# Isolation of a cDNA encoding a photoreceptor cell-specific actin-bundling protein: retinal fascin

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**Abstract** **We have isolated a novel retina-specific gene, retinal fascin, encoding a new member of actin-bundling protein gene family, from a bovine retina cDNA library. The cDNA encodes a 492 amino acid protein which shows 36–57% amino acid identity with three vertebrate fascins, echinoid fascin and *Drosophila* singed gene. Northern blot analysis revealed that retinal fascin mRNA was exclusively expressed in the eye and not seen in other tissues examined. In situ hybridization analysis indicated that retinal fascin mRNA signals were found only in the inner segment of the photoreceptor layer and outer nuclear layer, indicating that retinal fascin was specifically expressed in photoreceptor cells. As fascins are actin-bundling proteins important for constructing several intracellular structures, retinal fascin might play a pivotal role in photoreceptor cell-specific events, such as disk morphogenesis.**



*Key words:* Retinal fascin; Actin-bundling protein; Photoreceptor cell; Retina-specific gene; cDNA cloning

## 1. Introduction

Fascins are a widely distributed family of proteins that organize filamentous actin into bundles [1]. Kane first isolated echinoid fascin from cytoplasmic extracts of sea urchin eggs, which is required for producing a highly organized gel consisting of needle like structures [2,3]. The cDNA for echinoid fascin has been isolated subsequently, revealing that echinoid fascin shows homology with the *Drosophila* singed gene product responsible for gnarled bristle development and female sterility [4]. To date, members of fascin gene family have been also cloned from human, mouse and *Xenopus* [5–7]. Each of these proteins functions as an actin-bundling protein in many structures, such as microspikes and stress fibers of fibroblasts, filopodia of coelomocytes, bristles and nurse cells of Drosophila [1]. Their wide distribution suggests that fascins play important roles in morphogenesis of diverse intracellular structures.

In photoreceptor cells, actin is known to localize in the inner segment [8–12], synaptic region of rods [11–13], the connection between rod inner and outer segments [10,11], the lip of forming rod outer segment discs [10,14–16] and seems to be responsible for many functions, such as regulation of outer segment disk morphogenesis [18] and photoreceptor shortening during light or dark adaptation in lower vertebrates [19,20]. And these special functions of actin in photoreceptor cells raise the possibility that these cells might express their own actin-bundling proteins for regulating such structures. However, almost no information is available on actin-bundling proteins in the retina. In the course of our attempt to isolate retina-specific genes, we cloned a novel member of fascin gene family, retinal fascin, whose transcripts were exclusively expressed in photoreceptor cells. In the present study, we report its primary structure and expression pattern in the retina.

## 2. Materials and methods

### 2.1. cDNA library screening

A bovine retina cDNA library constructed in the Uni-ZAP XR vector (Stratagene) was screened by random primed labeling with [32P]dCTP. The probe was made from one of the clones randomly picked up from retina cDNA library and showing an eye-specific expression pattern by Northern blot analysis. Probes were hybridized to the phage DNA immobilized on nylon membranes (Hybond-N+, Amersham Corp.) in a solution containing 5×SSPE, 2×Denhardt's solution, 0.1% SDS, 40% formamide, 50 μg/ml denatured salmon sperm DNA. They were incubated at 37°C for 20 h and washed 2 times at 55°C each time in 1×SSPE, 0.4% SDS. The membranes were exposed to autoradiographic film (X-Omat AR, Eastman Kodak Co.) in cassettes overnight at room temperature. After second screening, positive clones were excised in vivo with R408 helper phage. We subcloned the cDNA inserts into pBluescript II SK(+).

### 2.2. DNA sequencing

DNA sequencing was performed on the dideoxy chain termination method (Applied Biosystems Inc.) using Ampli Taq DNA polymerase. It was analyzed with an automatic DNA sequencer (ABI). DNA sequencing was confirmed by both strands.

### 2.3. Northern blot analysis

mRNA was isolated from various rat organs with a Fast Track mRNA purification kit (Invitrogen). 3 μg of mRNA was electrophoresed in a 1.0% agarose formaldehyde gel and transferred onto a nylon membrane (Hybond-N+, Amersham Corp.). Partial clones of rat retinal fascin and rat GAPDH cDNA were labeled with [α-32P]dCTP (4500 Ci/mmol, ICN) using a random primer DNA labeling kit (Takara). Hybridization was performed at 42°C over night in a solution containing 5×SSPE, 5×Denhardt's solution, 1% SDS, 50% formamide, 200 μg/ml denatured salmon sperm DNA. The membrane was washed 2 times in 2×SSPE and 0.1% SDS at room temperature for 10 min and washed in 1×SSPE and 0.1% SDS at 65°C for 15 min and then washed in 0.1×SSPE and 0.1% SDS at 65°C for 10 min. The hybridization signals were analyzed with a bioimaging analyzer BAS 5000 (FUJIX, Tokyo, Japan).

### 2.4. In situ hybridization

The procedures were basically the same as those described previously [21]. Male wistar rats (150 g) were used in this study. In situ hybridization with [α-35S]UTP-labeled cRNA probes transcribed from partial rat retinal fascin cDNA was performed on retina sections from adult rats.

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**A**

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5'  AGG GGC CCC CGT CGG GGA CCT CAG CCA GGC CCG AAG ATG CCT ACA AAT GGC CTG   54
                                                M   P   T   N   G   L
    CAC CAG GTG CTG AAA ATC CAG TTT GGC CTC GTC AAT GAC ACT GAC CGC TAC CTG  108
     H   Q   V   L   K   I   Q   F   G   L   V   N   D   T   D   R   Y   L
    ACG GCC GAG AGC TTT GGC TTC AAG GTC AAT GCC TCA GCA CCC AGC CTC AAG CGG  162
     T   A   E   S   F   G   F   K   V   N   A   S   A   P   S   L   K   R
    AAG CAG ATG TGG GTG CTG GAG CCG GAC CCA GGG GAG GGC ACT GCC GTG CTG TTT  216
     K   Q   M   W   V   L   E   P   D   P   G   E   G   T   A   V   L   F
    CGC AGC AGC CAC CTG GGC CGT TAC CTG TCG GCC GAG GAG GAC GGG CGT GTG GCC  270
     R   S   S   H   L   G   R   Y   L   S   A   E   E   D   G   R   V   A
    TGC GAG GCG GAG CGG CCG GGT CGT GAC TGC CGC TTC CTG GTC CTG CCG CAG CCC  324
     C   E   A   E   R   P   G   R   D   C   R   F   L   V   L   P   Q   P
    GAT GGG CGC TGG GTG CTG CAG TCG GAA CCG CAC GGC CGC TTC TTT GGT GGC ACC  378
     D   G   R   W   V   L   Q   S   E   P   H   G   R   F   F   G   G   T
    GAG GAC CAG CTG TCC TGC TTC GCC ACG GCC ATC ACC CCA GCC GAG CTG TGG ACA  432
     E   D   Q   L   S   C   F   A   T   A   I   T   P   A   E   L   W   T
    GTG CAC CTG GCC ATC CAC CCG CAG GCC CAC CTG CTG AGC GTG AGC CGG CGG CGC  486
     V   H   L   A   I   H   P   Q   A   H   L   L   S   V   S   R   R   R
    TAC GCA CAC CTA TGC CCG CAG GAA GAT GAG ATC GCA GCG GAC AGC AAT ACG CCA  540
     Y   A   H   L   C   P   Q   E   D   E   I   A   A   D   S   N   T   P
    TGG GGT GTG GAC GCG CTT GTC ACG CTC ATC TTC CAG AAC CGG CAG TAC TGC CTC  594
     W   G   V   D   A   L   V   T   L   I   F   Q   N   R   Q   Y   C   L
    AAG TCC TGT GAC AGC CGC TAC CTG CGC AGC GAC GGC CGC CTC GTC TGG GAG CCC  648
     K   S   C   D   S   R   Y   L   R   S   D   G   R   L   V   W   E   P
    GAG GCT CGT GCC CGC TAC ACG CTT GAG TTC AAG GCG GGC AAG TTG GCC TTC AAG  702
     E   A   R   A   R   Y   T   L   E   F   K   A   G   K   L   A   F   K
    GAC TGC GAT GGC CAC TAC CTG GCA CCC GTG GGC CCC GCG GGC ACG CTC AGG GCG  756
     D   C   D   G   H   Y   L   A   P   V   G   P   A   G   T   L   R   A
    GGC CGC AAC ACA CGG CCT GGC AAG GAC GAG CTC TTC GAC CTG GAG GAG AGT CAC  810
     G   R   N   T   R   P   G   K   D   E   L   F   D   L   E   E   S   H
    CCA CAG GTG GTG CTG GTG GCC GCC AAC CAC CGC TAC GTG TCC GTG CGG CAA GGG  864
     P   Q   V   V   L   V   A   A   N   H   R   Y   V   S   V   R   Q   G
    GTC AAT GTC TCA GCC AAC CAA GAT GAA GAA CTG GAT CAC GAG ACC TTC TTG ATG  918
     V   N   V   S   A   N   Q   D   E   E   L   D   H   E   T   F   L   M
    CAA ATT GAC CAG GAG ACA AAG AAG TGC ACC TTC TAT TCC AGC ACT GGG GGC TAC  972
     Q   I   D   Q   E   T   K   K   C   T   F   Y   S   S   T   G   G   Y
    TGG ACC CTG GTC ACC CAC GGG GGC ATC CAG GCC ACA GCT ACA CAA GTT TCT GAG 1026
     W   T   L   V   T   H   G   G   I   Q   A   T   A   T   Q   V   S   E
    AAC ACC ATG TTT GAA ATG GAG TGG CGG GGC CGA CGG GTG GCC CTC AAG GCC AGT 1080
     N   T   M   F   E   M   E   W   R   G   R   R   V   A   L   K   A   S
    AAC GGG CGC TAT GTG TGC ATG AAG AAG AAT GGG CAG CTG GCG GCC ATC AGC GAT 1134
     N   G   R   Y   V   C   M   K   K   N   G   Q   L   A   A   I   S   D
    TTT GTG GGG GAG GAC GAG GAG TTC ACG CTC AAG CTT ATC AAC CGG CCC ATC CTG 1188
     F   V   G   E   D   E   E   F   T   L   K   L   I   N   R   P   I   L
    GTC CTG CGC GGC CTG GAC GGC TTC GTC TGC CAC CGA CGT GGC TCC AAC CAG TTG 1242
     V   L   R   G   L   D   G   F   V   C   H   R   R   G   S   N   Q   L
    GAC ACC AAC CGC TCG GTT TAC GAC GTG TTC CAC CTG AGC TTC AGC GAC GGC GCC 1296
     D   T   N   R   S   V   Y   D   V   F   H   L   S   F   S   D   G   A
    TAC CAG ATC CGA GGC CGC GGC GGC GGG TTC TGG CAC ACC GGC AGC CAC GGC AGC 1350
     Y   Q   I   R   G   R   G   G   G   F   W   H   T   G   S   H   G   S
    GTG TGC AGC GAC GGC GAG CGC GCC GAG GAC TTC CTG TTC GAG TTC CGG GAG CGC 1404
     V   C   S   D   G   E   R   A   E   D   F   L   F   E   F   R   E   R
    GGC CGC CTG GCC ATC CGG GCC CGG AGC GGC AAG TAC CTG CGT GGC GGC GCC TCG 1458
     G   R   L   A   I   R   A   R   S   G   K   Y   L   R   G   G   A   S
    GGG CTG CTG CGC GCG GAC GCG GAC GCG CCG GCT GGG GTT GCG CTC TGG GAA TAC 1512
     G   L   L   R   A   D   A   D   A   P   A   G   V   A   L   W   E   Y
    TGA GCG CCG CCC GTC TGT CCC CCA TTA AAC CGT GTC TGT GAA AAA AAA AAA AAA 1566
     *
    AAA AAA AAA AAA AAA AAA AAA AA 3'
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Fig. 1. A: Nucleotide and deduced amino acid sequence of retinal fascin. The translation termination codon is marked by an asterisk. This clone contains a cDNA insert of 1589 base pair with an open reading frame encoding a 492 amino acid protein. B: Alignment of amino acid sequences encoding retinal fascin, *Xenopus* fascin, human fascin, murine fascin, echnoid fascin and *Drosophila* singed gene. Dots indicate amino acids conserved in fascin gene family. C: Partial nucleotide sequence of rat retinal fascin cDNA.

**B**

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retinal fascin    1 MPTNGLHQVL KIQF------ ---------- ---GLVNDTD RYLTAESFGF   50
Xenopus           1 MSSGP----- -LQL------ ---------- ---GLVNCNN KYLTAEAFGF   50
human             1 MTANGTAEAV QIQF------ ---------- ---GLINCGN KYLTAEAFGF   50
murine            1 MTANGTAEAV QIQF------ ---------- ---GLISCGN KYLTAEAFGF   50
echinoid          1 MPAMN----- -LKY------ ---------- -KFGLVNSAG RYLTAEKFGC   50
Drosophila        1 MNGQG----- -CELGHSNGD IISQNQQKGW WTIGLINGQH KYMTAETFGF   50
                    *                                 **       * *** **

retinal fascin   51 KVNASAPSLK RKQMWVLEPD PGEGT-AVLF RSSHLGRYLS AEEDGRVACE  100
Xenopus          51 KINASASSLK KKQVWSLEPA GDDT--SAVL LRSHLGRFLS ADKDGKVSGE  100
human            51 KVNASASSLK KKQIWTLEQP PDEAGSAAVC LRSHLGRYLA ADKDGNVTCE  100
murine           51 KVNASASSLK KKQIWTLEQP PDEAGSAAVC LRTHLGRYLA ADKDGNVTCE  100
echinoid         51 KVNASGATLK ARQVWILEQE ESS---TISY LKAPSGNFLS ADKNGNVYCS  100
Drosophila       51 KLNANGASLK KKQLWTLEPS NTGE--SIIY LRSHLNKYLS VDQFGNVLCF  100
                    * **    **  * * **                 *      * *

retinal fascin  101 AERPGR--DC RFLVLP--QP DGRWVLQSEP HGRFFGGTED QLSCFATAIT  150
Xenopus         101 SD--TAGPEC RFLVSA--QG DGRWALQSEL FGRYFGGSED RISCFSPSVS  150
human           101 RE--VPGPDC RFLIVA--HD DGRWSLQSEA HRRYFGGTED RLSCFAQTVS  150
murine          101 RE--VPDGDC RFLVVA--HD DGRWSLQSEA HRRYFGGTED RLSCFAQSVS  150
echinoid        101 VEDRTEDADT GFEIEL--QP DGKWALKNVS HQRYLACNGE ELICSESSTS  150
Drosophila      101 SDERDAGTRG RFQISISEDG SGRWALKNES RGYFLGGTPD KLVCTAKTPG  150
                              *          * * *                 *

retinal fascin  151 -PAELWTVHL AIHPQAHLLS VSRRRYAHLC PQE----DEI AADSNTPWGV  200
Xenopus         151 -PAEKWGVHL AMHPQFTLYS VTRKRYRRL- --SA-SGDEL SVERDVPWGV  200
human           151 -PAEKWSVHI AMHPQVNIYS VTRKRYAHL- --SARPADEI AVDRDVPWGV  200
murine          151 -PAEKWSVHI AMHPQVNIYS VTRKRYAHL- --SARPADEI AVDRDVPWGV  200
echinoid        151 NPSANWTVQL AIHPQVCMKN VQHQRYAHLK TSEE-GEDSV VVDELVPWGA  200
Drosophila      151 -ASEFWTVHL AARPQVNLRS IGRKRFAHL- --SE-SQDEI HVDANIPWGE  200
                      * *   *  **          *   *         *          ***

retinal fascin  201 DALVTLIFQN RQYCLKSCDS RYLRS---DG RLVWEPEARA RYTLEFKAGK  250
Xenopus         201 DSLITLLFQ- E--NRYSIQT PDHRLLASDG SLREKPGPDT AYTLDISAGK  250
human           201 DSLITLAFQ- D--QRYSVQT ADHRFLRHDG RLVARPEPAT GYTLEFRSGK  250
murine          201 DSLITLAFQ- D--QRYSVQT SDHRFLRHDG RLVARPEPAT GFTLEFRSGK  250
echinoid        201 DSTLTLVYLG K--GKYGLEA FNGKFVQTDG QLAGTANEQT QFTLIFTSGH  250
Drosophila      201 DTLFTLEFRA EECGRYALHT CNNKYLNANG KLQVVCNEDC LFSAEYHGGH  250
                      *   **          *                 *  *           *

retinal fascin  251 LAFKDCDGHY LAPVGPAGTL RAGRNTRPGK DELFDLEESH PQVVLVAANH  300
Xenopus         251 VAFRGSDGRY LTSSGPSGTL KSGKNSKAGR DELFVLERSC PQVVLTAGNG  300
human           251 VAFRDCEGRY LAPSGPSGTL KAGKATKVGK DELFALEQSC AQVVLQAANE  300
murine          251 VAFRDCEGRY LAPSGPSGTL KAGKATKVGK DELFALEQSC AEVVLQAANE  300
echinoid        251 LVLRDNNGRH LGVDSGTRVL KSSKPG-LTK ANYFILEDSC PQGAFEFG--  300
Drosophila      251 LALRDRQGQY LSPIGSKAVL KSRSSS-VTR DELFSLEDSL PQASFIAGHN  300
                          *   *        *              * ** *

retinal fascin  301 R-YVSVRQGV NVSAN---QD EELDHETFLM QIDQETKKC- ----TFYSST  350
Xenopus         301 GT-CLQGKVL IFSAN---QD EESDQETFQL EINKDTKMC- ----AFRTHT  350
human           301 RN-VSTRQGM DLSAN---QD EETDQETFQL EIDRDTKKC- ----AFRTHT  350
murine          301 GN-VSTRQGM DLSAN---QD EETDQETFQL EIDRDTRKC- ----AFRTHT  350
echinoid        301 GKYASLKQGE DVSFKLLVDE DIEDTETFQL EFVETDKYAI RVCDPKKNSR  350
Drosophila      301 LRYVSVKQGV DVTAN---QD EVGENETFQL EYDWSAHRW- ----ALRTTQ  350
                                          ***

retinal fascin  351 GGYWTLVTHG GIQATATQVS EN-TMFEMEW RGRRVALKAS NGRYVCMKKN  400
Xenopus         351 GKYWTLSSNG GIQASASTLN SS-CYFEIDW CDRRITLKGV NGKYVTAKKN  400
human           351 GKYWTLTATG GVQSTASSKN AS-CYFDIEW RDRRITLRAS NGKFVTSKKN  400
murine          351 GKYWTLTATG GVQSTASTKN AS-CYFDIEW CDRRITLRAS NGKFVTAKKN  400
echinoid        351 DAKFWKTVAA GIQANGNSKD QTDCQFSVEY NGNDMHVRAP GGKYVSVRDN  400
Drosophila      351 DRYWCLSAGG GIQATGNRRC ADALFELIWH GDGSLSFRAN NGKFLATKRS  400
                          * *                                 *

retinal fascin  401 GQLAAISDFV GEDEEFTLKL INRPILVLRG LDGFVCHR-R GSNQLDTNRS  450
Xenopus         401 GQLSASVDTP GETELFLMKL INRPLIVLRG EHGFIGCR-K MTGTLDSNRS  450
human           401 GQLAASVETA GDSELFLMKL INRPIIVFRG EHGFIGCR-K VTGTLDANRS  450
murine          401 GHVAASVETA GDSELFLMKL INRPIIAFRG EHGFIACR-K VTGTLDANRS  450
echinoid        401 GHLFLQDSPK D----FIFRL LNRPKLVLKC PHGFVGMK-E GKAFVACNRS  450
Drosophila      401 GHLFATSESI EEIAKFYFYL INRPILVLKC EQGFVGYRTP GNLKLECNKA  450
                    *                 *  *  ***          **          *

retinal fascin  451 VYDVFHLS-F SDGAYQIRGR GGGFWHTGSH GSVCSDGERA EDFLFEFRER  500
Xenopus         451 IYDVFELE-F NDGAYSLKDA TGKYWTVGSD MSVTSRCHPG WS--LEFCDY  500
human           451 SYDVFQLE-F NDGAYNIKDS TGKYWTVGSD SAVTSSGDTP VDFFFEFCDY  500
murine          451 SYDVFQLE-F NDGAYNIKDS TGKYWTVGSD SSVTSSSDTP VDFFLEFCDY  500
echinoid        451 NFDVFTVT-Y KEGGYTIQDS CGKYWSCDDS SRIVLGEAAG -TFFFEFHEL  500
Drosophila      451 TYETILVERA QKGLVHLKAH SGKYWRIEGE SISVDADAPS DGFFLELREP  500
                            *          *                       *

retinal fascin  501 GRLAIRARS- GKYLRGGASG LLRADADAPA GVALWEY... .........  550
Xenopus         501 NKVAIQKV-N GLYLKGDHAG VLKANAENID STTLWEY... .........  550
human           501 NKVAI-KV-G GRYLKGDHAG VLKASAETVD PASLWEY... .........  550
murine          501 NKVAL-KV-G GRYLKGDHAG VLKACAETID PASLWEY... .........  550
echinoid        501 SKFAIRAESN GMLIKGEQSG LFTANGSEVS KDTLWEF... .........  550
Drosophila      501 TRICIRSQ-Q GKYLGATKNG AFKLLDDGTD SATQWEF... .........  550
                            *  *                    **
```

Fig. 1. (*Continued*).

C GTCCTGCTTTGCTACGGCCATCTCCCCGGCAGAGCTGTGGACCGTACACCTGGCCATCCACCCACAGGCTCACTTGCTGA 80

GTGTGAGCCGCCGGCGCTACGTGCACCTGTGCCTTCAGGAGGACGAGATGGCGGCAGATGGTGACATGCCGTGGGGTGTG 160

GACGCACTGCTCACCCTCATTTTCCAGAGCAGGCGGTACTGCCTCAAGTCTTATGACAGCCGCTACCTTCGAAGTGATGG 240

CCGCCTTGTCTGGGAGCCTGAAGCCCATGCCTGCTACACACTGGAGTTCAAGGCAGGCAAGCTGGCCTTCAAGGACTGTG 320

ACGGCCGATACCTGGCACCTGTGGGGCCTGCCGGCACGCTTAAGGCTGGCCGAAACACGAGGCCCGGCAAGGATGAACTC 400

ATCGACCTGGAGCAGAGTCACCCACAGGTGGTACTGGTAGCTGCCAACCACCGCTACGTCTCTGTGAGGCAAGGAATCAA 480

TGTCTCAGCC 490

Fig. 1. (*Continued*).

## 3. Results

### 3.1. Cloning of retinal fascin

In order to isolate retina-specific genes, we performed three different methods; differential display RT-PCR between retina and brain mRNA; subtraction hybridization of lambda ZAP cDNA libraries prepared from rat retina and brain; sequencing of 100 independent clones randomly picked up from a bovine retina cDNA library. We have selected several candidate clones from each methods. Northern blot screening revealed one of the clones (R4) randomly picked up from a bovine retina cDNA library showed an eye-specific expression pattern. By screening of a bovine retina cDNA library with R4 probe, we isolated eight positive clones. Restriction enzyme mapping and partial sequencing indicated that these clones encode a single protein. The largest clone contains a cDNA insert of 1589 base pair with an open reading frame encoding a 492 amino acid protein with a calculated relative molecular mass of 55070 Da. The nucleotide sequence and deduced amino acid sequence are shown in Fig. 1A. There is no N-terminal signal sequence. Hydropathy analysis of the primary amino acid sequence of the predicted protein shows absence of transmembrane domains. Computer analysis showed that there are nine potential sites for protein kinase C-dependent phosphorylation (Thr-20, Ser-39, Ser-147, Thr-237, Ser-272, Thr-300, Thr-375, Thr-404, Ser-465), two potential sites for protein kinase A-dependent phosphorylation (Thr-304, Ser-399), and two tyrosine kinase-dependent phosphorylation sites (Tyr-193, Tyr-228). Sequence analysis indicates that the deduced protein has 55% amino acid identity with murine fascin, 57% with human fascin, 52% with *Xenopus* fascin, 40% with *Drosophila* singed gene, 36% with echinoid fascin and shares most of the conserved motifs and conserved amino acids observed in the family (Fig. 1B). These findings indicate that retinal fascin is a novel member of fascin gene family.

### 3.2. Northern blot analysis

Since rat is more convenient for analyzing the distribution of retinal fascin, we performed PCR to obtain rat retinal fascin. Rat retinal fascin cDNA had been partially cloned and sequenced by using the reverse transcription-polymerase chain reaction (RT-PCR) method. The degenerate oligonucleotide primers used in this reaction were designed from bovine fascin. The 5′ primer [5′-TT(C/T)GGTGGCACCGA(A/G)GA(C/T)CA(A/G)(C/T)T-3′] corresponded to bp 367–389 of bovine retinal fascin sequence, and the 3′ primer [5′-TGATCCAG(T/C)TC(T/C)TC(G/A)TC(T/C)TG(G/A)TT-3′] corresponded to bp 880–902. We obtained a PCR fragment (Fig. 1C), which showed about 90% amino acid identity with bovine fascin, indicating that this clone is a partial fragment of rat retinal fascin. Northern blot analysis using rat retinal fascin as a probe revealed that a major band of approximately 1.5 kilobases and several minor bands were observed only in the eye but not in other tissues such as brain, lung, heart, liver, spleen, testis and skeletal muscle, suggesting that retinal fascin is specifically expressed in the eye and there are several splicing variants (Fig. 2).

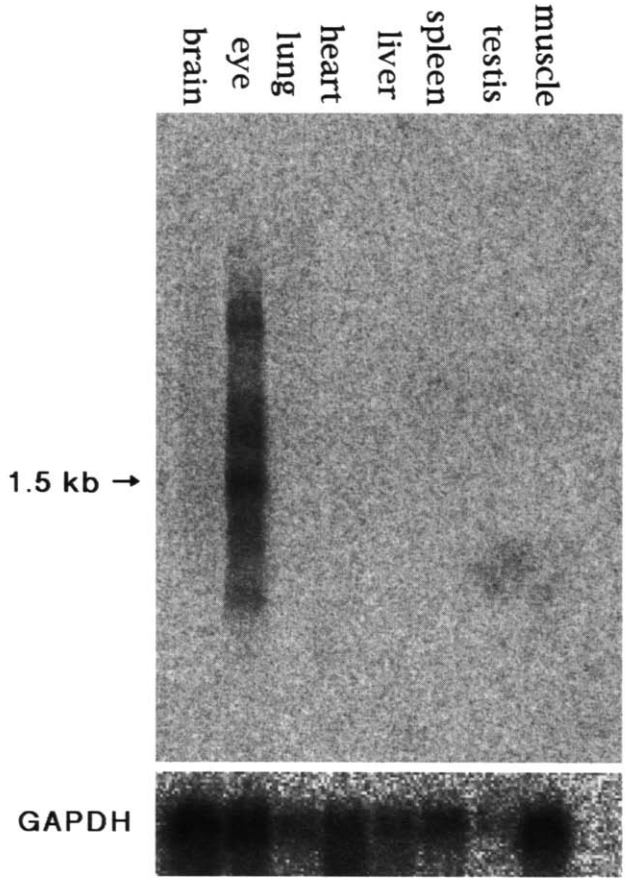


Fig. 2. Northern blot analysis of retinal fascin mRNA. 3 μg of mRNA from brain, eye, lung, heart, liver, spleen, testis and skeletal muscle.

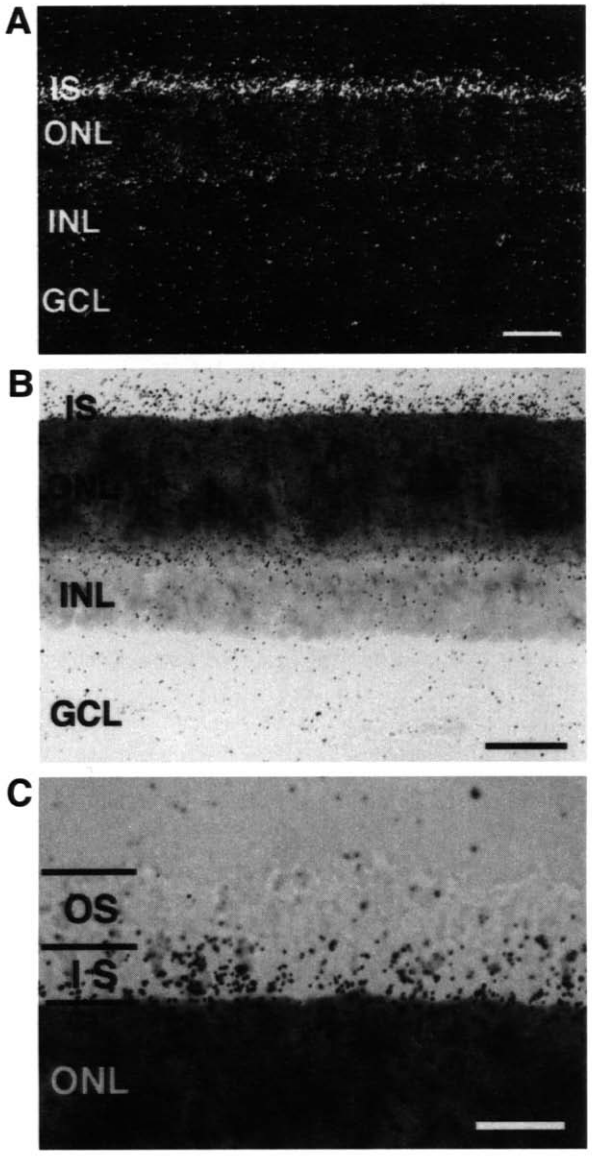


Fig. 3. Expression of retinal fascin mRNA in the adult rat retina. A: Dark-field photomicrograph. B: Bright-field photomicrograph. C: Bright-field photomicrograph of the photoreceptor cells. IS, inner segment; ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer. A: Bar = 20 μm. B, C: Bar = 50 μm.

### 3.3. *In situ hybridization*

To determine the more detailed distribution of retinal fascin in the rat retina, we performed in situ hybridization using a 35S-labeled cRNA probe. The specificity of the hybridization signals was determined by hybridizing consecutive sections with either antisense or sense probe. No specific signal was detected in sections hybridized with sense probe. In the rat retina, intense hybridization signals for retinal fascin mRNA were found in the inner segment of the photoreceptor cell layer (Fig. 3A and B). In addition, weak hybridization signals were detected in the outer nuclear layer (ONL) (Fig. 3A and B). At the high-power magnification bright field microscopic level, the signals for retinal fascin mRNA were concentrated in the inner segment (IS) of the retina (Fig. 3C), indicating that retinal fascin was exclusively expressed in photoreceptor cells. Similar photoreceptor cell-specific expression pattern was also observed in the bovine retina by in situ hybridization with bovine retina fascin cRNA probe (data not shown).

## 4. Discussion

We describe the isolation and characterization of a novel cDNA which is exclusively expressed in the photoreceptor cells. Sequence analysis reveals that it is a novel member of fascin gene family, actin-bundling proteins. Thus, we named it retinal fascin. To date, members of fascin gene family have been cloned from sea urchin [4], *Drosophila* [22], *Xenopus* [7], mouse [6] and human [5]. They share three conserved regions [1]. The largest region is a 28 residue block near the N-terminus, 15 residues of which are invariant with the remainder being largely conservative substitutions. Retinal fascin has also the same region, in which the 15 conserved amino acids are completely identical. The other two regions are amino acids ETFQLE in the middle of the sequence and GKYW near the C-terminus. In these regions, retinal fascins also showed 50% identity. As a whole, retinal fascin shares 92% of the conserved amino acids in the family, indicating that it is a member of the family. In addition, although amino acid sequences showed 95% identity between human fascin and murine fascin, bovine retinal fascin shares only 57% amino acid identity with human fascin and 55% identity with murine fascin, revealing that retinal fascin is not a bovine homolog of human fascin and murine fascin, but a novel member of fascin gene family.

Mutation analysis of *Drosophila* fascin has shown that substitution glycine 409 with glutamic acid results in partial inactivation of fascin, and that substitution serine 289 with asparagine almost completely inactivates fascin in vivo [23]. These two amino acids are well conserved in the fascin family, and retinal fascin also has both two amino acids, suggesting that these regions are also functionally important in retinal fascin. Yamakita et al. has reported that phosphorylation by protein kinase C activator, TPA, greatly reduced the actin binding ability of human fascin, and discussed the physiological significance of phosphorylation of fascin in microfilament organization and cell motility [24]. They have identified one of the sites of human fascin phosphorylation as Ser-39. The site is well conserved among many fascins, including human, mouse *Xenoups*, and *Drosophila*. Retinal fascin also shares the corresponding Ser-39. In addition, computer analysis showed that retinal fascin may contain many putative phosphorylation sites for protein kinase A, protein kinase C, and tyrosine kinase, suggesting that retinal fascin is also regulated by phosphorylation.

The functions of fascins have been well characterized mainly in invertebrates. Fascins are thought to act as actin-bundling proteins in many structures, such as microspikes and stress fibers of fibroblasts, filopodia of coelomocytes, bristles and nurse cells of Drosophila [1]. Reconstitution experiments has shown that vertebrate and invertebrate fascin-actin bundles have different structures. The echinoid and singed bundles have an 11 nm transverse periodicity which is perpendicular to the long axis of the bundle, while murine bundles contain a transverse repeat every 36 nm which lies at a 60° angle to the bundle axis [1]. These differences in packing are considered to be due to fascin, not actin, raising the possibility that different actin bundles might require their own fascins.

In photoreceptor cells, actin is known to localize in the inner segment [8–12], synaptic region of rods [11–13], the connection between rod inner and outer segments [10,11], the lip of forming rod outer segment discs [10,14–16], and the basal end of the rod outer segment axis [15,17], and seems to be responsible for many functions, such as regulation of outer segment disk morphogenesis [18] and photoreceptor shortening during light or dark adaptation in lower vertebrates [19,20]. For example, in the presence of cytochalasin D, a drug that disrupts actin filaments, new disks are produced, but the new disks are fewer, larger and profoundly disordered in shape [18]. It has been, thus, speculated that actin and actin-associated proteins at the inner segment outer segment junction may be the key regulatory component of disk morphogenesis. Retinal fascin mRNA is exclusively expressed in the photoreceptor cells. Thus, it is quite possible that retinal fascin is also involved in disk morphogenesis.

In addition, although we did not show the existence of retinal fascin homologues in lower vertebrates, it is noteworthy that actin-dependent contraction in Teleost retinal rods depends on the intracellular concentration of cAMP [20]. The author discussed that cAMP might activate rod contraction by phosphorylation of some components of the contractile machinery. As mentioned above, retinal fascin shares two putative protein kinase A-dependent phosphorylation sites. Thus, it is quite possible that retinal fascin homologues in lower vertebrates might be involved in photoreceptor shortening during light or dark adaptation. Further studies using the specific antibody against retinal fascin is needed to know where in the photoreceptor cells it acts, and in which mechanisms it is involved.

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