are indicated (R1–R3), and the proline-rich region (residues 837–878) is boxed. One chicken embryo vinculin cDNA (cVin5) analyzed by Price *et al.* (15) lacks 123 bp encoding residues 167–207. The

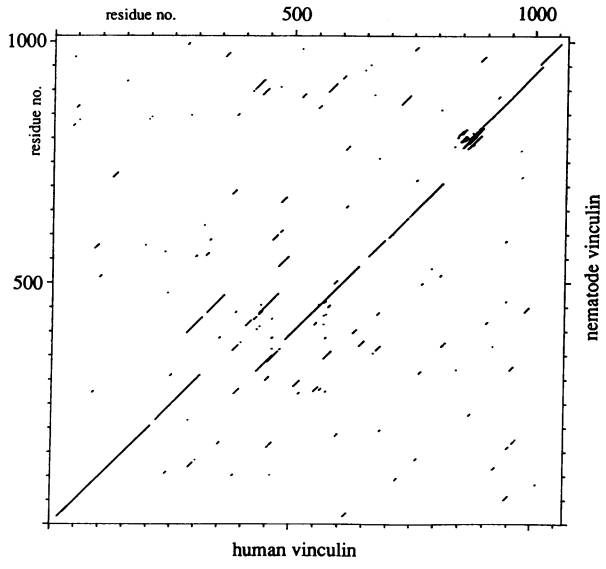

FIG. 3. Dot matrix comparison of human and nematode vinculin amino acid sequences using the University of Wisconsin Genetics Computing Group programs COMPARE and DOTPLOT, with window and stringency values of 30 and 8, respectively.

RESULTS

The relationship between the eight cDNA clones from which we have determined the complete sequence of human vinculin is shown in Fig. 1. Sequence analysis shows that the cDNAs span 44 base pairs (bp) of 5′ untranslated sequence, the complete coding sequence (3198 bp), and 1852 bp of 3′ untranslated sequence (Fig. 2). Comparison of the coding sequence of human vinculin with that of chicken embryo vinculin (14–16) shows a high level of sequence identity both at the DNA (82%) and amino acid (95%) levels, although the 5′ and long 3′ untranslated sequences are divergent. Human vinculin contains 1066 amino acids, as does the chicken embryo protein, and has a deduced molecular mass of 116,732 Da. Most of the amino acid sequence divergence between the human and chicken embryo vinculin sequences occurs in the central region of the protein, which contains three repeats of approximately 112 amino acids (residues 259–589) of unknown function (14). There are 32 amino acid sequence differences in this repeat region, which spans 330 residues. The region N-terminal to the repeats, which contains a talin-binding site (23), is more highly conserved with only 5 differences in 258 amino acids. Similarly, there are only 13 sequence differences in the 476 amino acids C-terminal to the repeats. This region contains a proline-rich sequence (residues 837–878) thought to be important in separating the globular head of vinculin from its extended tail (14, 15).

The sequence of nematode vinculin also has been determined (17), and similarities with the partial chicken embryo vinculin sequence (14) were noted. Interestingly, there are only two central repeats in nematode vinculin, which accounts for its lower molecular mass estimated by SDS/PAGE. Dot matrix analysis of the complete human and nematode sequences (Fig. 3) confirms that the N- and C-terminal domains of vinculin are the most highly conserved regions of the protein (54% and 61% sequence identity, respectively), and the alignments of these regions are shown in Fig. 4.

Southern Blot Analysis of the Human Vinculin Gene. Southern blot analysis of human DNA was carried out with a

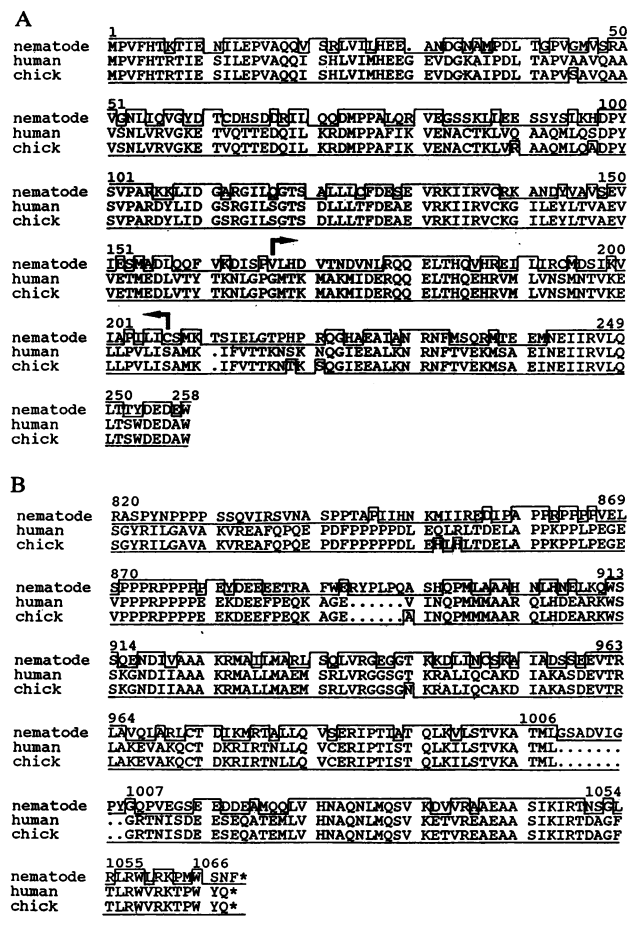

FIG. 4. Alignment of the N- and C-terminal regions of human, chicken embryo, and nematode vinculin. Amino acids are numbered relative to the human sequence. Alignments were determined by using the University of Wisconsin Genetics Computing Group program GAP. Residues identical to the human sequence are boxed. (*A*) N-terminal region, residues 1–258. Arrows denote the boundaries of the 41-amino acid region (residues 167–207) absent from the chicken embryo cDNA cVin5 (15). (*B*) C-terminal region, residues 820–1066.

number of vinculin cDNA fragments as probes (Fig. 5). When blots were hybridized with fragments spanning the entire coding sequence and part of the 3′ untranslated region, a relatively complex pattern of hybridization was obtained (data not shown). In contrast, short fragments encoding residues 447–539 and 704–773 as well as a 499-bp fragment from the 3′ untranslated sequence gave rise to single hybridizing bands (Fig. 5 *b*, *c*, and *e*), strongly suggesting that there is a single human vinculin gene. However, a 596-bp fragment consisting of the 5′ untranslated region and coding up to residue 182 and a 367-bp fragment encoding residues 943–1065 hybridized to multiple bands in most enzyme digests (Fig. 5 *a* and *d*). This result suggests that the organization of the 5′ and 3′ ends of the gene may be relatively complex. Alternatively, the probes could be detecting pseudogenes or other vinculin-related sequences, although the high stringency at which hybridization was carried out makes this latter possibility unlikely.

Chromosome Assignment of the Human Vinculin Gene. Southern blots of *Eco*RI-digested somatic cell hybrid DNAs were hybridized with the 1.5-kb human vinculin cDNA HV1 (see Fig. 1), which detects four major fragments in human

origin of this heterogeneity remains to be established. However, both the human vinculin cDNAs that span this region (HV2 and HV7) contain this sequence. The chicken embryo vinculin sequence published by Price *et al.* (GenBank accession no. Y00312; refs. 14 and 15) contains an error: residues 442–447 should now read Thr-Ala-Lys-Leu-Ser-Asp, in agreement with the sequence published by Coutu and Craig (GenBank accession no. J04126; ref. 16).

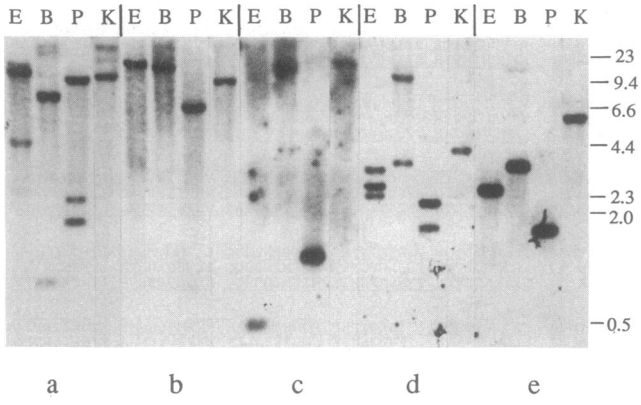

FIG. 5. Analysis of the human vinculin gene by Southern blotting. Human genomic DNA was cleaved with the restriction endonucleases *Eco*RI (E), *Bam*HI (B), *Pst* I (P), and *Kpn* I (K). DNA fragments were separated in a 0.8% agarose gel and transferred to Hybond N (Amersham), and the blot was hybridized at 65°C in 0.45 M NaCl/0.045 M sodium citrate (pH 7) with five different vinculin cDNA fragments in succession. Posthybridization washes were at 65°C in 0.03 M NaCl/0.003 M sodium citrate (pH 7). The filters were stripped between each hybridization, and the efficiency of probe removal was checked by autoradiography. (*a*) *Eco*RI–*Sac* I fragment (596 bp) from clone HV2, including the 5′ untranslated sequence and the coding sequence up to residue 182. (*b*) *Bgl* II fragment (279 bp) from HV1 encoding residues 447–539. (*c*) *Cla* I–*Eco*RI fragment (208 bp) from HV1 encoding residues 704–773. (*d*) *Kpn* I fragment (367 bp) from HV6 encoding residues 943–1065. (*e*) *Nco* I–*Eco*RI fragment (499 bp) from HV6 containing only the 3′ untranslated sequence. Migration positions of size markers (phage λ *Hin*dIII fragments) are shown in kb.

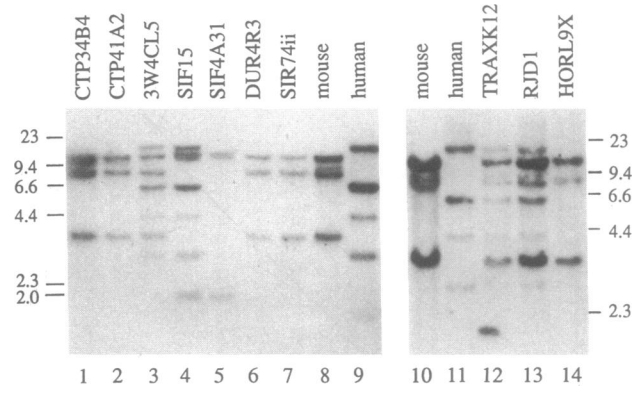

FIG. 6. Chromosome assignment of the human vinculin gene. Southern blot of *Eco*RI-cleaved somatic cell hybrid DNAs (lanes 1–7 and 12–14) and mouse (lanes 8 and 10) and human (lanes 9 and 11) genomic DNA hybridized with a 1559-bp human vinculin cDNA probe (HV1, see Fig. 1). Hybrids in lanes 4 and 5 are human–rat. Migration positions of size markers (phage λ *Hin*dIII fragments) are shown in kb.

DNA of approximately 12, 6, 4, and 3 kb (Fig. 6, lanes 9 and 11). In mouse DNA, this probe detects fragments of 10, 8, and 3.5 kb, (Fig. 6, lanes 8 and 10) and in rat DNA of 11 kb and 2 kb (Fig. 6, lane 5). Typical results obtained from screening seven hybrids, two of which were found to contain the human vinculin gene, are shown (Fig. 6, lanes 1–7). A total of 17 human–rodent cell hybrids were screened, and results from the analysis are shown in Table 1. The only chromosome to show complete concordance with the presence of the human vinculin gene is chromosome 10 (Table 1). The hybrid TRAXK12 (a gift from P. J. Goodfellow, Imperial Cancer Research Fund, London), which contains a chromosome X/10 translocation t(X;10)(p22.3;q11.2) was positive for the human vinculin gene (Fig. 6, lane 12), thus allowing further localization to chromosome 10q11.2–qter.

DISCUSSION

Comparison of the human, chicken embryo, and nematode vinculin sequences shows that the regions N- and C-terminal to the central repeat region are highly conserved and presumably contain domains important to the function of the protein. In the case of the N-terminal region, we have provided evidence that residues 167–207 are important to the talin-binding activity of chicken embryo vinculin (23). It is also apparent that this region of the molecule is important for the ability of the expressed protein to localize to cell–matrix junctions (23, 24). Furthermore, we have shown that the deletion of contiguous regions of as little as 7 amino acids in the sequence spanning residues 167–201 dramatically reduce the talin-binding activity of the N-terminal 398 amino acids of vinculin (23). Although residues 167–207 are identical in human and chicken embryo vinculin, this is a region of

Table 1. Assignment of the human vinculin gene to chromosome 10

	Chromosome																							
Hybrid name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	*VINC*
3WCL5	−	−	−	−	−	−	+	−	−	+	+	+	−	+	+	−	+	−	−	−	+	−	+	+
SIF15	−	+	−	−	−	+	+	−	−	+	−	−	−	+	+	−	−	−	−	+	−	−	+	+
DT1.2	−	−	+	−	−	−	−	−	−	+	+	−	+	−	+	−	+	+	−	+	+	+	+	+
HORP9.5	−	−	−	−	−	−	−	−	−	+	+	+	−	+	−	−	−	−	−	−	−	+	+	+
MOG34A4	+	−	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−	+	+	−	+	−	+	+
FG10	−	+	−	−	+	−	−	+	−	+	−	−	−	−	+	−	−	+	−	−	+	−	+	+
FST9/10	−	−	+	+	−	+	−	+	−	+	−	+	+	−	+	−	−	+	−	+	−	+	+	+
DT1.2.4	−	−	+	−	−	−	−	−	−	+	+	−	−	−	+	−	+	+	−	+	+	−	+	+
RJD1†	−	−	+	−	−	−	+	−	−	+	−	−	−	−	−	−	−	+	+	+	−	−	+	+
TRAXK12‡	−	−	−	−	−	−	−	−	−	*	+	−	−	−	+	+	−	−	−	−	+	+	*	+
SIR74ii	+	+	+	+	−	−	−	−	−	−	−	+	+	+	−	−	+	t	−	−	+	t	+	−
DUR4R3	−	−	+	−	+	−	−	+	−	−	+	+	+	+	−	−	+	−	−	+	+	−	−	−
SIF4A31	−	−	+	+	+	+	−	−	−	−	−	−	−	+	−	+	−	−	−	−	−	−	+	−
CTP41A2	−	+	−	−	−	+	+	−	−	−	−	−	−	+	−	−	+	−	−	−	−	−	+	−
CTP34B4	+	+	+	−	+	+	+	+	−	−	−	+	−	+	−	+	+	+	−	−	−	−	+	−
TWIN19-D12	+	−	+	+	−	+	−	+	−	−	+	+	−	+	−	+	+	+	−	+	−	−	−	−
HORL9X	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−

+, Chromosome present; −, chromosome absent; t, trace; *, hybrids containing chromosome fragments; *VINC*, presence (+) or absence (−) of human vinculin gene.

†Human Genetic Resources, Imperial Cancer Research Fund.

‡Gift from P. J. Goodfellow. For details of other hybrids, see ref. 22.

considerable divergence in the nematode sequence (17). However, it is striking that residues 178–185 are identical in all three species. The C-terminal region of vinculin (the last 170 amino acids) apparently contains a site involved in targeting the molecule to cell–matrix junctions (24). This is a highly conserved region of the protein, which has also been suggested to be involved in the ability of vinculin to self-associate (25). It has been calculated that the C-terminal region of chicken embryo vinculin (residues 858–1066) has a pI of 9.7, compared with a pI of 5.9 for the intact protein (16). This may account for the apparent ability of vinculin to interact directly with phospholipids (26).

Vinculin has been reported to contain a short region of sequence homology with actin (27). Thus, residues 300–307 in the first repeat of chicken embryo vinculin (Arg-Gln-Ile-Leu-Asp-Glu-Ala-Gly) can be aligned with residues 359–366 in α-actin (Lys-Gln-Glu-Tyr-Asp-Glu-Ala-Gly) (27). A number of actin-binding proteins also contain a similar sequence (reviewed in ref. 27). The human and chicken embryo vinculin sequences are identical over this region, but the nematode sequence is divergent, although it does contain a different short stretch of sequence homology with actin (17). Despite an earlier report, there is no evidence that vinculin binds directly to actin (28). There is no significant sequence similarity between vinculin and any other protein in the current data base. Vinculin does not contain the SH3 domain found in a number of other proteins associated with the membrane cytoskeleton (29).

There is at least one additional isoform of vinculin, termed metavinculin, which has a higher apparent molecular weight than vinculin and is expressed specifically in muscle (30–33). The close similarity between these isoforms suggests that they are generated by alternative splicing of transcripts from a single gene (34). Southern blots of chicken embryo genomic DNA cleaved with a variety of restriction endonucleases and hybridized with a short vinculin cDNA fragment containing only the 5′ untranslated sequence detected a single band in each digest, consistent with the existence of a single vinculin gene in chicken (15). The more extensive Southern blot analysis of the human vinculin gene reported here is also consistent with a single functional gene. By using the HV1 cDNA as a probe, the human vinculin gene has been assigned to chromosome 10 in the region q11.2–qter. It is interesting to note that chromosome mapping of the mouse vinculin locus with the same probe has led to assignment of the mouse gene to chromosome 14, which is syntenic to human chromosome 10 (M. Strobel, personal communication). It should be of interest to define more accurately the position of the human vinculin gene, as a number of loci of interest map to the long arm of chromosome 10. These include the cell cycle control gene *CDC2* (22, 35) and the disease locus for multiple endocrine neoplasia types 2A and 2B (36, 37). The data on mapped loci on the long arm of chromosome 10 are summarized in the recent Human Gene Mapping Workshop (38).

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