



ACADEMIC
PRESS

Available online at www.sciencedirect.com

SCIENCE @ DIRECT®

Genomics 82 (2003) 401–405

GENOMICS

www.elsevier.com/locate/ygeno

The centrosomal proteins pericentrin and kendrin are encoded by alternatively spliced products of one gene

Mark R. Flory^a and Trisha N. Davis^{a,b,*}

^a *Molecular and Cellular Biology Program, University of Washington, Seattle, WA 98195, USA*

^b *Department of Biochemistry, Box 357350, University of Washington, Seattle, WA 98195, USA*

Received 5 December 2002; accepted 11 February 2003

Abstract

Pericentrin, a critical centrosome component first identified in mouse, recruits factors required for assembly of the mitotic spindle apparatus. A similar yet larger human protein named kendrin was recently identified, but its relationship to pericentrin was not clear. Extensive sequence homology between the mouse chromosome 10 region encoding pericentrin and the human chromosome 21 region encoding kendrin indicates that these proteins are encoded by syntenic loci. However, comparison of the published mouse pericentrin cDNA sequence to mouse genomic DNA sequences revealed two important differences: the stop codon present in the published mouse pericentrin cDNA is not found in the mouse genomic sequence, and the 3' end of the published mouse pericentrin cDNA is a fragment from a different mouse chromosome. To resolve these discrepancies, we sequenced a mouse expressed sequence tag (EST) that corresponds to the 3' end for a 7.1-kb mouse pericentrin RNA encoded on chromosome 10. Extensive northern blot analysis revealed that the pericentrin gene displays a complex expression pattern in both mouse and human: a 10-kb kendrin transcript is found in most tissues, whereas smaller transcripts are detected in a limited subset of tissues. These analyses demonstrate that pericentrin and kendrin are encoded by one gene, correct the previously published pericentrin cDNA sequence, and describe the complex expression pattern for a gene important for centrosome function in normal and transformed cells.

© 2003 Elsevier Science (USA). All rights reserved.

Keywords: Pericentrin; Kendrin; Centrosome; Microtubules

Pericentrin is a critical component of the centrosome, the organelle that organizes mitotic spindle microtubules to segregate chromosomes during cell division. Pericentrin interacts with γ -tubulin [1], which is required for microtubule nucleation. Although pericentrin was originally cloned from mouse, the cross-reactivity of mouse pericentrin antibodies with centrosomes from many vertebrates suggests that pericentrin is highly conserved. Two transcripts of 9.5 and 7.0 kb were described in whole-mouse embryos for mouse pericentrin, and the published mouse pericentrin cDNA encodes a 7.0-kb transcript [2].

A human protein related to pericentrin was recently identified. This protein, named kendrin or pericentrin-B, shares strong overall homology with mouse pericentrin in the N-

terminal 2000 amino acid residues [3,4]. However, kendrin also contains a unique C-terminal calmodulin-binding region, making it much larger than mouse pericentrin (380 kDa versus 220 kDa). Northern blot analysis using probes made by standard conditions revealed low-abundance transcripts with homology to kendrin of 10 and 7.5 kb [3]. The 7.5-kb transcript shares homology with the 10-kb transcript at its 5' end [3]. A 10.5-kb cDNA encoding the 380-kDa kendrin was recently reported [4].

We show here that mouse pericentrin and human kendrin are encoded by syntenic loci, *Pcnt2(MMU)* and *PCNT2(HSA)*, with complex expression patterns. We propose that the different isoforms be identified by suffixes reflecting the size of the predicted protein product. Thus, kendrin or pericentrin-B would be referred to as pericentrin-380, whereas the pericentrin already characterized in mouse (pericentrin-A) would be referred to as pericentrin-220 and in the corrected form (see later) as pericentrin-250.

* Corresponding author. Fax: +1-206-685-1792.

E-mail address: tdavis@u.washington.edu (T.N. Davis).

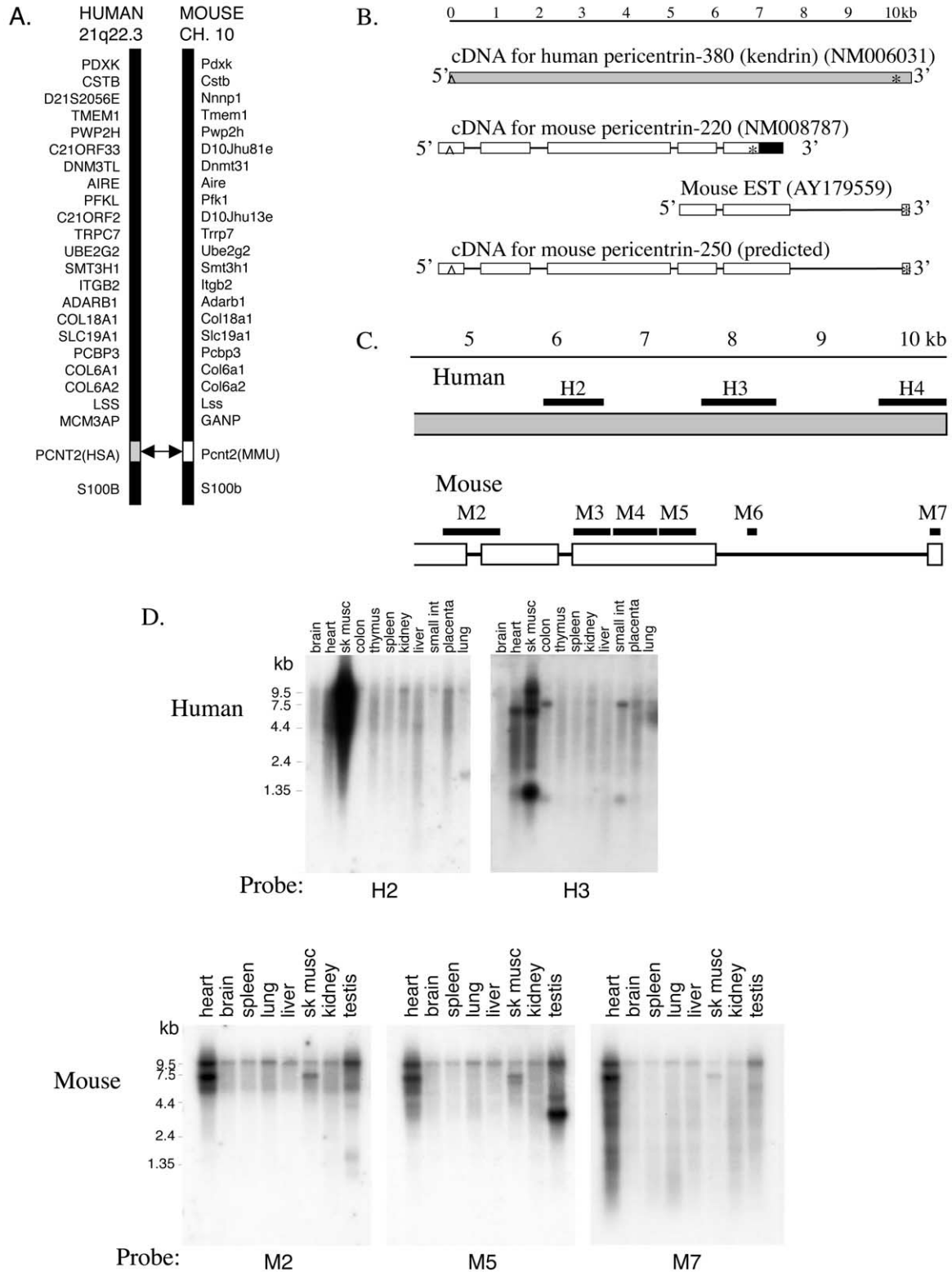


Fig. 1. (A) Synteny map showing related regions of human chromosome 21 (21q22.3) and mouse chromosome 10 (derived from NCBI). Human pericentrin (*PCNT2(HSA)*) and mouse pericentrin (*Pcnt2(MMU)*) are flanked on both sides by homologous genes (*MCM3AP/GANP* and *S100B/S100b*) and are thus contained within syntenic genomic regions. (B) Isolated cDNAs homologous to pericentrin in mouse and human. Boxes indicate the exons present in each cDNA. Lines between the boxes indicate exons found in the cDNA for pericentrin-380 but not found in the other cDNAs. Carets (^) indicate the start codons; asterisks (*) indicate the stop codons. The black box indicates the chimeric portion of the mouse pericentrin-220 cDNA encoded on mouse chromosome 11. Note that the stop codon in the pericentrin-220 cDNA is not present in the mouse genomic DNA. The mouse EST AY179559 predicts a new stop codon and a new 3' end for a 7.1-kb message in muscle and heart encoding pericentrin-250 (see northern blots). (C) Expanded views of the 3' ends of the cDNAs

There is extensive synteny between the human and mouse genomic regions containing the *PCNT2(HSA)* and *Pcnt2(MMU)* genes, respectively, indicating that these genes are orthologs. *PCNT2(HSA)* maps to human chromosome 21 (21q.22.3) [5,6], a completely sequenced human chromosome [7]. The human chromosome 21.q22.3 region containing *PCNT2(HSA)* shares synteny with a large segment of mouse chromosome 10 spanning 1.1 cM (positions 41.0–42.1), as shown by MegaBLAST analysis. These syntenic genomic regions contain 24 homologous genes arranged in the same order (Fig. 1A). This region of mouse chromosome 10 contains the *Pcnt2(MMU)* gene in the exact location predicted by the position of *PCNT2(HSA)* in the corresponding syntenic human chromosome 21 region. *PCNT2(HSA)* and *Pcnt2(MMU)* are directly flanked on both sides by homologous genes (5', *MCM3AP/GANP*; 3', *S100B/S100b*; Fig. 1A) and are thus contained within the interval of synteny.

Comparison of the published 7.0-kb mouse pericentrin cDNA with the genomic sequence of mouse chromosome 10 revealed two major differences. First, the reported TAG stop codon at nucleotides (nt) 6054–6056 in the pericentrin cDNA is a TGG codon in the mouse genomic sequence (Fig. 1B). The rest of the exon encodes an open reading frame and matches the mouse genomic sequence exactly. Thus, the initially reported stop codon is most likely an error. The second difference is that nucleotides from 6418 to the end of the pericentrin cDNA were found to be encoded by mouse chromosome 11, indicating that the published pericentrin cDNA is chimeric (Fig. 1B). In the absence of a genomic sequence from mouse chromosome 10, this chimeric 3' fragment led to the erroneous conclusion that mouse pericentrin and human kendrin were encoded by related but different genes [3].

To unambiguously identify a 3' end of mouse pericentrin, we examined the mouse EST database for ESTs homologous to the region of the pericentrin upstream of base 6418. One EST was sequenced in entirety (AY179559). The EST has a poly(A) tail, is 99.7% identical to bases 5227–6417 of the mouse pericentrin cDNA, and extends 707 bases beyond base 6417 (Fig. 1B). The whole of this EST (including the 707 extra bases) is identical to the mouse pericentrin locus on chromosome 10. The EST sequence lacks the earlier discussed stop codon at bases 6054–6056 and

has the same TGG codon found in the mouse genome. There are several other minor differences between the mouse pericentrin cDNA (NM_008787) and EST AY179559: GG at 5463–5464 in NM_008787 is AA in the EST, C at 5493 is G, C at 5602 is G, and T at 6406 is A. These result in the following amino acid residue changes: G1724N, Q1734E, and S1770C. Correcting the mouse pericentrin cDNA (NM_008787) with EST AY179559 predicts a 7.1-kb cDNA encoding a protein of 250 kDa. The cDNA is encoded by 31 exons from the 92-kb pericentrin locus on mouse chromosome 10 (using the MCGSV3 database). Note that the penultimate exon is separated from the exon encoding the 3'-untranslated region (3' UTR) by 29.6 kb of genomic DNA.

The original human kendrin cDNA (U52962) contained several differences from the human genomic DNA sequence. NCBI now lists a corrected cDNA (NM_006031) encoding human pericentrin-380. Pericentrin-380 is encoded by 47 exons from the 122-kb *PCNT2* genomic locus on human chromosome 21. Exon 19 is duplicated in the genome, and exon 24 is only 20 nt long. A map of this region can be found at <http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?1=5116>.

To discover the expression pattern of pericentrin in mouse and human tissue, we carried out a series of northern blots (Figs. 1C and 1D; Table 1). We found that probes made by standard techniques gave faint signals on northern blots, so we modified the labeling conditions to produce probes of very high specific activity (see Fig. 1D legend). In mouse, these probes hybridized to a 10-kb message in all tissues tested. A 7-kb message corresponding to pericentrin-250 was detected only in heart and skeletal muscle. Mouse heart and testis had a complex pattern of expression (Fig. 1D). Pericentrin messages are very abundant in human skeletal muscle and range in size from 1.3 to 10 kb. Probes H1 and H2 hybridized to a low-abundance 10-kb message in most tissues and a 7.4-kb message found in colon, kidney, small intestine, placenta, and lung. In both mouse and human, a probe encompassing the 3' UTR recognized many different-sized transcripts, suggesting that many of the alternatively spliced forms of pericentrin share the same 3' UTR (Table 1; Fig. 1D).

These results have important implications of the relatedness of mouse and human pericentrin, and for the relationship between these proteins and the *Saccharomyces cerevisiae*

indicating the probes used in the northern blot analysis. (D) Northern blot analysis. The northern blots were purchased from Clontech (Palo Alto, CA). Probing with the control probe (β -actin) provided by the manufacturer indicated that each lane had an equal loading of RNA, and no degradation was detected (not shown). The experimental probes were labeled by PCR in the presence of [α - 32 P]dATP using *Taq* polymerase (Promega). Probes H1, M1, M6 and M7 were amplified from synthetic oligos, approximately 100 nt in length. To label these short probes, the 30- μ l reaction mix contained: 3 μ l 10 \times PCR buffer (Promega), 1.8 μ l 25 mM MgCl₂ (Promega), 0.3 μ l each primer (100 μ M stock), 0.1 pmol template, 1 μ l nucleotide mix, 10 μ l [α - 32 P]dATP (3000 Ci/mmol, 10 mCi/ml), and 0.5 μ l *Taq* polymerase (Promega). The mouse probes M3, M4, and M5 were amplified from restriction fragments generated from 1 μ g of plasmid AI747881 (clone ID no. IMAGE:2064994, purchased from ATCC) and then purified by electrophoresis on a low-melting-point agarose gel (FMC Bioproducts). After visualizing the band by staining with ethidium bromide, it was cut out and melted at 65°C. The 30- μ l PCR reaction mix was the same as for the short probes except it contained 2 μ l of template (in melted agarose) and 2 μ l nucleotide mix. The amplification primers were designed to amplify the entire restriction fragment. The mouse probe M2 was amplified from a purified restriction fragment from pericentrin clone 4B (gift of E. Halilovic and S. Doxsey). Human probes H2, H3, and H4 were amplified from purified restriction fragments of plasmid pMF35, which is plasmid pBlueScript II KS minus containing a partial kendrin cDNA [3]. Nucleotide mix contains 1.25 mM each dCTP, dGTP, and dTTP, and 0.125 mM dATP.

Table 1
Summary of northern blot analysis in mouse and human^a

Human														
Probe	Nucleotides NM_006031 encoding human pericentrin-380 (exon no.)	Brain	Heart	Muscle	Colon	Thymus	Spleen	Kidney	Liver	Small intestine	Placenta	Lung		
H1	1396–1505 (exon 9)	10	10	10	10	nd ^b	nd ^b	10	nd ^b	10	10	10		
H2	5851–6492 (exons 28–30)	10	10	5.2, 6.5, 7.5, 10	10	nd ^b	nd ^b	10	5.2	10	10	10		
H3	7639–8495 (exons 35–38)	nd ^b	1.3, 6.5, 10	1.3, 6.5, 7.5, 10	1.1, 7.4 , 10	nd ^b	nd ^b	7.4, 10	0	1.1, 7.4	7.4	7.4		
H4	9684–10501 (exons 44–47, includes 3' UTR)	Many faint bands	Many faint bands	1.5, 2.9, 4.4 , 6.5, 7.5, 10	Many faint bands	Many faint bands	Many faint bands	Many faint bands	Many faint bands	Many faint bands	Many faint bands	Many faint bands	Many faint bands	
Mouse														
Probe	Nucleotides in NM_008787 encoding mouse pericentrin-220	Nucleotides in predicted mouse. pericentrin-250 cDNA (exon no.)	Corresponding region in human NM_0060301	Brain	Heart	Muscle	Colon	Thymus	Spleen	Kidney	Liver	Small intestine	Testes	Lung
M1	1255–1369	1255–1369 (exon 8)	1395–1509	10	1.6 , 4.7, 10	10	na ^c	na ^c	10	10	4.7	na ^c	6.6, 10	10
M2	4325–4791	4325–4791 (exons 21–23)	4759–5381	10	5.9, 7.5 , 10	7.5 , 10	na ^c	na ^c	10	10	10	na ^c	1.5, 4.3, 6.1, 10	10
M3	5532–5854	5532–5854 (exon 24)	6377–6717	10	4.5, 5.9, 7.5 , 10	7.5 , 10	na ^c	na ^c	10	10	10	na ^c	1.4, 6.1, 10	10
M4	5872–6309	5872–6309 (exons 24–26)	6735–7178	10	1.5, 4.5, 5.9, 7.5 , 10	7.5 , 10	na ^c	na ^c	1.4, 10	1.5, 10	1.6, 10	na ^c	1.4, 3.7 , 5.1, 6.1, 10	1.4, 10
M5	Not present	6321–6762 (exons 26–29)	7178–7604	10	4.5, 5.9, 7.5 , 10	7.0, 7.5 , 10	na ^c	na ^c	10	10	10	na ^c	3.7 , 5.1 , 10	10
M6	Not present	Not present	8188–8286	10	10	10	na ^c	na ^c	0	10	10	na ^c	3.7 , 10	10
M7	Not present	7004–7125 (exon 31, is 3' UTR)	3' UTR	10, many faint bands	7.5 , 10, many faint bands	7.5 , 10, many faint bands	na ^c	na ^c	10, many faint bands	10, many faint bands	10, many faint bands	na ^c	6.1, 10 , many faint bands	10, many faint bands

^a Message sizes are shown in kb. The bold type indicates the major species.

^b No transcript detected.

^c Tissues not included in the northern blot.

siae centrosome protein Spc110p. Our data show that mouse and human pericentrin exhibit complex expression patterns; however, a large 10-kb message encoding the calmodulin-binding pericentrin-380 (pericentrin-B) isoform is found in most tissues in both organisms. The smaller isoform corresponding to pericentrin-250 (or pericentrin-A) is detected in a subset of tissues. Like pericentrin-380, the *S. cerevisiae* centrosome protein Spc110p and two other recently identified fungal centrosome proteins related to Spc110p contain extensive coiled-coil secondary structure and a C-terminal calmodulin-binding domain [3,8]. This family of proteins may promote mitotic spindle formation by attaching γ -tubulin to the centrosome in a calmodulin-dependent manner, as does Spc110p in *S. cerevisiae* [9–11]. Pericentrin-380 works in concert with another centrosomal protein, CG-NAP/AKAP450, to mediate the attachment of γ -tubulin to the centrosome in human cells [12], and the

C-terminal, calmodulin-binding PACT (pericentrin-AKAP450 centrosomal targeting) domain of these proteins directs them to the centrosome [13].

The high levels and complex pattern of pericentrin transcript expression in muscle, heart, and testis suggests additional functions for pericentrin in these tissues. The abundant unique 3.7-kb transcript in testis could be involved in formation of the specialized microtubule structures assembled during spermiogenesis, the manchette or the axoneme. The abundance of transcripts in muscle could be related to the extensive reorganization of microtubule nucleating sites to the surface of nuclei during myogenesis [14]. Finally, the 10-kb transcript encoding pericentrin-380 is overexpressed in carcinoma cell lines [3], many of which contain centrosomes of abnormal size and number [15,16]. Thus, pericentrin-380 may be a contributor to aneuploidy and a potential target for anticancer therapeutics.

Acknowledgments

We thank Tess Yoder and Ray Monnat for helpful discussions.

References

- [1] J.B. Dichtenberg, et al., Pericentrin and γ -tubulin form a protein complex and are organized into a novel lattice at the centrosome, *J. Cell Biol.* 141 (1998) 163–174.
- [2] S.J. Doxsey, P. Stein, L. Evans, P.D. Calarco, M. Kirschner, Pericentrin, a highly conserved centrosome protein involved in microtubule organization, *Cell* 76 (1994) 639–650 [see comments].
- [3] M.R. Flory, M.J. Moser, R.J. Monnat Jr., T.N. Davis, Identification of a human centrosomal calmodulin-binding protein that shares homology with pericentrin, *Proc. Natl. Acad. Sci. USA* 97 (2000) 5919–5923.
- [4] Q. Li, et al., Kendrin/pericentrin-b, a centrosome protein with homology to pericentrin that complexes with pcm-1, *J. Cell Sci.* 114 (2001) 797–809.
- [5] H. Chen, A. Gos, M.A. Morris, S.E. Antonarakis, Localization of a human homolog of the mouse pericentrin gene (*pcnt*) to chromosome 21qter, *Genomics* 35 (1996) 620–624.
- [6] V. Lapenta, et al., Construction of a 2.5-Mb integrated physical and gene map of distal 21q22.3, *Genomics* 49 (1998) 1–13.
- [7] M. Hattori, et al., The DNA sequence of human chromosome 21. The chromosome 21 mapping and sequencing consortium, *Nature* 405 (2000) 311–319 [see comments].
- [8] M.R. Flory, M. Morphew, J.D. Joseph, A.R. Means, T.N. Davis, Pcp1p, an Spc110p-related calmodulin target at the centrosome of the fission yeast *Schizosaccharomyces pombe*, *Cell Growth Differ.* 13 (2002) 47–58.
- [9] M. Knop, E. Schiebel, Spc98p and Spc97p of the yeast γ -tubulin complex mediate binding to the spindle pole body via their interaction with Spc110p, *EMBO J.* 16 (1997) 6985–6995.
- [10] T. Nguyen, D.B.N. Vinh, D.K. Crawford, T.N. Davis, A genetic analysis of interactions with Spc110p reveals distinct functions of Spc97p and Spc98p, components of the yeast γ -tubulin complex, *Mol. Biol. Cell* 9 (1998) 2201–2216.
- [11] H.A. Sundberg, L. Goetsch, B. Byers, T.N. Davis, Role of calmodulin and Spc110p interaction in the proper assembly of spindle pole body components, *J. Cell Biol.* 133 (1996) 111–124.
- [12] M. Takahashi, A. Yamagiwa, T. Nishimura, H. Mukai, Y. Ono, Centrosomal proteins cg-nap and kendrin provide microtubule nucleation sites by anchoring γ -tubulin ring complex, *Mol. Biol. Cell* 13 (2002) 3235–3245.
- [13] A.K. Gillingham, S. Munro, The PACT domain, a conserved centrosomal targeting motif in the coiled-coil proteins akap450 and pericentrin, *EMBO Rep.* 1 (2000) 524–529.
- [14] A.M. Tassin, B. Maro, M. Bornens, Fate of microtubule-organizing centers during myogenesis in vitro, *J. Cell Biol.* 100 (1985) 35–46.
- [15] W.L. Lingle, W.H. Lutz, J.N. Ingle, N.J. Maihle, J.L. Salisbury, Centrosome hypertrophy in human breast tumors: implications for genomic stability and cell polarity, *Proc. Natl. Acad. Sci. USA* 95 (1998) 2950–2955.
- [16] G.A. Pihan, et al., Centrosome defects and genetic instability in malignant tumors, *Cancer Res.* 58 (1998) 3974–3985.