Cloning and characterization of subtracted cDNAs from a human ciliary body library encoding *TIGR*, a protein involved in juvenile open angle glaucoma with homology to myosin and olfactomedin

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Abstract A group of cDNAs isolated from a subtractive ciliary body library of a normal human eye donor revealed 100% identity with *TIGR* a candidate gene responsible for juvenile open zangle glaucoma [Science 275 (1997) 668–670]. Several structural features of the deduced human protein have been noted: a cleavable N-terminal signal peptide, a periodic repetition at the N-terminus of leucine and arginine residues at every seventh and eleven position respectively in helix conformation (leucine zipper-like motif) exhibiting homology with myosin, and with olfactomedin in the C-terminus. The mRNA for *TIGR* is abundantly expressed in the ciliary body, iris, heart and skeletal muscle.

Key words: Subtracted cDNAs; Human ocular ciliary body; TIGR gene; Myosin; Olfactomedin; mRNA expression

1. Introduction

The first candidate gene associated to Juvenile Open Angle Glaucoma (JOAG) has been recently identified [1]. This gene, called *TIGR* (trabecular meshwork-induced glucocorticoid protein) encodes a novel protein that is inducible in trabecular meshwork cells in culture when challenged with glucocorticoids [2]. Searching for homology in the GenBank database revealed that *TIGR* do not share homology with any known gene, except with four expressed sequence tags (CBS-424; CBS-582; CBS-591 and CBS-670) isolated in our laboratory from a subtracted cDNA library generated from the ocular ciliary body of a pair of eyes of a human donor [3]. These subtracted clones were enriched after subtractive hybridization of single-stranded DNA from a ciliary body library (target), to biotinylated RNA prepared from a library of a cell line used as driver [3]. Although many subtracted clones were identified, many remain unknown since they did not exhibit significant homology to any cDNA sequence when searched in the nucleic acid databases. In order to obtain additional information on the ciliary body genes homologous to *TIGR* we have determined their nucleotide and amino acid sequences, and investigated the main structural features of the protein that they encode. Finally, we researched their tissue distribution by Northern blot analysis and RT-PCR on human ocular and non-ocular tissues and cells in culture.

2. Materials and methods

2.1. Tissues and cell lines

Human eyes were obtained from cadavers within 24 h after enucleation through the National Disease Research Interchange (Philadelphia, PA). Under a dissecting microscope the cornea, iris, lens, ciliary body, ciliary muscle, retinal pigment epithelium (RPE) and retina were microdissected and stored in liquid nitrogen until further analysis. Human cells lines established from cat iris sphincter smooth muscle (SV-CISM-2) [4], human ocular ciliary epithelium [5] and human trabecular meshwork [6] were also used in this work.

2.2. RNA preparation and Northern blot analysis

Total RNA from ocular dissected tissues was prepared by the guanidium isothiocyanate method [7], size-fractionated by electrophoresis on denaturing 1% agarose-formaldehyde gels, and transferred to Nytran filters (Schleicher and Schuell, Keene, NH) as described [3]. A premade Northern blot containing RNA from eight different normal human tissues was purchased from Clontech Lbs, Inc. (Palo Alto, CA). A 1.7-kb DNA insert was generated from clone CBS-670 after digestion with *Eco*RI/*Xho*I restriction enzymes, and labeled with ^{32}P-dCTP (Amersham Corp.) by random priming [3]. Hybridization, and subsequent washing of the filters was carried out at high stringency. Filters were autoradiographed at −70°C with intensifying screens.

2.3. RT-PCR

The PCR method of Saiki et al. 1985 [8] was used to synthesize cDNA in vitro from 5 μg of total RNA using a RT-PCR kit (Stratagene, La Jolla, CA). After the reverse transcriptase (RT), was inactivated at 94°C, an aliquot of the RT reaction was amplified by annealing oligonucleotide primers (at 1 μM), corresponding to the CBS-670 nucleotide sequence (forward: 5′-ACGGGTGCTGTGGTGTA-3′ and reverse: 5′-ATAGCGGTTCTTGAATGGGATGGT-3′), to cDNA in a final volume reaction of 100 μl in MicroAmp tubes, (Perkin Elmer, Emeryville, CA). The reaction was carried out in 10 mM Tris-HCl (pH 9); 50 mM KCl, 0.1% Triton X-100; and 1.5 mM $MgCl_2$ as buffering components; 200 μM (each) deoxynucleoside triphosphates; and 5 U of *Taq* polymerase (Promega, Co). PCR was done in a Perkin Elmer DNA Thermal Cycler 480 (Perkin Elmer Cetus, Norwalk, CT). Each PCR cycle consisted of a denaturation step at 94°C for 1 min, annealing for 1 min at the optimal temperature of 58°C and 1 min of polymerization at 72°C. This cycle was repeated 30 times, the final polymerization step was extended an additional 5 min. The amplified DNA product (441 bp) was resolved on a 1% agarose gel and sequenced directly using the method based on a Sequenase PCR sequencing kit (United States Biochemical, Cleveland, OH).

2.4. Nucleotide sequence of human subtracted ciliary body clones homologous to TIGR

Four human ciliary body subtracted clones, CBS-670, CBS-591, CBS-582, CBS-424 (GenBank accession numbers: R95491; R95447; R95443; and R47209) previously isolated [3] sharing homology to TIGR (GenBank accession number: U85257), were sequenced. The nucleotide sequence of the largest of the above clones CBS-670 (insert of 1.7 kb) was obtained from both strands using fluorescent dideoxynucleotides at the nucleotide sequencing facility at Yale University. Since CBS-670 lacks approximately 110 amino acids of coding region at its 5′end we used RT-PCR combined with direct nucleotide se-

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quence to obtain the 5′-end of this gene. For RT-PCR two sets of oligonucleotide primers were used: forward 5′-GAAGCCTCACCAAGCCTCTGC-3′, and reverse: 5′-TCTCGGGTCTGGGGACACTGG-3′. This set of primers were annealed to cDNA template prepared from human ciliary body total RNA. The conditions of PCR were exactly the same as indicated above with the exception of the annealing temperature that was 60°C.

2.5. Search for homologies

The programs Blast and Fasta were used to search for nucleotide, and protein sequence similarity respectively in GenBank/EMBL and Swiss-Prot databases. The programs Motif, at the World Wide Web Baylor College of Medicine Search Launcher, and Protean from Lasergene (DNASTAR, Wisconsin), were used to search for protein sequence motifs and structural features. Finally, the Bestfit program of the Wisconsin Software Package was used for amino acid alignment.

2.6. Nucleotide sequence accession number

The nucleotide sequence data reported in this work has been submitted to GenBank/EMBL and assigned accession number AF001620.

3. Results and discussion

Four subtracted cDNA clones CBS-670, CBS-591, CBS-582 and CBS-424, (GenBank accession numbers R95491, R95447, R95443, R47209) isolated from a human ciliary body library in λ Uni-ZAP XR [3], have been found to share homology with the *TIGR* gene, a candidate gene for JOAG recently identified in trabecular meshwork (TM) cells [1]. To determine the extend of homology of these ciliary body subtracted clones with *TIGR*, their entire nucleotide sequence was obtained and compared. The four cDNAs overlapped to each other, and their nucleotide sequences exhibited 100% homology to *TIGR*. The largest of the four clones, CBS-670 1.7 kb in length, lacks approximately 360 bp from the initiation codon ATG. We determined the nucleotide sequence of the 5′-end of this clone by RT-PCR combined with direct sequencing. For this purpose two sets of oligonucleotide primers were used, the forward: 5′-GAAGCCTCACCAAGCCTCTGC-3′ corresponding to the 5′-end untranslated region of the *TIGR* gene (nucleotides 27–47) (GenBank accession number U85257) and the reverse: 5′-TCTCGGGTCTGGGGACACTGG-3,′ corresponding to a nucleotide sequence present at the 5′-end of clone CBS-670 (nucleotides 571 to 590). A DNA product of 590 bp was obtained and sequenced. The nucleotide sequences of the RT-PCR 5′-end and of CBS-670 overlapped and were arranged using the multialignment program of the Lasergen program (DNASTAR, Madison, WI). (Fig. 1).

The ciliary body cDNA homologous to the *TIGR* gene contains an open reading frame of 1512 nucleotides, and a 509-nucleotide 3′-untranslated sequence with poly(A)$^+$ tail and three polyadenylation signals, AATAAA, at positions 1736, 1887, and 2026. The open reading frame encodes a polypeptide of 504 amino acids with a M_r of 56976 (Fig. 1). The deduced amino acid sequence of CBS-670 when compared with that of *TIGR* (GenBank accession number U85257) exhibited 100% identity, indicating that the ciliary body CBS-670 and the trabecular meshwork *TIGR* cDNA encoded the same protein.

Searching for structural motifs in the amino acid sequence of CBS-670, using various programs, the following main features were found: (i) At the N-terminus, a hydrobobic region with a signal peptide cleavable at position 32: VGA↓RT. This will result in a putative secretory protein of *M*r 53445. (ii) A leucine rich region ranging from residue 117 to 166 was to contain an α-helical conformation, using both Chou-Fasman and Garnier-Robson methods (Protean program, DNASTAR, Madison, WI). In this region a leucine (L) residue is positioned at every seventh position eight times (Fig. 1). These leucine repeats, are forming an apolar region, and reassembles the leucine zipper motifs in proteins lacking the basic DNA binding region [9]. (iii) There are five arginine (*R*) residues positioned at every 11 residues over a range of 44 amino acids (starting at position 125) within the leucine zipper-like region. When this region of the protein (from residue 123 to 172) was represented as a helical wheel using the Protean program (DNASTAR, Madison, WI), the five arginine residues were found to be positioned on the opposite side of the helix to the leucine repeats, forming a positively charged region (Fig. 2). This type of conformation may suggest that both type of repeats (leucine and arginine) may be involved in protein-protein interactions.

Searching for homology in the protein databases indicated that the protein encoded by CBS-670 or *TIGR* had not apparent homology to any known protein registered in SwissProt database. However, a detailed analysis of this search resulted in two important observations, (i) a 25% identity, at the N-terminus (between residues 72 and 179), with the heavy chain of myosin (GenBank accession number X52889) and (ii) 40% identity, at the C-terminus (between residues 246 and 501), with olfactomedin (GenBank accession number U79299) (Fig. 2). Although the homology with myosin at the N-terminus is relatively low, the homology with olfactomedin at the C-terminus is significant. Olfactomedin has been determined to be a major component of the extracellular matrix of the olfactory neuroepithelium [10]. The identity of the protein encoded by CBS-670 with olfactomedin, made provide a clue on the possible function(s) of this putative secretory protein found to cause JOAG. Interestingly, the different point mutations described in the *TIGR* gene in patients with JOAG [1] are present over a region of 274 nucleotides (from 1060 to 1334) (Fig. 1). This region is within the coding region that shares homology to olfactomedin.

Next, we determined the tissue distribution of CBS-670 by Northern blot analysis in human ocular (Fig. 3A) and non-ocular tissues (Fig. 3B). A blot containing total RNA from ocular tissues of a human eye donor was hybridized with the 1.7-kb cDNA of CBS-670, as indicated in Experimental procedures. A main transcript of about 2.2 kb in size was found to hybridize most abundantly in iris and ciliary body, and in

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Fig. 1. Nucleotide and deduced protein sequence of the human ciliary body subtracted clone CBS-670, homologous to *TIGR*. Nucleotides are numbered on the left; amino acids (single-letter code) are numbered on the right, starting at the initiating methionine as +1. The deduced amino acid sequence is given under the open reading frame. Three polyadenylation signals are underlined. Set of oligonucleotides used to determine the 5′-end of CBS-670 by RT-PCR is underlined by double lines. Leucine (L) residues (total of eight) at every seventh position in an alpha-helix conformation (leucine zipper-like motif) are highlighted in shadow and single underlined. Arginines positioned at 11 positions are double underlined. Vertical arrow at residue 32, indicates the predicted signal peptide cleavage site (VGA↓R). Underlined are also the codons with the nucleotide in bold, where a point mutation have been determined in JOAG. Start indicates stop codon TGA.

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1    GAAGCCTCACCAAGCCTCTGCAATGAGGTTCTTCTGTGCACGTTGCTGCAGCTTTGGGCC
                          M  R  F  F  C  A  R  C  C  S  F  G  P    13
61   TGAGATGCCAGCTGTCCAGCTGCTGCTTCTGGCCTGCCTGGTGTGGGATGTGGGGGCCAG
      E  M  P  A  V  Q  L  L  L  L  A  C  L  V  W  D  V  G  A↓R    33
121  GACAGCTCAGCTCAGGAAGGCCAATGACCAGAGTGGCCGATGCCAGTATACCTTCAGTGT
      T  A  Q  L  R  K  A  N  D  Q  S  G  R  C  Q  Y  T  F  S  V    53
181  GGCCAGTCCCAATGAATCCAGCTGCCCAGAGCAGAGCCAGGCCATGTCAGTCATCCATAA
      A  S  P  N  E  S  S  C  P  E  Q  S  Q  A  M  S  V  I  H  N    73
241  CTTACAGAGAGACAGCAGCACCCAACGCTTAGACCTGGAGGCCACCAAAGCTCGACTCAG
      L  Q  R  D  S  S  T  Q  R  L  D  L  E  A  T  K  A  R  L  S    93
301  CTCCCTGGAGAGCCTCCTCCACCAATTGACCTTGGACCAGGCTGCCAGGCCCCAGGAGAC
      S  L  E  S  L  L  H  Q  L  T  L  D  Q  A  A  R  P  Q  E  T   113
361  CCAGGAGGGGCTGCAGAGGGAGCTGGGCACCCTGAGGCGGGAGCGGGACCAGCTGGAAAC
      Q  E  G  N  Q  R  E  L  G  T  N  R  R  E  R  D  Q  N  E  T   133
421  CCAAACCAGAGAGTTGGAGACTGCCTACAGCAACCTCCTCCGAGACAAGTCAGTTCTGGA
      Q  T  R  E  N  E  T  A  Y  S  N  N  L  R  D  K  S  V  N  E   153
481  GGAAGAGAAGAAGCGACTAAGGCAAGAAAATGAGAATCTGGCCAGGAGGTTGGAAAGCAG
      E  E  K  K  R  N  R  Q  E  N  E  N  N  A  R  R  L  E  S  S   173
541  CAGCCAGGAGGTAGCAAGGCTGAGAAGGGGCCAGTGTCCCCAGACCCGAGACACTGCTCG
      S  Q  E  V  A  R  L  R  R  G  Q  C  P  Q  T  R  D  T  A  R   193
601  GGCTGTGCCACCAGGCTCCAGAGAAGTTTCTACGTGGAATTTGGACACTTTGGCCTTCCA
      A  V  P  P  G  S  R  E  V  S  T  W  N  L  D  T  L  A  F  Q   213
661  GGAACTGAAGTCCGAGCTAACTGAAGTTCCTGCTTCCCGAATTTTGAAGGAGAGCCCATC
      E  L  K  S  E  L  T  E  V  P  A  S  R  I  L  K  E  S  P  S   233
721  TGGCTATCTCAGGAGTGGAGAGGGAGACACCGGATGTGGAGAACTAGTTTGGGTAGGAGA
      G  Y  L  R  S  G  E  G  D  T  G  C  G  E  L  V  W  V  G  E   253
781  GCCTCTCACGCTGAGAACAGCAGAAACAATTACTGGCAAGTATGGTGTGTGGATGCGAGA
      P  L  T  L  R  T  A  E  T  I  T  G  K  Y  G  V  W  M  R  D   273
841  CCCCAAGCCCACCTACCCCTACACCCAGGAGACCACGTGGAGAATCGACACAGTTGGCAC
      P  K  P  T  Y  P  Y  T  Q  E  T  T  W  R  I  D  T  V  G  T   293
901  GGATGTCCGCCAGGTTTTTGAGTATGACCTCATCAGCCAGTTTATGCAGGGCTACCCTTC
      D  V  R  Q  V  F  E  Y  D  L  I  S  Q  F  M  Q  G  Y  P  S   313
961  TAAGGTTCACATACTGCCTAGGCCACTGGAAAGCACGGGTGCTGTGGTGTACTCGGGGAG
      K  V  H  I  L  P  R  P  L  E  S  T  G  A  V  V  Y  S  G  S   333
1021 CCTCTATTTCCAGGGCGCTGAGTCCAGAACTGTCATAAGATATGAGCTGAATACCGAGAC
      L  Y  F  Q  G  A  E  S  R  T  V  I  R  Y  E  L  N  T  E  T   353
1081 AGTGAAGGCTGAGAAGGAAATCCCTGGAGCTGGCTACCACGGACCAGTTCCCGTATTCTTG
      V  K  A  E  K  E  I  P  G  A  G  Y  H  G  Q  F  P  Y  S  W   373
1141 GGGTGGCTACACGGACATTGACTTGGCTGTGGATGAAGCAGGCCTCTGGGTCATTTACAG
      G  G  Y  T  D  I  D  L  A  V  D  E  A  G  L  W  V  I  Y  S   393
1201 CACCGATGAGGCCAAAGGTGCCATTGTCCTCTCCAAACTGAACCCAGAGAATCTGGAACT
      T  D  E  A  K  G  A  I  V  L  S  K  L  N  P  E  N  L  E  L   413
1261 CGAACAAACCTGGGAGACAAACATCCGTAAGCAGTCAGTCGCCAATGCCTTCATCATCTG
      E  Q  T  W  E  T  N  I  R  K  Q  S  V  A  N  A  F  I  I  C   433
1321 TGGCACCTTGTACACCGTCAGCAGCTACACCTCAGCAGATGCTACCGTCAACTTTGCTTA
      G  T  L  Y  T  V  S  S  Y  T  S  A  D  A  T  V  N  F  A  Y   453
1381 TGACACAGGCACAGGTATCAGCAAGACCCTGACCATCCCATTCAAGAACCGCTATAAGTA
      D  T  G  T  G  I  S  K  T  L  T  I  P  F  K  N  R  Y  K  Y   473
1441 CAGCAGCATGATTGACTACAACCCCCTGGAGAAGAAGCTCTTTGCCTGGGACAACTTGAA
      S  S  M  I  D  Y  N  P  L  E  K  K  L  F  A  W  D  N  L  N   493
1501 CATGGTCACTTATGACATCAAGCTCTCCAAGATGTGAAAAGCCTCCAAGCTGTACAGGCA
      M  V  T  Y  D  I  K  L  S  K  M  *                          504
1561 ATGGCAGAAGGAGATGCTCAGGGCTCCTGGGGGGAGCAGGCTGAAGGGAGAGCCAGCCAG
1621 CCAGGGCCCAGGCAGCTTTGACTGCTTTCCAAGTTTTCATTAATCCAGAAGGATGAACAT
1681 GGTCACCATCTAACTATTCAGGAATTGTAGTCTGAGGGCGTAGACAATTTCATATAATAA
1741 ATATCCTTTATCTTCTGTCAGCATTTATGGGATGTTTAATGACATAGTTCAAGTTTTCTT
1801 GTGATTTGGGGCAAAAGCTGTAAGGCATAATAGTTTCTTCCTGAAAACCATTGCTCTTGC
1861 ATGTTACATGGTTACCACAAGCCACAATAAAAAGCATAACTTCTAAAGGAAGCAGAATAG
1921 CTCCTCTGGCCAGCATCGAATATAAGTAAGATGCATTTACTACAGTTGGCTTCTAATGCT
1981 TCAGATAGAATACAGTTGGGTCTCACATAACCCTTTACATTGTGAAATAAAATTTTCTTA
2041 CCCAAAAAAAAAAAAAAAAA
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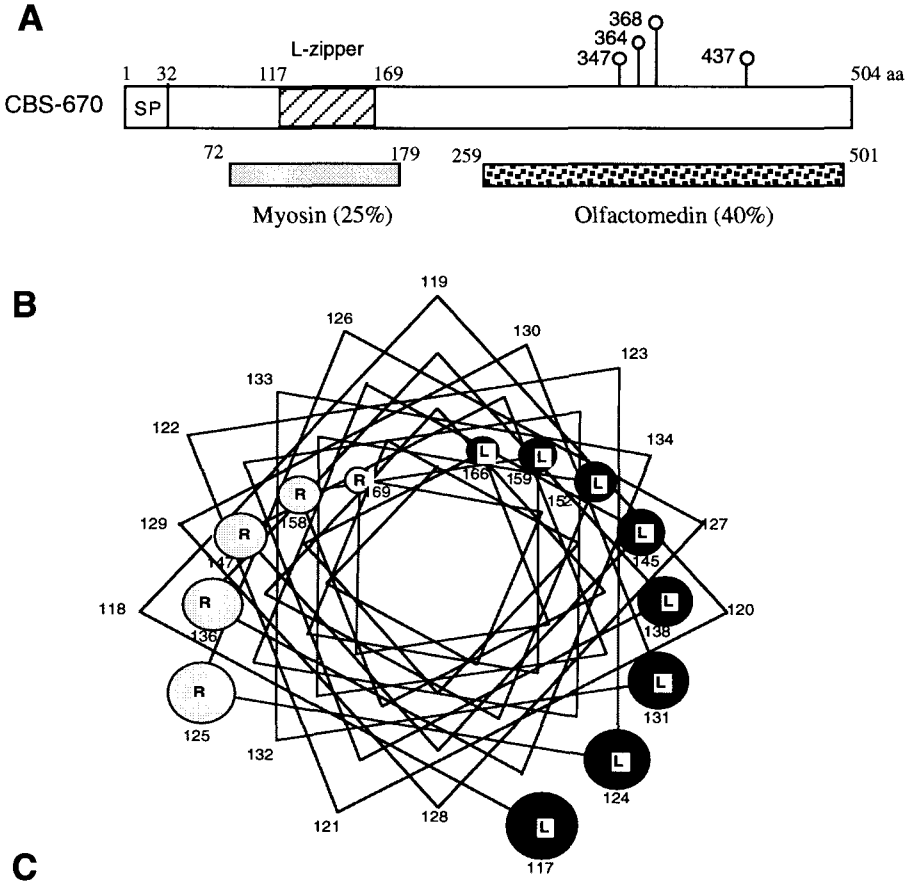


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                 * .*   ..* ** **    *       * .*    . * * **  .  **        * *
HU-OLFT  11      TVKTSGSRFGSWMTDPLA--PEGDNRVWYMDGYHNN-RFVREYKSMVDFMNTDNFTSHRL   67
CBS-670  259     TAETITGKYGVWMRDPKPTYPYTQETTWRIDTVGTDVRQVFEYDLISQFMQGYPSKVHIL  318

                 * *   ** ***.**.**   .*  .**..* ***.    . .   ***    . *.***..*
HU-OLFT  68      PHPWSGTGQVVYNGSIYFNKFQSHIIIRFDLKTETILKTRSLDYAGYNNMYHYAWGGHSD  127
CBS-670  319     PRPLESTGAVVYSGSLYFQGAESRTVIRYELNTETVKAEKEIPGAGYHGQQFPYSWGGYTD  378

                 *** ***.*** .*.*..  * **.*.*.* .*.  *** *.  *.*   *********
HU-OLFT  128     IDLMVDESGLWAVYATNQNAGNIVVSRLDPVSLQTLQTWNTSYPKRSAGEAFIICGTLYV  187
CBS-670  379     IDLAVDEAGLWVIYSTDEAKGAIVLSKLNPENLELEQTWETNIRKQSVANAFIICGTLYT  438

                 . * *    * .** *      . . ***.*.* . **.**** .. *.**.*    . *.
HU-OLFT  188     TNGY-SGGTKVHYAYQTNASTYEYIDIPFQNKYSHISMLDYNPKDRALYAWNNGHQILYN  246
CBS-670  439     VSSYTSADATVNFAYDTGTGISKTLTIPFKNRYKYSSMIDYNPLEKKLFAWDNLNMVTYD  498

                 . *
HU-OLFT  247     VTL
CBS-670  499     IKL
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Fig. 2. (A) Schematic diagram of the open reading frame of CBS-670 (*TIGR*), with structural features and homologous regions to Myosin and Olfactomedin. The signal peptide (sp) and the leucine zipper-like motif are indicated. The position of amino acid residues indicated are approximately positioned relative to the main features described in the text. Mutated residues found in JOAG gene [1] are indicated by pin marks. (B) Helical configuration of the leucine zipper-like motif and arginine rich motif between residues 117 and 169 of the coding region of CBS-670. The Protean program (DNASTAR, Madison, WI) was used. Only leucine (L) and arginine (R) residues are highlighted. (C) Comparison of amino acid sequences of CBS-670 and Olfactomedin (HU-OLFT). Amino acids are indicated by single-letter code, and the numbers for the numbers for the amino acid residues are shown on both sides. Identical residues between sequences are indicated with asterisks and amino acid substitutions with a single dot. Dashes indicate gaps introduced into the alignment to allow best fit. Residues outlined and underlined in CBS-670 sequence are the corresponding amino acids where mutations have been detected in the *TIGR* gene with JOAG [1].

low or undetectable levels in cornea, lens, retinal pigment epithelium and retina. Interestingly, when the 1.7-kb probe from CBS-670 was hybridized to a Northern containing poly A^+ RNA from non-ocular tissues, it hybridized also to a 2.2-kb transcript preferentially to heart and skeletal muscle RNA, and undetectable in brain, placenta, lung, liver, kidney and pancreas. These results strongly suggested that CBS-670 is restricted preferentially to muscle in ocular and non-ocular tissues. In this regard, the sphincter muscle and the dilator muscle of the iris, as well as the ciliary muscle of the ciliary body are smooth muscle, and they are richly innervated by sympathetic and parasympathetic fibers [11]. It is known that iris muscles, for example, contract (miosis) or dilate (mydriasis) in response to adrenergic or cholinergic agonists. The

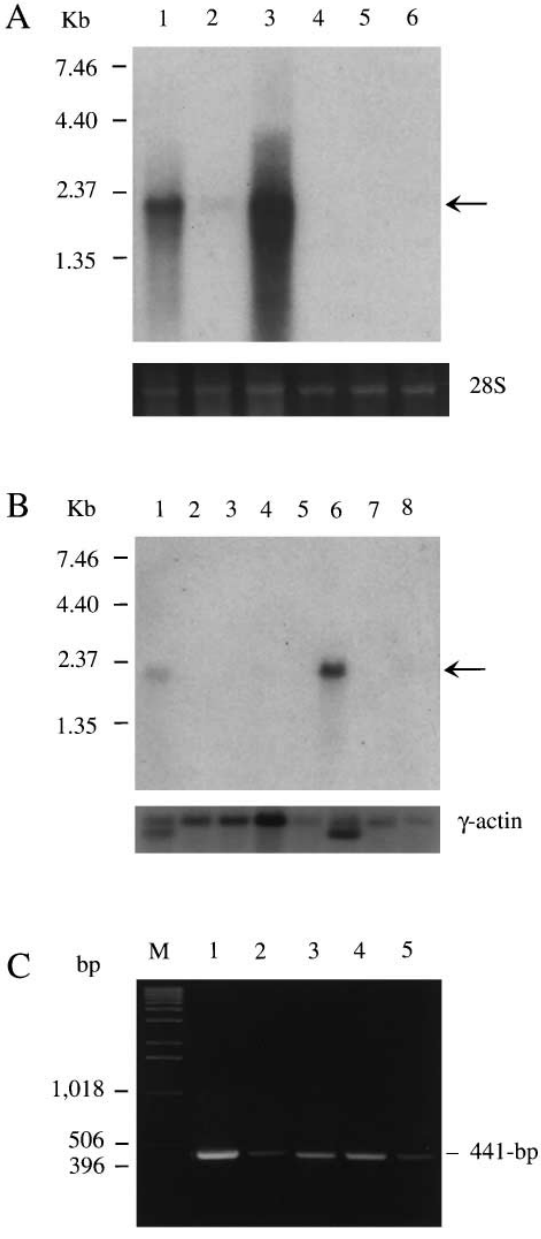

Fig. 3. Expression of the CBS-670 gene in human tissues and cells in culture. Northern blot analysis in ocular (A) and non-ocular (B) tissues. In (A), total RNA (20 μg/lane) from: ciliary body (lane 1); cornea (lane 2); iris (lane 3); lens (lane 4); retinal pigment epithelium (lane 5), and retina (lane 6), were hybridized in a blot with a 1.7-kb DNA probe of CBS-670. In (B), a premade blot (Clontech Labs., CA) containing poly A^+ mRNA (2 μg/lane) from: heart (lane 1); brain (lane 2); placenta (lane 3); lung (lane 4); liver (lane 5); skeletal muscle (lane 6); kidney (lane 7); and pancreas (lane 8), was hybridized with same DNA probe as in A. In both, A and B, arrow at right indicates the position of the main transcript (2.2 kb) hybridized. RNA molecular weight markers (kb), ribosomal 28S or γ-actin are also indicated. In (C), detection of CBS-670 expression, by RT-PCR, in human cultured cells: ciliary muscle (lane 1); trabecular meshwork (lane 2); non-pigmented ciliary epithelial cell line (lane 3); transformed trabecular meshwork cell line (lane 4); and transformed iris sphincter smooth muscle cell line (lane 5). cDNA templates were synthesized in vitro from RNA prepared from above cells, amplified and resolved on 1% agarose gel as described in experimental procedures.

ciliary muscle of the ciliary body can also contract or relax in response to cholinergic or adrenergic activation.

Since *TIGR* was originally identified in cultured TM cells, we determined whether its ciliary body homologue, CBS-670, was also expressed in cultured TM cells. For this purpose, we performed RT-PCR, since not detectable levels of expression were obtained by conventional Northern blot analysis (data not shown). Total RNA from primary cultures (TM) or established cell lines (ciliary smooth muscle, iris sphincter, TM and ciliary epithelial) was prepared, cDNA synthesized in vitro, and annealed to a set of oligonucleotide primers corresponding to the CBS-670 nucleotide sequence, and amplified as described in experimental procedures. The RT-PCR product (441 bp) was resolved in an agarose gel and directly sequenced (Fig. 3C). This result indicates that CBS-670, homologous to *TIGR*, is also expressed in cultured TM cells, muscle or ciliary epithelial cells of the human eye. The possibility that TIGR gene might be restricted to cells in the anterior segment of the eye with contractile properties is supported by the observation that these cells contract in vitro in response to cholinergic or adrenergic agonists [12,13].

This work establishes a basis to further investigate the protein to protein interaction that is predicted in the novel putative secretory protein found to cause JOAG.

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