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# Expression of Centrin Isoforms in the Mammalian Retina

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**Centrin is a calcium-binding phosphoprotein of centrosomes, mitotic spindle poles, and flagellar basal apparatus. Indirect immunofluorescence studies in human and rat retinas reveal centrin localization in two distinct cellular structures: at centrosomes of nonciliated neuronal cells as well as in basal bodies, and in larger amounts in the highly modified cilium—the connecting cilium—of photoreceptor cells. Western blot analyses of mammalian retinal proteins show two closely migrating centrin bands at about 20 kDa, the previously described molecular weight of centrins. Using isoform specific primers in PCR, the expression of two related but distinct forms of centrin (centrin 1 and centrin 2), can be identified in the retina of human and rat as well as in the mammalian testis, tissues where cilia are present. However, only one isoform (centrin 2) is expressed in nondifferentiated, nonciliated retinal cells (retinoblastoma cells), as well as in rat liver, skeletal muscle, and cardiac muscle. These observations suggest centrin 2 message may be universally expressed while centrin 1 message may be restricted to retina and testis which contain cells that have differentiated cilia or flagella, or their modifications.** © 1998 Academic Press

***Key Words:*** **retina; photoreceptor cells; cytoskeleton; $Ca^{2+}$-binding proteins; centrin; mammals; RT-PCR.**

## INTRODUCTION

Centrin is a low molecular weight calcium-binding protein that is found in centrosomes and mitotic spindle poles of all eukaryotic cells and at the flagellar basal apparatus of ciliated and flagellated cells [see reviews: 6, 24, 32]. Centrin plays an essential role in centrosome duplication and separation during the cell cycle [24]. In addition, in certain cells, centrin mediates the process of microtubule severing during flagellar excision [29, 30]. Sequence analysis of cDNA clones encoding centrin has demonstrated that centrin is a member of the EF-hand superfamily of calcium-binding proteins [9, 15, 20]. Previously, cDNA clones encoding two highly related, yet distinct, human centrin isoforms have been identified. One isoform, human centrin 1 (HsCen1), was isolated from a human testis cDNA library [9], and the other, human caltractin, or human centrin 2 (HsCen2), was isolated from two human cDNA libraries constructed from primary cultured umbilical vein endothelia mRNA and from T-cell lymphoplastic leukemia (MOLT-4) mRNA, respectively [15]. Additionally, a mouse cDNA encoding mouse centrin 1 (Mcen1) was isolated from a testis cDNA library [20]. The deduced protein products of these cDNA clones are each 172 amino acids in length and show approximately 85–90% sequence identity. Sequence analyses suggest that the two testis-derived products (HsCen1 and MmCen1) are more closely related to one another than they are to the cell line-derived product (HsCen2).

In this work, we have addressed the question of tissue-specific expression of centrin isoforms with particular emphasis on the retina. The mammalian retina has a complex organization of well-defined cellular layers, including the outermost layer, the photoreceptor cells. Photoreceptor cells are highly specialized ciliary cells whose nonmotile ciliated structure is restricted to the connecting cilium which joins the light-sensitive outer segment and the metabolically active inner segment. In bovine, centrin has been previously localized at the connecting cilium of photoreceptor cells [45]. Present immunofluorescence microscopy demonstrates this unique localization of centrin within the connecting cilium of photoreceptor cells in the human and rat retina, in addition to the localization of centrin at the centrosome of nonciliated neuronal cells.

Using PCR primers that discriminate between the two isoforms of human centrin (HsCen1 and HsCen2), we have identified both isoforms amplified by RT-PCR in both human and rat retina. This is the first demonstration of both centrin isoforms expressed in the same tissue and also shows this dual expression in mammals other than human. Using the same primer combinations, analysis of cDNA from rat testis yields both centrin isoforms, while expression in liver and skeletal and cardiac muscle is restricted to the centrin 2 isoform. Furthermore, using a human undifferentiated, noncili-

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ated retinal cell line (retinoblastoma cell line Y79), we have been successful in amplifying only the HsCen2 isoform. These results suggest that while both centrin isoforms are expressed in the retina, one of these (centrin 1) may be restricted to particular cells.

## MATERIALS AND METHODS

*Antibodies.* Two different anti-centrin antibodies were used for this study: The polyclonal rabbit antiserum 26/14-1 (MC1) and the monoclonal antibody clone 20H5, raised against bacterially expressed *Chlamydomonas* centrin [2, 4, 9]. Antibodies against $\gamma$-tubulin were kindly provided by Drs. H. D. Joshi (Atlanta, GA, U.S.A.) and S. Rensch (EMBL, Heidelberg, Germany).

*Immunoprecipitation.* Rat tissues were isolated, lysed as described by Wolfrum [45], and centrifugated (15 min; 15,000 rpm; 4°C). The soluble protein fraction was diluted in immunoprecipitation buffer (IB) A (190 mM NaCl, 1 mM $CaCl_2$, 2.5% Triton X-100, 50 mM Tris, pH 7.4) containing a protein inhibitor cocktail (0.1 mM phenylmethylsulfonyl fluoride (PMSF), 0.42 g/ml leupeptin, 0.083 g/ml pepstatin A, 0.83 g/ml aprotinin obtained from Sigma). For immunoprecipitation, anti-centrin serum MC1 was added and the samples were rocked at 4°C overnight. After adding protein A/G–Sepharose beads (Pierce, Rockford, IL, U.S.A.), samples were incubated for at least 2 h at 4°C. Immunocomplexes were collected by centrifugation and analyzed by SDS–PAGE followed by Western blotting. As controls, lysed tissues were incubated with protein A/G–Sepharose beads without prior antibody incubation.

*SDS–PAGE and Western blot.* SDS–polyacrylamide gel electrophoresis (SDS–PAGE) was performed as described in Wolfrum [45], using 15% polyacrylamide gels. Gels were transferred onto Immobilon PVPF membranes (Millipore Corp., Bedford, MA, U.S.A.). For transfer, gels were soaked for 15 min in transfer buffer ($KH_2HPO_4$ buffer, pH 7.0) and transferred at 20 V overnight 4°C using a Hoeffer TE Transpor unit (Hoeffer Sci. Inst., San Francisco, CA, U.S.A.) onto prewetted membranes. After transfer, membranes were fixed for 45 min with 0.2% (v/v) glutaraldehyde in transfer buffer, washed in TBS (10 mM Tris–HCl, 150 mM NaCl, 1 mM $CaCl_2$) containing 0.05% Tween 20 (v/v) (washing buffer), and blocked with 5% nonfat dry milk in TBS for 2 h. After three rinses in washing buffer, membranes were incubated with anti-centrin antibodies in nonfat dry milk (2 h at 37°C or overnight at 4°C), washed, and incubated with alkaline phosphatase-conjugated goat anti-rabbit IgG (Lot No. 57424, Cappel Oreganon Teknika Corp., Durham, NC, U.S.A.) diluted in nonfat dry milk for 2 h at 37°C. The antibody–alkaline phosphatase complex was visualized by the reaction with BCIP/NBT (Sigma FastTM, Sigma, St. Louis, MO, U.S.A.).

*Fluorescence staining of retinal cryosections.* Isolated retinas were cryofixed in melting isopentane and cryosectioned as described in Wolfrum [42, 43]. Sections were placed on coverslips precoated with 0.05% aqueous poly-L-lysine (Sigma) and stored at 0–4°C until immunolabeling: Cryosections were incubated first with 0.01% Tween 20 in 0.15 M phosphate-buffered saline (PBS), pH 7.3, for 10 min and washed in PBS. After blocking with 0.5% fish gelatin (Sigma) plus 0.1% ovalbumin (Sigma) in PBS (blocking buffer) for 10 min, one of the primary antibodies in blocking buffer was placed on each section for 12 h at 4°C. Unbound antibodies were subsequently washed out three times with PBS and specific secondary antibodies, fluorescein-isothiocyanate (FITC)- or rhodamine-conjugated anti-rabbit-IgG and anti-mouse-IgG antibodies (Lot Nos. 55666 and 55646, Cappel Oreganon Teknika Corp.) in blocking buffer were placed on each section for 1 h at room temperature in the dark. After washing in PBS, the sections were mounted in Vectashield (Vector Laboratories, Burlingame, CA, U.S.A.). Controls included: (i) the omission of primary or secondary antibody, (ii) a secondary antibody against antibodies differing from the primary antibody being used, and nonimmunoserum or preimmunoserum from rabbit used in place of the primary antibodies. In no case was a reaction observed in the controls. The retinal sections were examined and photographed with a Nikon Microphot FXA microscope. Kodak Ectachrome 400 slide film (exposed at 900 ASA) was used for photographic documentation.

*Tissue isolation and tissue culture.* Retinas, testis, liver, skeletal, and cardiac muscle were dissected from adult Sprague–Dawley rats or mice of the strain C57Bl/6J that had been kept on a 12 h light/12 h dark cycle. All treatments in this study conformed to the National Institute of Health and the ARVO statement for the use and care of animals in research. The human retina used was obtained from a 54-year-old male eye donor 18 h postmortem. The tenets of the Declaration of Helsinki were followed. Retinoblastoma Y79 cells (American Type Culture Cell, Rockville, MD, U.S.A.) were grown as suspension cultures in RPMI 1640, 15% fetal calf serum using standard tissue culture techniques.

*RNA extraction, reverse transcription, and PCR.* Total RNA was isolated from Retinoblastom cells (Y79), mouse and rat tissues using the TRI reagent (Molecular Research Center, Inc.) or using TRIZOL reagent (Life Technologies, U.S.A.). Samples of purified total RNA were DNase treated for 15 min to remove genetic DNA. Poly-dT primed cDNA synthesis (reverse transcriptase reaction (RT)) was performed using the Invitrogene cDNA Cycle kit and 5 $\mu$g total RNA as per directions. In control preparations, samples of total RNA isolation DNase treated or untreated, were used in PCR without prior cDNA synthesis. Human retina cDNA, poly-dT primed and purified to remove interfering RNA and genomic DNA, was purchased from Clonetech (Quick-Clone Human retina cDNA; Lot No. 24317). PCR was performed in a volume of 50 $\mu$l using 2 $\mu$l of prepared cDNA as per directions, 5 ng of purchased human cDNA, and 0.25 $\mu$g of each primer per reaction. As positive controls in PCR, amplifications of HsCen1 from an approximate 1100-bp *Eco*RI fragment of a bluescript human centrin 1 clone [6] or of glyceralaldehyde-3-phosphate dehydrogenase (GAPDH) were performed. Cycling conditions were 30 cycles at 94°C for 1 min, 55°C for 2 min, 72°C for 3 min, followed by a 10-min 72°C extension. PCR product lengths were determined on 0.8% agarose gels. As DNA markers, a 1-kb DNA ladder or $\phi$X-174 *Hae*III marker obtained from GibcoBRL Life Technologies (Gaithersburg, MD, U.S.A.) were used. Sequencing (Applied Biosystems sequencers) of PCR products was performed in the Mayo Clinic Molecular Biology Core. For sequence comparisons and oligonucleotide generation the computer program DNAsis (Hitachi Software Engineering Co., San Bruno, CA, U.S.A.) was used.

*PCR Primers used for RT-PCR and sequencing.* (1) Primers specific for human centrin isoforms: HsCen1-primers, the forward primer HsCen1-R1 (5′-CGGGAAGCATTTGACCTCTTCGAC-3′) and the reverse primer HsCen1-R3 (5′-GCTGGTCTTCTTCATGATCCG-3′). HsCen2-primers, the forward primer HsCen2-R1 (5′-CGGGAAGCTTTTGATCTTTTCGAT-3′) and the reverse primer HsCen2-R3 (5′-GCTGGTCTTTTTCATGATGCG-3′).

(2) Primers specific for mouse caltractin or MmCen1-primers: the forward primer MmCen1-R1 (5′-CGGGAAGCCTTTGACCTCTTCGAT-3′) and the reverse primer MmCen1-R3 (5′-GTTGGTCTTTTTCATGATCTT-3′). HsCen2 primers amplified human cDNAs as well as from other vertebrate and mammalian species. HsCen1 primers did not amplify cDNA from mammalian species other than human. Since the published sequence of mouse centrin (MmCen1) [20] is more closely related to HsCen1 than HsCen2, MmCen1-specific primers were generated to similar regions as the human centrin primers. MmCen1 and HsCen 2 primers were used in PCR to distinguish between centrin isoforms in mouse and rat tissues.

(3) Following primer for glyceralaldehyde-3-phosphate dehydrogenase (GAPDH) where used in control PCR amplifications of a 249-bp product: the forward primer (5′-TGATGACATCAAGAAGGTGGTGAAG-3′) and the reverse primer (5′-TCCTTGGAGGCCATGTAGGCCAT-3′).

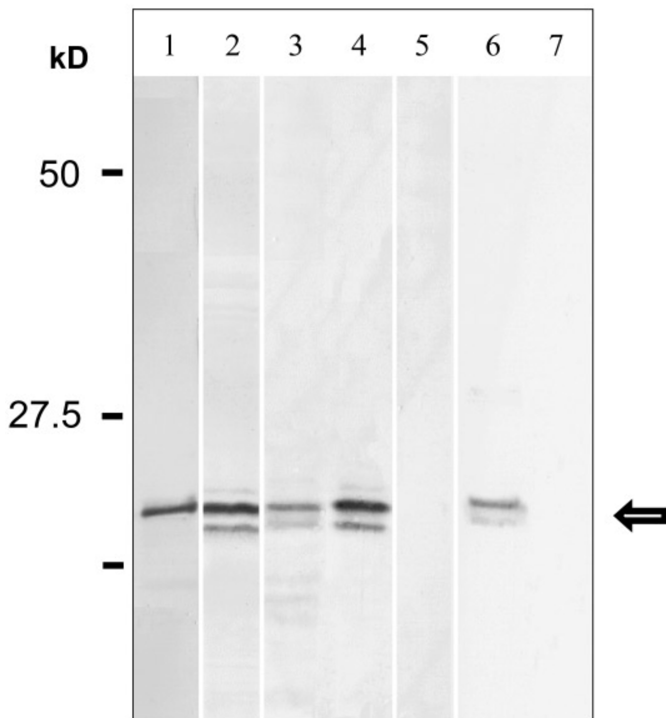


**FIG. 1.** Anti-centrin Western blot analysis. Lane 1: Bacterially expressed *Chlamydomonas* centrin labeled with monoclonal antibody 20H5. Lane 2: mouse retina with polyclonal anti-centrin antibodies (MC1). Lane 3: human retina labeled with 20H5 lane 4: rat retina labeled with 20H5. Lane 5: rat retina incubated with 20H5 preadsorbed with purified *Chlamydomonas* centrin. Lane 6: rat cardiac muscle (heart). Lane 7: Control precipitation of lysed rat cardiac muscle without antibodies. In all preparations except the controls, centrin antibodies recognize bands at about 20 kDa (arrow).

## RESULTS

### *Identification and Immunocytochemical Localization of Centrin in the Mammalian Retina*

Western blot analysis reveals that anti-centrin antibodies recognize protein bands at a molecular weight of about 20 kDa, the previously described molecular weight of known centrins [24]. In human and rodent retinas, anti-centrin antibodies stain polypeptides at 20 kDa which comigrate with purified bacterially expressed *Chlamydomonas* centrin [4] (Fig. 1; lanes 1–4). The labeling of the bands is inhibited when anti-centrin antibodies were preincubated with *Chlamydomonas* centrin (Fig. 1, lane 5). The monoclonal anti-centrin antibody, clone 20H5, also immunoprecipitates centrin from lysed other rat tissues, e.g., cardiac muscle (Fig. 1, lane 6). The presence of centrin in retinas has been also verified in all mammalian species investigated (e.g., ground squirrel, bovine [45]).

Indirect immunofluorescence was applied to immunocytochemically localized centrin in tissue sections of human and rodent retinas. Staining with anti-centrin antibodies reveals that centrin is localized in the retinas in two basically different cellular structures (Fig. 2): At the centrosomes and at the basal bodies of retinal cells it is colocalized with $\gamma$-tubulin (Figs. 2D and 2E). Interestingly, in contrast to $\gamma$-tubulin antibodies, which react equally with both centrioles, the antibodies to centrin stain one centriole brighter than the other. The brighter centriole is probably the maternal centriole. Identical differences in the centrin staining pattern show spindle poles during mitosis [24]. However, centrin is present more intensely in the connecting cilium of the photoreceptor cells (Fig. 2 and [45]). This nonmotile cilium links the light-sensitive outer segment of the photoreceptor cell to the metabolically active inner segment, with all intracellular transport between both segments occuring through this slender connecting cilium.

### *Differential Amplification and Sequence Comparison of Centrin Isoform cDNAs in Mammalian Retinas*

In previous studies, it has been shown that two distinct centrin isoforms are expressed in different human cell types. A single isoform has been described in mouse. We were therefore interested in determining whether the expression of centrin isoforms is tissue or cell specific, whether one or more centrin isoforms are expressed in mammalian retinas, and whether distinct centrin isoforms are expressed in mammalian species other than human. To address these questions, isoform-specific primers were synthesized for PCR amplification using the published sequence for human centrin 1 (HsCen1) isolated from a testis cDNA library [9] or human centrin 2 (HsCen2), also known as human caltractin, isolated from cDNA libraries constructed from primary cultured umbilical vein endothelia and from T-cell lymphoplastic leukemia (MOLT-4) [15]. Using the computer program DNAsis (Hitachi America Ltd., San Bruno, CA, U.S.A.), the sequences of both human centrin isoforms were screened for divergent sequence regions. These divergent regions were used to generate sense and anti-sense primers specific for either HsCen1 or HsCen2.

To determine whether human centrin isoforms HsCen1 and HsCen2 are both expressed in human retinas, 2 $\mu$l of purchased human retinal cDNA were amplified by PCR using primers specific for either HsCen1 or HsCen2. Each primer combination generated a product of similar length (Fig. 3); however, upon sequence analysis, those products generated with HsCen1 primers showed 100% identity to the published human centrin 1 sequence, and those generated with HsCen2 primers were 100% identical to the published human centrin 2 sequence.

Interestingly, amplification of cDNA generated from

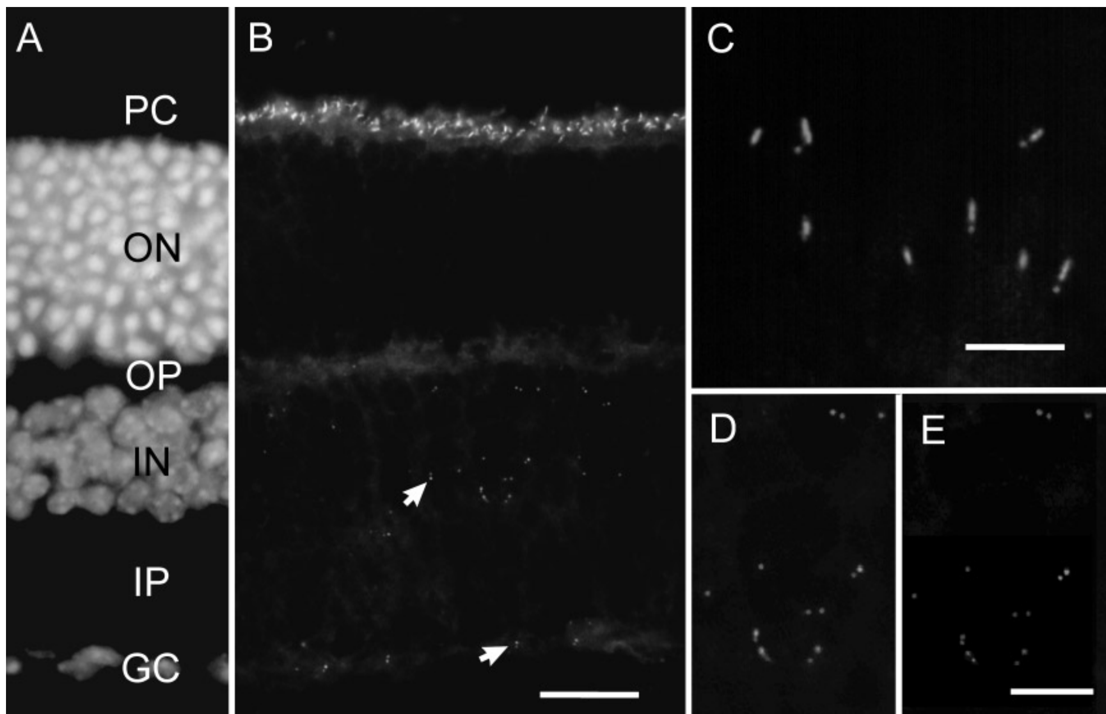


**FIG. 2.** Anti-centrin immunofluorescence in sections of rat and human retina. (A) DAPI-staining of a longitudinal cryosection through the rat retina. Staining of nucleae DNA demonstrates the retinal layers: PC, layer of outer and inner segments of photoreceptor cells; ON, outer nuclear layer where nuclei of photoreceptors are localized; OP, outer plexiform layer; IN, inner nuclear layer; IP, inner plexiform layer; GC, ganglian cell layer. (B) Indirect anti-centrin immunofluorescence in the section through rat retina. Anti-centrin antibodies predominantly react within the photoreceptor cell layer at the joint between the inner and out segment of the photoreceptors. In addition, indirect immunofluorescence is present in dot pairs in the inner nuclear layer and ganglion cell layer (arrows). (C) shows higher magnifications of stained structures in the photoreceptor layer of a human retina: A slender stripe and a dot—the connecting cilium and the basal bodies—are stained in photoreceptor cells of cryosectioned human retina. (D and E) Higher magnification of immunofluorescent double staining with antibodies against centrin (D) and $\gamma$-tubulin (E) in the inner nuclear layer of the section shown in B. Additional $\gamma$-tubulin labeling reveals that centrin and $\gamma$-tubulin are colocalized at centrosomes. Note that as a rule one centriole of a single centrosome shows brighter anti-centrin immunofluorescence. Bars in B: (A) 20 $\mu$m; (C) 7 $\mu$m; (D, E) 9 $\mu$m.

the total RNA extracted from retinoblastoma cells (Y79), which are undifferentiated, nonciliated retinal cells, was restricted to the primer combinations specific for human centrin 2 and for the control gene of GAPDH (Fig. 3). Repeated amplifications using HsCen1 primer pairs failed to produce a product. Sequence analysis of the retinoblastoma PCR product generated with HsCen2 primers confirmed 100% identity to human centrin 2.

Using reverse transcriptase reactions, DNased total RNA from isolated retinas of rats and mice were transcribed into cDNA. In controls of total RNA preparations, genomic DNA was not amplified. However, amplification of synthesized rat cDNAs using either MmCen1 or HsCen2 primer combinations generated PCR products of similar length (Fig. 4). Sequence analysis of these products revealed two different centrin isoforms. The sequence of the PCR products generated from rat retina cDNA using MmCen1 primers was more related to HsCen1 and to the published sequence of mouse caltractin (MmCen1), and has been designated as rat centrin 1 (RnCen1). The sequence of HsCen2-amplified PCR products from rat and mouse retina were more related to HsCen2 and have designed as rat centrin 2 (RnCen2) and mouse centrin 2 (MmCen2). The comparison of the DNA sequences of the PCR products amplified from human and rat as well as mouse transcripts are summarized in Table 1.

*Tissue-Specific Expression of Centrin Isoforms*

Finally, to address the question of whether dual expression of centrin isoforms is restricted to the retina, or also occurs in other tissues, RT-PCR using our isoform-specific primer combinations was performed on total RNA extracted from adult rat tissues. Comparisons were made between retina and testis which both contain ciliated cells and tissues where ciliated cells are not found, including liver and skeletal and cardiac muscle. In the testis as well as in the retina both centrin isoforms, RnCen1 and RnCen2, were expressed (Fig. 4). In contrast, in the nonciliated cell tissues (i.e.,

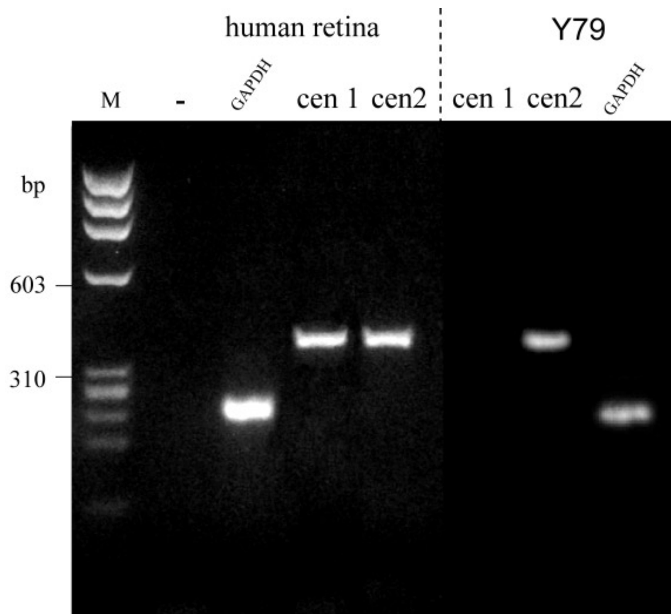


**FIG. 3.** PCR amplification of centrin isoform from purcased human retina cDNA and reverse transcriped RNA from human Retinoblastoma cells (line Y79). Specific primers for HsCen1 and HsCen2 amplified PCR-products of similar length (420 bp). Both isoforms are amplified from human retina cDNA. In retinoblastoma cells, expression of human centrin isoforms is restricted to centrin 2. HsCen2 primers amplified a centrin 2 product while specific primers for HsCen1 do not amplify a PCR-product. As controls 249-bp fragments of human *GAPDH* were amplified. − Control was done without template. Marker (M), $\phi$X-174 *Hae*III base ladder: 1353, 1078, 872, 603, 310, 271, 281, 234, 194, 118, and 72 bp.

liver and skeletal and cardiac muscle), only the centrin 2 isoform (RnCen2) was expressed (Fig. 4). The results are compared in Table 2.

## DISCUSSION

Centrin was first described as a 20-kDa phosphoprotein of lower eukaryotes in green algae [24, 25, 27, 28, 35]. In mammals, however, there are contradictory reports on the molecular mass of the centrins or centrin-like proteins. Western blot analyses in different cells and tissue show a range in molecular weight from 165 kDa, 62–64 kDa to about 20 kDa [1, 9, 15, 19, 21], the molecular weight of centrins and centrin-like proteins in lower eukaryotes [e.g., 11, 25, 38]. Here we demonstrate by SDS–PAGE and Western blot analysis that in the neuronal retina of different mammals, centrin has a molecular weight of about 20 kDa [see also: 45]. This result is in agreement with the predicted and estimated molecular mass of the two cloned human centrin isoforms, human centrin 2 or human caltractin (HsCen2, $M_r$ = 19,570), as well as human centrin 1 (HsCen1, $M_r$ = 19,738) [9, 15]. Since the deduced protein sequence is 84% identical between HsCen1 and HsCen2, the antibodies which were used to not discriminate between the centrin isoforms. Therefore, it cannot be inferred that the two major bands which are visible in anti-centrin Western blots represent two distinct isoforms of centrin. However, staining of identical bands in control tissue which do not consist of ciliated cells indicate that they are probably the result of protein degradation or posttranslational modifications (e.g., phosphorylation [21]).

### *Dual Expression of Centrin Isoforms in the Mammalian Retina*

Since the two cDNA clones of human centrin isoforms, HsCen1 and HsCen2, have been isolated in two distinct cDNA libraries [9, 15], it remained unclear whether the centrin isoforms are tissue specific or are coexpressed in a single tissue. Here we demonstrate for the first time the dual expression of the two centrin isoforms in a single well-defined tissue, the mammalian retina. We duplicated and verified this result in testis, which also has the common feature of containing ciliated cells. In contrast, in nonciliated precursor cells of the retina, and in tissues where ciliated cells are absent, this coexpression of centrin isoforms is not observed. In these cases, only the centrin 2 isoform is present. These results suggest that centrin 2 is a universally expressed isoform of centrin, while centrin 1 may be restricted to specialized cells, such as ciliated cells. Alternatively, centrin 1 may be expressed at very low levels undetectable by the highly sensitive RT-PCR method of analysis used here or it may be expressed at restricted times during the cell cycle.

Isoforms and isotypes are well known for cytoskeletal proteins, e.g., for actin and for $\alpha$- and $\beta$-tubulin, which

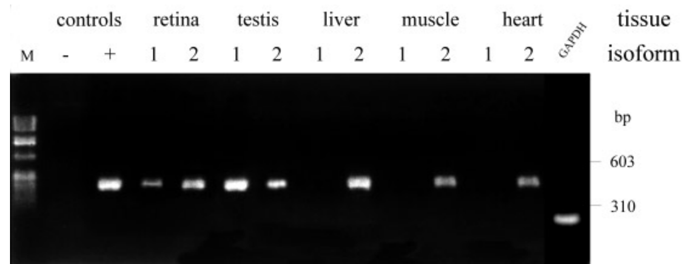


**FIG. 4.** RT-PCR analysis of centrin isoforms in rat tissues. In retina and testis—tissues containing ciliated cells—centrin isoform-specific primers amplified 420-bp products of rat centrin 1 and rat centrin 2. Using the same primers in liver, skeletal muscles (muscle), and cardiac muscle (heart) only a PCR-product for rat centrin 2 product is detected. + Control: HsCen1 amplification from a 953-bp fragment of bluescript human centrin 1 clone. As an additional control, 249-bp fragments of *GAPDH* were amplified from rat tissues; the amplified product of heart cDNA is shown as an example. − Control no template; marker (M), 1-kb DNA ladder: 12,216–1,018, 506, 517, 396, 344, 298, and 220–75 bp.

**TABLE 1**

Interspecies Comparison of Nucleotide Sequences Obtained from PCR Products of Centrin Isoforms

| | HsCen1 | HsCen2 | MmCen1 | MmCen2 | RnCen1 | RnCen2 |
|---|---|---|---|---|---|---|
| HsCen1 | # | | | | | |
| HsCen2 | 80% | # | | | | |
| MmCen1 | 85% | 80% | # | | | |
| MmCen2 | 76% | 89% | 79% | # | | |
| RnCen1 | 85% | 80% | 94% | 78% | # | |
| RnCen2 | 79% | 88% | 80% | 90% | 81% | # |

show cell and tissue specificity but also coexpression [e.g., 13, 18, 36, 37, 39]. Here we show that isoforms of another cytoskeletal protein, centrin, also coexpress. Although in lower eukaryotes only one form of centrin is present, in mammals, two centrin isoforms have been described for human, while in the mouse animal model, only one centrin was known [9, 15, 20, 24]. Here we clearly show that in mouse, rat, and human, two related but distinct centrin isoforms exist which are also coexpressed in specialized tissue containing ciliated cells. We suggest that all mammals may have at least two centrin isoforms. Protein isoforms can be the products of separate gene expression or posttranslational modifications of a single gene product. In human, the expression of centrin isoforms arises from the expression of different gene products from genes which map to chromosome 18 and chromosome X for HsCen 1 and HsCen 2, respectively [9, 35]. Additional posttranslational modifications of centrin may also occur in each isoform. Centrins bind Ca-ions and have several putative phosphorylation sites which are suggested to play important roles in the cellular function of centrins [24, 32, *et loc. cit.*].

*Immunohistological Localization of Centrin in Two Basical Distinct Structures*

In mammalian cell lines, centrin was previously described as a component of the spindle poles, the centrioles of centrosomes, and their pericentriolar lattice or satellites [2, 3, and for review see 24, 32]. Although the function of centrin in vertebrates is not yet known, centrin might play an essential role in centrosome duplication [24] and in microtubule severing [29, 30]. The latter possibility leads to the suggestion that at centrosomal structures, centrin is involved in the release of *de novo* polymerized microtubules from the microtubule organization center [9, 31, 46]. However, in the neuronal retina of mammals, the localization of centrin is not restricted to centrosomes or centrosomal satellites [44, 45]. Immunocytochemistry for light and electron microscopy clearly reveals that centrin is localized in the mammalian retina in two basically distinct structures: the centrosomes and the basal bodies and in the modified connecting cilium of photoreceptor cells [present study and 45]. Although anti-centrin antibodies cannot distinguish between the two centrin isoforms, the finding that the expression of centrin 1 is restricted to ciliated cells together with the cellular localization of centrin within the connecting cilium suggests that the centrin isoform which is present at the cilium of the ciliated cells is probably centrin 1.

The connecting cilium shows the features of a nonmotile cilium. It links the inner segment with the light-sensitive outer segment of the photoreceptor cells [7, 10, 23]. The cilium is localized at a strategic point of the cell, at the slender joint between the inner and the outer segment of the photoreceptor cell through which a high bidirectional transport must occur [7, 8, 17, 22, 41]. The localization of centrin in two basically different cellular structures within the mammalian retina may also implicate distinct cellular functions of centrin, and it is possible that centrin may also participate in the transport through the connecting cilium of the photoreceptor cells [45]. However, centrins may also regulate, like calmodulin enzymatic activities in a $Ca^{2+}$-dependent matter [21]. Differential localization of centrin isoforms using isoform-specific antibodies should provide more evidence for the deduction of specific cellular

**TABLE 2**

Tissue-Specific Expression of Centrin Isoforms

| Tissue | Retina | Testis | Liver | Muscle | Heart | Y79 cells |
|---|---|---|---|---|---|---|
| Centrin | Cen1 | Cen1 | — | — | — | — |
| Isoforms | Cen2 | Cen2 | Cen2 | Cen2 | Cen2 | Cen2 |

functions of each centrin isoform. Additional studies during various stages of development may also show differential expression or modification of centrin isoforms and hence may further elucidate the functions of centrin isoforms.

Centrin, as a cytoskeletal protein, in combination with its unique localization within the ciliated structure of the sensory cells and the current finding of differential isoform expression, could be an interesting protein for further study in the development of disease affecting ciliary sensory structures. Genes encoding proteins of the connecting cilium in photoreceptor cells have been implicated as points of possible defects leading to the development of Usher syndrome, a hereditary disease which causes combined blindness and deafness and may lead to disorders in olfaction [5, 12, 47]. Defects in the gene for an unconventional myosin, myosin VIIA, is responsible for one type of Usher, Usher syndrome 1B [40]. Recently, it has been shown that centrin and myosin VIIa are both localized at the connecting cilium of mammalian photoreceptor cells [16, 33]. Therefore, future studies into the function of centrins in higher eukaryotes may also lead to better understanding of diseases based on ciliated structures.

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