Vinculin gene is non-essential in *Drosophila melanogaster*

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Abstract Vinculin is thought to be an important cytoskeletal protein in the linkage between actin cytoskeleton and integrin transmembrane receptors. We identified *Vinculin* (*Vinc*) gene in the *X* chromosome of *D. melanogaster*. *Drosophila* vinculin is highly homologous in its N- and C-terminal domains both to mammalian and nematode vinculins, and contains internal repeats and proline-rich region typical for vinculins. The *X* chromosome rearrangement *In(1LR)pn2a* was found to disrupt *Vinc* so that the coding sequence is interrupted by the $(AAGAG)_n$ satellite DNA. Northern analysis revealed that the *Vinc* transcript is completely absent in the *In(1LR)pn2a* homozygous flies. Surprisingly, these *Vinc* flies are viable and fertile. This finding highlights plasticity and adaptive capacity of cellular cytoskeletal and anchorage system.

Key words: Protein homology; Vinculin function; Chromosome rearrangement

1. Introduction

Vinculin is a cytoskeletal protein of eukaryotic cells found at the sites of F-actin attachment to plasma membrane [1]. It is present in adherent-type cell-cell and cell-extracellular matrix junctions [2,3]. It was demonstrated that activated vinculin displays high affinity for talin and can bind F-actin [4] suggesting a role of vinculin in microfilament attachment through talin and integrins to extracellular matrix. The complete sequences of human [5], chicken [6–8] and nematode [9] vinculins was determined, and it was shown that vinculins is highly conserved in evolution.

In the nematode, *Caenorhabditis elegans*, mutations in the vinculin gene are lethals [10]. On the other hand, recent findings show that cultured mammalian vinculin-deficient cells form proper integrin-based focal adhesions [11,12] and indicate that vinculin gene may be not vitally important in mammals.

Studies of the intergrin-based adhesion complexes in *Drosophila* revealed that the integrins are required for different cell layers to adhere to each other during development (reviewed in ref. [13]). Much less is known about adapter molecules which provide the link between the integrins and the actin cytoskeleton in *Drosophila*. Main candidates for the adapter molecules in vertebrates are α-actinin, talin and vinculin. The gene encoding α-actinin has been identified in *Drosophila* to date, and it was found that its null alleles are lethals [14].

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This paper reports the structure of the single copy gene encoding *Drosophila* homolog of the cytoskeletal protein vinculin. The flies carrying disrupted *Vinc* gene as a result of the *X* chromosome inversion are viable and fertile suggesting that the *Vinc* gene is not of vital importance in *Drosophila*.

2. Materials and methods

2.1. DNA clones and sequence analysis

A Lambda ZAP II *Drosophila* ovarian cDNA library (Stratagene) was plated and screened according to the Stratagene protocol. The 6.6-kbp and 2.2-kbp *Eco*RI fragments from pA334 cosmid [15] (Fig. 1*b*) were used as probes. DNA sequencing of genomic and cDNA clones was performed using a Sequenase Version 2.0 Kit (Amersham) according to the supplier's instructions. Sequences were analyzed using the GENEBEE program [16].

2.2. Drosophila DNA isolation and Southern hybridization

Genomic DNA was prepared using a modification of phenol deproteinization method [15]. Restriction analysis, blotting and hybridization were carried out by standard techniques [17]. DNA probe was labelled by random priming method.

2.3. Drosophila RNA isolation and Northern hybridization

Total cellular RNA was isolated from adult flies by guanidinium thiocyanate extraction [18]. RNA blotting and hybridization were performed according to standard methods [17]. ^{32}P-labelled antisense RNA probe was used for hybridization.

3. Results and discussion

Genetic and molecular structure of the *Pgd-K10* region of the *Drosophila melanogaster X* chromosome has been determined in numerous studies (Fig. 1*a*). Earlier we described *X* chromosome inversion *In(1LR)pn2a* with breakpoints in the euchromatic *Pgd-K10* region and centromeric heterochromatin which leads to position effect variegation of genes from *l(1)2Ea* to *Pgd* [23,24,15]. To find new transcriptional units (genes) in the vicinity of the *In(1LR)pn2a* breakpoint in the *Pgd-K10* region was screened *Drosophila* cDNA library with the 6.6-kbp and 2.2-kbp *Eco*RI fragments (Fig. 1*b*). Seven independently obtained cDNA clones were further analyzed. Comparison of cDNA sequences allowed us to arrange clones into contig and to deduce a complete cDNA sequence. Cloned cDNAs were mapped to a fragment of the genomic DNA of about 8 kbp localized just distal to the *pcx* locus [25]. To establish direction of transcription and exon-intron structure of the gene we determined nucleotide sequence of this fragment and compare genomic and cDNA sequences (Fig. 2). It was found that the mRNA is synthesized in the direction from centromere to telomere and comprised of six exons. The introns range in size between 59 and 2262 bp. All donor and acceptor sites conform to the *Drosophila* consensus sequences [26] with the exception of the 5′ splice sites of introns 2 and 3. Analysis of the transcribed sequence revealed a single 2886-bp

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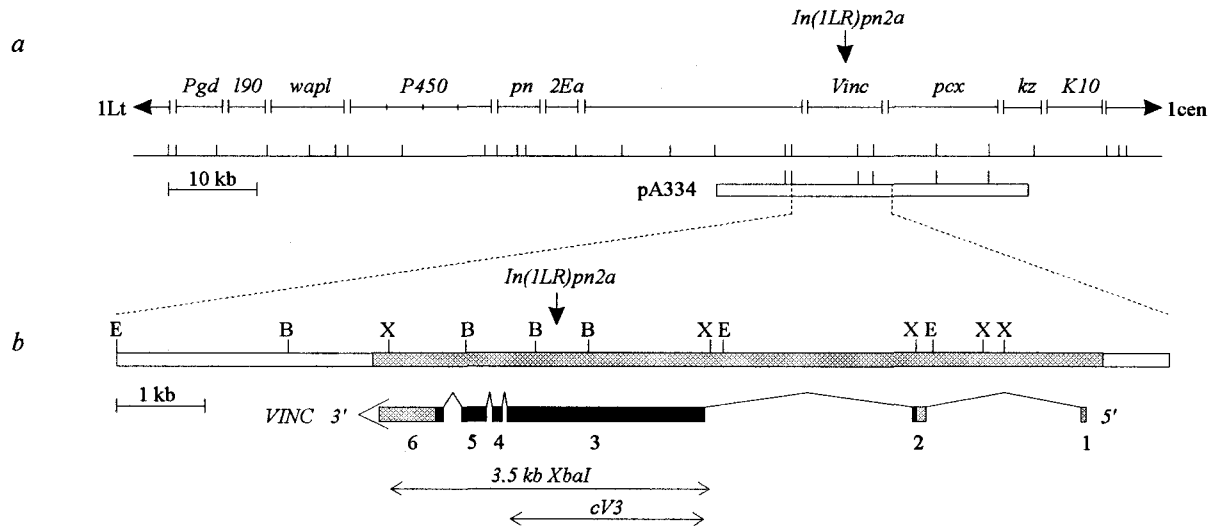

Fig. 1. *Vinculin* gene localization and structure. (*a*) Genetic and restriction map of the *Pgd-K10* region. Orientation of the *X* chromosome is from the left arm telomere (1Lt) to the centromere (1cen). For details of the genetic loci, see [19,20]. Vertical arrow shows location of the *In(1LR)pn2a* euchromatic breakpoint. Cleavage sites for *Eco*RI are indicated on restriction map [21,22]. Cosmid pA334 [15] is positioned below the map. (*b*) Molecular map of the *Vinc* gene. E, X, B indicate cleavage sites for *Eco*RI, *Xba*I, *Bam*HI, respectively. Sequenced genomic DNA fragment is shown as shaded box (EMBL Database accession No. X96601; sequence of the coding strand has been deposited). Structure and direction of the *Vinc* transcript is shown below the restriction map, exons are shown by boxes (1 to 6) with the black area representing protein-coding sequences.

open reading frame. The translation start site is in exon 2, and the stop codon is in exon 6 (Fig. 2). The sequence upstream of the proposed initiation codon matches consensus sequence for *Drosophila* translation initiation site [27]. Conceptual translation of the open reading frame yields a 962 amino acid polypeptide with a strong similarity to vertebrates and nematode vinculins (Fig. 3).

Drosophila vinculin has a deduced molecular mass of 107 kDa and contains two divergent internal repeats of 106 amino acids in the central part (Fig. 3*a*), as does the nematode protein [9]. Vertebrate vinculins contain three internal repeats, which account for its higher molecular mass [5,8]. An alignment of the predicted sequence of *Drosophila* vinculin with other vinculins confirmed the earlier data that the most of the amino acid sequence divergence between different vinculins occurs in the central region. Like all the other vinculins *Drosophila* protein contains a proline-rich region which spans about 50 residues preceding the C-terminal domain. The most highly conserved regions of vinculin are the N- and C-terminal domains [5]. The N- and C-terminal regions of *Drosophila* and nematode vinculin show 67% and 62% sequence identity, respectively. The N- and C-terminal domains of *Drosophila* and human proteins are 56% and 70% identical, respectively. Multiple alignment reveals numerous motifs of amino acids which are completely identical in the all known vinculins (Fig. 3*b*).

Several lines of evidence suggest that the *Vinc* gene is unique in *Drosophila* genome. A single band of hybridization was detected in the 2E region of the wild type *X* chromosome when the cosmid pA334 containing the *Vinc* gene were used as a probe for in situ hybridization to polytene chromosomes [15]. Southern hybridizations of the *Vinc* specific probes were carried out to several overlapping cosmids covered about 100 kbp of the *Pgd-K10* region, and only the bands corresponding to the restriction fragments containing the *Vinc* gene from pA334 were detected. This excludes a possibility of existence of tandem copies of the *Vinc* gene. Fig. 4 demonstrates Southern analysis of *Drosophila* genomic DNA digested with *Bam*HI and probed with 3.50-kbp *Xba*I DNA fragment. The same sizes of hybridization bands (14 kbp, 1.8 kbp, 0.8 kbp and 0.6 kbp) are detected both in the genomic DNA and pA334, suggesting that the *Vinc* is a single copy gene in *Drosophila* genome.

It was shown that the euchromatic breakpoint of the *In(1LR)pn2a* inversion falls into the 0.6-kbp *Bam*HI fragment (see Fig. 1*b*). Inverse PCR with the *Vinc* specific primers was used to clone an eu-heterochromatic junction in the rearranged chromosome (E.V. Tolchkov et al., submitted). The sequence of cloned fragment is shown on Fig. 5. It was found that AAGAG repeats are fused to the 3′-half of the *Vinc* gene, adjacent to the third exon sequence coding GLY_{573}. Three independent clones which we analyzed have the same structure at the eu-heterochromatic junction. Thus, in the rearranged chromosome the sequence of the *Vinculin* gene is interrupted by the $(AAGAG)_n$ satellite DNA.

Transcription of the *Vinc* gene was studied by Northern analysis of RNA isolated from the wild type flies and the *In(1LR)pn2a* hetero- and homozygous females (Fig. 6). The 2-kbp cDNA cV3 containing the most of the coding region (Fig. 1*b*) was used as hybridization probe. The only *Vinc* specific transcript was detected in the wild type females RNA. Its size corresponds well with that of the poly(A)-less cDNA (3622 bp). Transcription level is reduced in females heterozygous for the *In(1LR)pn2a*, and the *Vinc* transcript

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Fig. 2. Predicted amino acid sequence of the *Drosophila* vinculin. Numeration of nucleotides is given according to determined genomic sequence (EMBL Database accession No. X96601). Nucleotides present in both cDNA and genomic sequences are shown by uppercase letters. Lowercase letters represent genomic sequence. Sequence corresponding to the consensus of the *Drosophila* translation initiation site [27] is underlined. Nucleotide at the site of gene disruption in the *In(1LR)pn2a* is shown in shaded box.

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 151 ccaatcgaaatctttttttagtattgaaagaacagtcgggcaagaaagtt
 201 ggcGCGCAGCAAAATCGAGCCAGACAAAgtaaat...(intron 1)...
1901 .........................cttttagCTCACTACAGATCTGGC
1951 CCACGAGAAAGAGAGAGGAACAATAAACTCTGAGACAACCAAACTGAAAA
2001 CAGGAATCAGACGAAAAGCGCAGCCAAAATGCCCGTCTTTCACACAAAAA
                           M  P  V  F  H  T  K  T

2051 CCATCGAGAGCATTCTAGATCCTGTTGCCCAACAGgaatga.........
      I  E  S  I  L  D  P  V  A  Q  Q

4301 ...(intron 2).............................cggcagGTA
                                                    V

4351 TCTCGTCTGGTGATCCTGCACGAGGAGGCCGAGGATGGCAACGCGATGCC
     S  R  L  V  I  L  H  E  E  A  E  D  G  N  A  M  P

4401 GGACCTCAGTCGACCGGTGCAGGTGGTGTCGGCGGCAGTGGCTAATCTCG
     D  L  S  R  P  V  Q  V  V  S  A  A  V  A  N  L  V

4451 TGAAGGTGGGCCGCGATACGATCAACAGCTCCGACGACAAGATCCTGCGC
     K  V  G  R  D  T  I  N  S  S  D  D  K  I  L  R

4501 CAGGACATGCCCTCGGCATTGCACCGCGTGGAGGGTGCCTCGCAGCTGCT
     Q  D  M  P  S  A  L  H  R  V  E  G  A  S  Q  L  L

4551 GGAGGAGGCGTCCGATATCGTCCGCTCCGACCCGTACTCGGGTCCGGCGC
     E  E  A  S  D  I  V  R  S  D  P  Y  S  G  P  A  R

4601 GCAAGAAGCTAATCGAGGGGAGCCGCGGCATTCTGCAGGGCACCTCCTCG
     K  K  L  I  E  G  S  R  G  I  L  Q  G  T  S  S

4651 CTGCTGCTTTGCTTCGACGAGAGTGAGGTGCGGAAGATCATCCAGGAGTG
     L  L  L  C  F  D  E  S  E  V  R  K  I  I  Q  E  C

4701 CAAGAGGGTACTGGACTACTTGGCCGTGGCGGAGGTGATAAACACCATGG
     K  R  V  L  D  Y  L  A  V  A  E  V  I  N  T  M  E

4751 AACAGCTGGTGCAGTTCCTCAAGGACCTCTCGCCCTGTCTGAGCAAGGTG
     Q  L  V  Q  F  L  K  D  L  S  P  C  L  S  K  V

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     H  R  E  V  G  A  R  E  K  E  L  T  H  Q  V  H  S

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     E  I  L  V  R  C  L  E  Q  V  K  T  L  A  P  I  L

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     I  C  S  M  K  V  Y  I  H  I  V  E  Q  Q  G  R

4951 GGAGCTGAGGAAGCGGCTGAGAACAGGAACTACCTGGCCGCCAGGATGAG
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5001 CGACGAGCTGCAGGAGATCATCCGCGTGCTGCAGCTGACTACCTACGATG
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5051 AGGACACCAGCGAGCTGGACAACCTCACTGTGCTGAAGAAGCTCTCCAAT
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5101 GCCATTTCCAACAAAATGGAGCAGGCCAACGAGTGGCTATCGAATCCCTA
     A  I  S  N  K  M  E  Q  A  N  E  W  L  S  N  P  Y

5151 TGCCTTGCGAGGCGGCGTGGGCGAGAAGGCTCTCCGCCAAGTGATCGATA
     A  L  R  G  G  V  G  E  K  A  L  R  Q  V  I  D  N

5201 ATGCTACGGAGATCTCAGAGCGCTGTCTGCCCCAGGACTCTTATCCCATC
     A  T  E  I  S  E  R  C  L  P  Q  D  S  Y  P  I

5251 CGCAAGCTGGCTGATGAGGTGACGGCTATGGCCAACACCTTGTGCGAGCT
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5301 GCGCCAGGAGGGCAAGGGCCAGAGTCCCCAAGCGGAGTCCCTGGTCAGGG
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5601 TGACGAAGTGGAGCGCCTCAAACGCCAGGCAGCCGGATCCGGCCCTGCGG
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     P  G  R  E  Q  N  F  N  Q  K  S  N  N  L  Q  A
                                    •
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     F  S  D  R  A  S  K  T  S  R  M  V  A  A  G  G  A

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6051 GTACGCGGATACCGTGCTGCGCATGCGCACCCTCTGCGACCAGGCCACGG
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6101 ACCCGGCGGACTTCATTAAAACATCCGAGGAGCACATGCAGGTTTACGCC
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6201 CAACACCTCGAACATTGCCCGCCTGATCAATAGGGTCTTGTTGGTAGCCA
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                                    •
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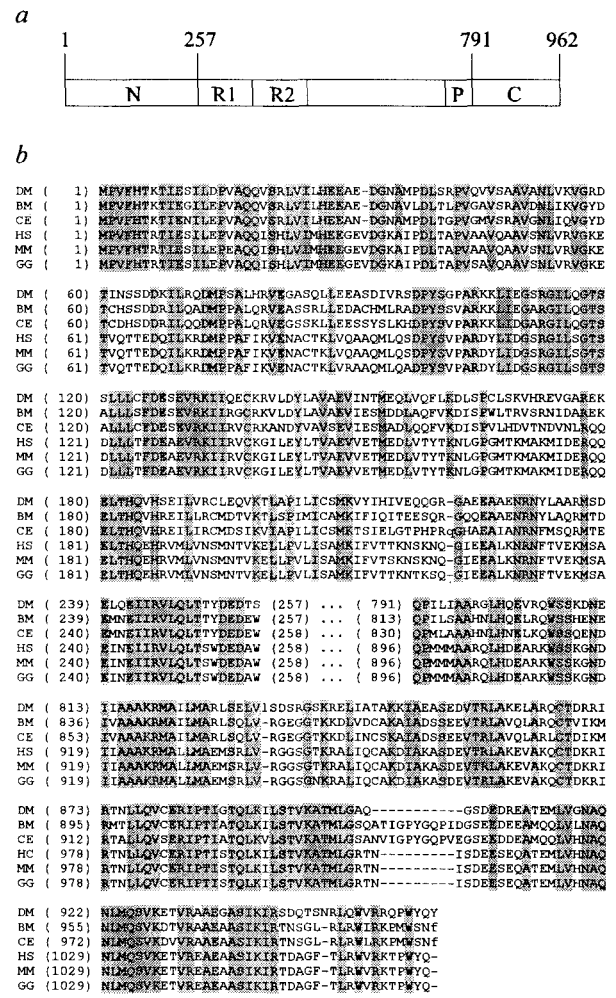

Fig. 3. Structural homology of *Drosophila* vinculin with other vinculins. (*a*) Domain structure of the *Drosophila* vinculin. Drosophila vinculin is represented by N-terminal domain (N), central domain containing repetitive (R1 and R2) and proline-rich region (P), and C-terminal domain (C). (*b*) Multiple alignment of amino acid sequences for the N- and C-terminal regions of vinculins from *Drosophila melanogaster* (DM, accession No. X96601), *Brugia malayi* (BM, accession No. U07023), *Caenorhabditis elegans* (CE, accession No. J04804), *Homo sapiens* (HS, accession No. M33308), *Mus musculus* (MM, accession No. L18880), *Gallus gallus* (GG, accession No. J04126) is shown. Amino acids identical in all vinculins are enclosed in shaded boxes.

completely vanishes in females homozygous for the *In(1LR)pn2a* (Fig. 6).

Thus, a single copy *Vinc* gene is disrupted in the *In(1LR)pn2a* chromosome, and its transcription is undetectable. Surprisingly, these flies carrying inactive *Vinc* gene are viable and fertile. These data suggests that the *Vinculin* gene is not of vital importance in *Drosophila*. The earlier genetic data also points to the fact that there are no lethal loci in the region where the *Vinc* gene was localized [25].

Vinculin knock-out experiments in cultured mammalian F9 and ES cells revealed an effects on cell adhesion and locomotion, however vinculin deficient cells were capable of forming focal adhesions, and other junctional molecules were properly localized [11,12]. According to our data it seems plausible that vinculin is not of vital importance not only for cultured cells but for development of the whole organism.

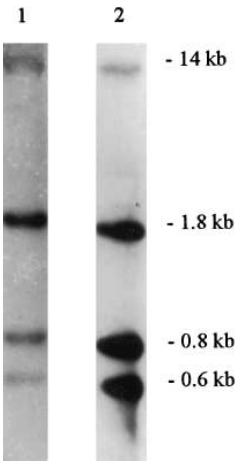

Fig. 4. *Vinculin* is a single copy gene in the *Drosophila* genome. Southern hybridization of the ^{32}P-labelled 3.5-kbp *Xba*I DNA fragment to the *Bam*HI digested DNA from wild type flies (1) and cosmid pA334 (2).

This conclusion is in contrast with an earlier one drawn from the analysis of the vinculin mutants in nematode *Caenorhabditis elegans* [10]. The mutants were paralyzed and had

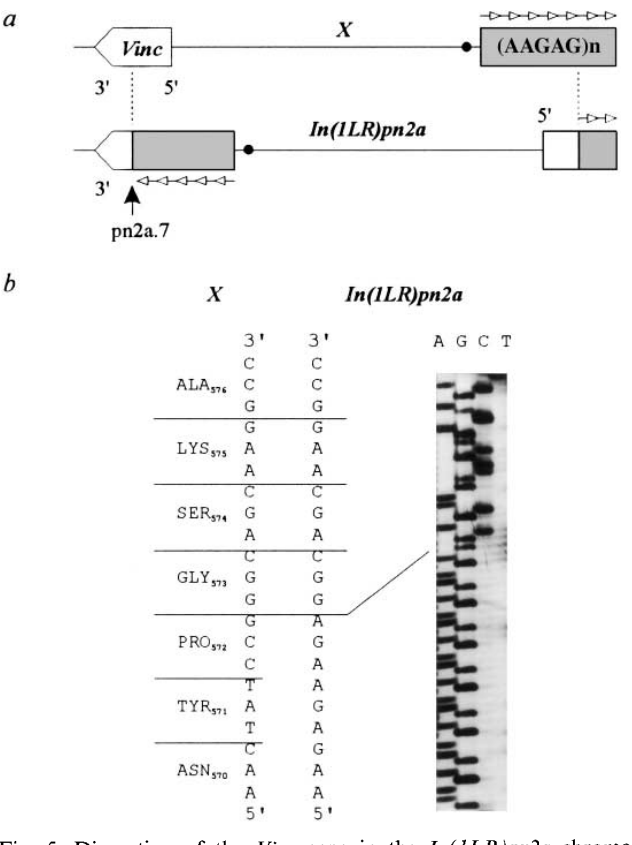

Fig. 5. Disruption of the *Vinc* gene in the *In(1LR)pn2a* chromosome. (*a*) *In(1LR)pn2a* origin and gross structure. The *In(1LR)pn2a* rearrangement is a pericentric inversion of the *X* chromosome [24]. *In(1LR)pn2a* breakpoints lie within the *Vinc* gene and the 1.2-Mbp block of the $(AAGAG)_n$ satellite DNA which is situated in the heterochromatic right arm of the *X* chromosome [28]. Position of the pn2a.7 clone carrying one of the eu-heterochromatic junctions of the *In(1LR)pn2a* is shown by black arrow. (*b*) Sequence of the pn2a.7 clone at the eu-heterochromatic junction. The *Vinc* coding region is interrupted by $(AAGAG)_n$ satellite DNA.

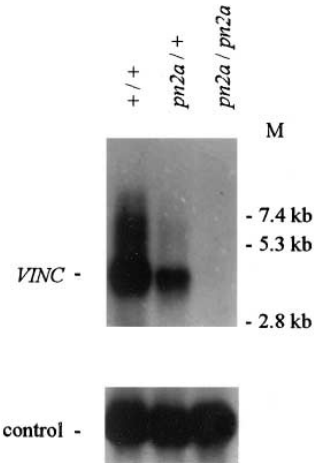

Fig. 6. Transcription of the *Vinc* gene. Northern hybridization of the labelled cDNA cV3 to RNA from wild type (+/+), *In(1LR)pn2a* heterozygous (*pn2a*/+) and homozygous (*pn2a*/*pn2a*) females. Lower panel represents the same blot after hybridization with the tubulin specific probe pBSBT1 [29] as a control for equal loading of different RNAs.

disorganized muscle suggesting that vinculin is essential for muscle function in nematode. Our multiple alignment shows the only significant difference in vinculin structures in nematodes *Caenorhabditis elegans* and *Brugia malayi*, namely the 11 amino acids sequence insertions in the highly conserved C-terminal domain (Fig. 2*b*). This sequence (–NVIGPYGQPVE– for the *Caenorhabditis elegans* vinculin) is strictly encoded by unusually small exon 15 of the nematode gene [9]. In accordance with Gilbert's [30] and Blake's [31] hypotheses one can suggest that it is precisely this exon that encodes vitally important discrete structural element of nematode vinculin. The loss of this sequence in the higher organisms may account for the loss of vital importance of vinculin.

In the last years a large number of the knockout mutations affecting cell adhesion were described (reviewed in ref. [32]). In many cases effects of deletion of genes are less severe that would have been predicted. The possibility which can provide an explanation for the obtained results is an existence of overlapping function and compensation mechanism in higher organism [32]. Vinculins share considerable structural similarity with α-catenins which are involved in adherents-type cell-cell junctions [33]. Taking into account a similarity in structure and site of location, α-catenin could be considered as a candidate molecule substituting for vinculin.

On the other hand, it seems reasonable to suggest that some molecules with important structural and signalling roles in cellular cytoskeletal and anchorage system may be functionally redundant. Such a redundancy would provide high reliability of the system thus giving a selective advantage in unusual situation. In particular, integrin-based complexes may be assembled and exist without vinculin, and possible functions of vinculin are acceleration of assembly/disassembly of complexes, stabilization of their structures and/or improvement of complex-associated processes of signal transduction. In such a case vinculin would be non-essential, but improve fitness and provide some selective advantages on the level of a whole organism that in turn could lead to preservation vinculin structure and functions in evolution.

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References

[1] Geiger, B., Tokuyasu, K.T., Dutton, A.H. and Singer, S.J. (1980) Proc. Natl. Acad. Sci. USA 77, 4127–4131.
[2] Burridge, K., Fath, K., Kelly, T., Nuckolls, G. and Turner, C. (1988) Annu. Rev. Cell Biol. 4, 487–525.
[3] Geiger, B., Ginsberg, D., Salomon, D. and Volberg, T. (1990) Cell Differ. Dev. 32, 343–354.
[4] Johnson, R.P. and Craig, S.W. (1995) Nature 373, 261–264.
[5] Weller, P.A., Ogryzko, E.P., Corben, E.B., Zhidkova, N.I., Patel, B., Price, G.J., Spurr, N.K., Koteliansky, V.E. and Critchley, D.R. (1990) Proc. Natl. Acad. Sci. USA 87, 5667–5671.
[6] Price, G.J., Jones, P., Davison, M.D., Patel, B., Eperon, I.C. and Critchley, D.R. (1987) Biochem. J. 245, 595–603.
[7] Price, G.J., Jones, P., Davison, M.D., Patel, B., Bendori, R., Geiger, B. and Critchley, D.R. (1989) Biochem. J. 259, 453–461.
[8] Coutu, M. and Craig, S.W. (1988) Proc. Natl. Acad. Sci. USA 85, 8535–8539.
[9] Barstead, R.J. and Waterston, R.H. (1989) J. Biol. Chem. 264, 10177–10185.
[10] Barstead, R.J. and Waterston, R.H. (1991) J. Cell Biol. 114, 715–724.
[11] Coll, J.-L., Ben-Ze'ev, A., Ezzell, R.M., Rodriguez Fernandez, J.L., Baribault, H., Oshima, R.G. and Adamson, E.D. (1995) Proc. Natl. Acad. Sci. USA 92, 9161–9165.
[12] Volberg, T., Geiger, B., Kam, Z., Pankov, R., Simcha, I., Sabanay, H., Coll, J.-L., Adanson, E. and Ben-Ze'ev, A. (1995) J. Cell Sci. 108, 2253–2260.
[13] Brown, N.H. (1993) BioEssays 15, 383–390.
[14] Fyrberg, E., Kelly, M., Ball, E., Fyrberg, C. and Reedy, M.C. (1990) J. Cell Biol. 110, 1999–2011.
[15] Alatortsev, V.E. (1988) Genetika 24, 23–33.
[16] Brodksy, L.I., Ivanov, V.V., Kalaydzidis, Ya.L., Leontovich, A.M., Nikolaev, V.K., Feranchuk, S.I. and Drachev, A. (1995) Biochemistry (Moscow) 60, 923–928.
[17] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[18] Chomczynski, A. and Sacchi, G. (1987) Anal. Biochem. 162, 156–159.
[19] Lindsley, D. and Zimm, G. (1992) The Genome of Drosophila melanogaster, Academic Press, San Diego, CA.
[20] Frolov, M.V. and Alatortsev, V.E. (1994) DNA Cell Biol. 13, 663–668.
[21] Haenlin, M., Steller, H., Pirrotta, V. and Mohier, E. (1985) Cell 40, 827–837.
[22] Dura, J.M., Randsholt, N.B., Deatrick, J., Erk, I., Santamaria, P., Freeman, J.D., Freeman, S.J., Weddel, D. and Brock, H.W. (1987) Cell 51, 829–839.
[23] Alatortsev, V.E., Tolchkov, E.V., Slobodyanyuk, C.Y. and Gvozdev, V.A. (1982) Genetika 18, 13–23.
[24] Tolchkov, E.V., Balakireva, M.D. and Alatortsev, V.E. (1984) Genetika 20, 1846–1856.
[25] LaBonne, S.G., Sunita, I. and Mahowald, A.P. (1989) Dev. Biol. 136, 1–16.
[26] Mount, S.M., Burks, K., Hertz, G., Stormo, G.D., White, O. and Fields, C. (1992) Nucleic Acids Res. 20, 4255–4262.
[27] Cavener, D.R. (1987) Nucleic Acids Res. 15, 1353–1361.
[28] Lohe, A.R., Hilliker, A.J. and Roberts, P.A. (1993) Genetics 134, 1148–1174.
[29] Bialojan, S., Falkenburg, D. and Renkawitz-Pohl, R. (1984) EMBO J. 3, 2543–2548.
[30] Gilbert, W. (1978) Nature 271, 501.
[31] Blake, C.C.F. (1978) Nature 273, 267.
[32] Hynes, R.O. (1996) Dev. Biol. 180, 402–412.
[33] Kemler, R. (1993) Trends Genet. 9, 317–321.