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The sequence of human caveolin reveals identity with VIP21, a component of transport vesicles

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Caveolin is a protein present in membrane specializations termed caveolae where it may play a structural role. Previous studies on the cDNA sequence of chicken caveolin demonstrated that it is an integral membrane protein without significant homology to any known protein. The cDNA sequence of human lung caveolin is presented here. A striking sequence homology is observed with the chicken protein, as well as a very recently reported protein termed VIP21 [(1992) J. Cell Biol. 118, 1003-1014], a putative vesicle transport protein isolated from MDCK cells. Genomic Southern blots suggest that caveolin is present in a single copy, and Northern blots confirm that the caveolin mRNA is elevated in muscle.

Membrane transport; Tyrosine kinase; Oncogene; DNA sequence

1. INTRODUCTION

Caveolin is a 22 kDa protein first identified with monoclonal antibodies to tyrosine-phosphorylated proteins derived from Rous sarcoma virus-transformed chick embryo fibroblasts [1,2]. The function of caveolin has been elusive and the significance of its tyrosine phosphorylation is unknown.

A possible role for caveolin comes from studies of its subcellular distribution. Initial immunofluorescence microscopy revealed a punctate staining pattern underlying the plasma membrane in many instances concentrated in large patches and at the margins of cells [2]. More recent studies have shown that this represents the staining of caveolae, small, non-clathrin-coated invaginations of the plasma membrane, hence the protein was termed caveolin [3]. Caveolae structures are abundant in endothelium and muscle but may be present in all cells [4–6]. Caveolin is known to be particularly abundant in muscle.

We have recently reported the sequence of chicken caveolin based on PCR products and genomic clones [7]. The predicted cDNA sequence perfectly matched the sequence of peptides generated from gizzard caveolin. A unique integral membrane protein was predicted with an extensive hydrophobic region near the C-terminus. We have now used the chicken clones to screen a human cDNA library. The sequence of one of these clones is reported here. Human caveolin, like the chicken protein, is predicted to be 178 amino acids in length, having extensive sequence homology with both chicken caveolin and a recently reported protein, termed VIP21, and thought to be involved in membrane transport [8].

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2. MATERIALS AND METHODS

A human lung cDNA 5′-stretch library in λGT11 was purchased from Clontech and plated on Y1090 *E. coli* cells. Approximately 5×10^5 plaques were screened with a 500 bp fragment from the chicken caveolin cDNA [7] and labeled with ^{32}P using random DNA primers (USB). Phage DNA was adsorbed to nylon membranes in duplicate and hybridization was performed at 37°C by standard methods using 40% formamide. After hybridization, filters were washed 1 h with 1 × SSC with 0.5% SDS and exposed to film for 6 h at −70°C. Three positives were detected and re-cloned two additional times. Phage DNA was prepared and subjected to Southern blotting after digestion with *Eco*RI. Two of the DNA inserts were then amplified by PCR with λ sequences outside the RI sites and sequenced by the cycle sequencing method (BRL). Both strands were sequenced first with λ primers then with primers unique to the inserts. The full sequence of one (termed λC) is reported here.

For genomic Southern blots, human DNA was purified from human fibroblasts by standard methods and 50 μg aliquots were digested with the specified restriction enzymes. DNA was resolved by agarose gel electrophoresis, transferred to nylon membrane and probed with the λC (described above) DNA at 42°C in 50% formamide. Filters were washed first with 2 × SSC, 1% SDS then with 0.1 × SSC, 1% SDS. A Northern blot with human poly(A)$^+$ RNA from a variety of tissues was purchased from Clontech and probed as above.

3. RESULTS

A 500 bp cDNA probe encoding chicken caveolin was used to screen a human lung library. Caveolin is known to be expressed at high levels in lung [1]. Three caveolin clones were detected out of 5×10^5 phage tested and the

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1      GGA GTT TTC ATC CAG CCA CGG GCC AGC ATG TCT GGG GGC AAA TAC GTA GAC TCG GAG GGA
                                          Met Ser Gly Gly Lys Tyr Val Asp Ser Glu Gly

61     CAT CTC TAC ACC GTT CCC ATC CGG GAA CAG GGC AAC ATC TAC AAG CCC AAC AAC AAG GCC
       His Leu Tyr Thr Val Pro Ile Arg Glu Gln Gly Asn Ile Tyr Lys Pro Asn Asn Lys Ala

121    ATG GCA GAC GAG CTG AGC GAG AAG CAA GTG TAC GAC GCG CAC ACC AAG GAG ATC GAC CTG
       Met Ala Asp Glu Leu Ser Glu Lys Gln Val Tyr Asp Ala His Thr Lys Glu Ile Asp Leu

181    GTC AAC CGC GAC CCT AAA CAC CTC AAC GAT GAC GTG GTC AAG ATT GAC TTT GAA GAT GTG
       Val Asn Arg Asp Pro Lys His Leu Asn Asp Asp Val Val Lys Ile Asp Phe Glu Asp Val

241    ATT GCA GAA CCA GAA GGG ACA CAC AGT TTT CAC GGC ATT TGG AAG GCC AGC TTC ACC ACC
       Ile Ala Glu Pro Glu Gly Thr His Ser Phe His Gly Ile Trp Lys Ala Ser Phe Thr Thr

301    TTC ACT GTG ACG AAA TAC TGG TTT TAC CGC TTG CTG TCT GCC CTC TTT GGC ATC CCG ATG
       Phe Thr Val Thr Lys Tyr Trp Phe Tyr Arg Leu Leu Ser Ala Leu Phe Gly Ile Pro Met

361    GCA CTC ATC TGG GGC ATT TAC TTC GCC ATT CTC TCT TTC CTG CAC ATC TGG GCA GTT GTA
       Ala Leu Ile Trp Gly Ile Tyr Phe Ala Ile Leu Ser Phe Leu His Ile Trp Ala Val Val

421    CCA TGC ATT AAG AGC TTC CTG ATT GAG ATT CAG TGC ACC AGC CGT GTC TAT TCC ATC TAC
       Pro Cys Ile Lys Ser Phe Leu Ile Glu Ile Gln Cys Thr Ser Arg Val Tyr Ser Ile Tyr

481    GTC CAC ACC GTC TGT GAC CCA CTC TTT GAA GCT GTT GGG AAA ATA TTC AGC AAT GTC CGC
       Val His Thr Val Cys Asp Pro Leu Phe Glu Ala Val Gly Lys Ile Phe Ser Asn Val Arg

541    ATC AAC TTG CAG AAA GAA ATA TAA ATG ACA TTT CAA GGA TAG AAG TAT ACC TGA TTT TTT
       Ile Asn Leu Gln Lys Glu Ile ***

601    TTC CTT TTA ATT TTC CTG GTG CCA ATT TCA AGT TCC AAG TTG CTA ATA CAG CAA CGA ATT

661    TAT GAA TTG AAT TAT CTT GGT TGA AAA TAA AAA GAT CAC TTT CTC AGT TTT CAT AAG TAT

721    TAT GTC TCT TCT GAG CTA TTT CAT CTA TTT TTG GCA GTC TGA ATT TTT AAA ACC CAT TTA

781    TAT TTC TTT CCT TAC CTT TTT ATT TGC ATG TGG ATC AAC CAT CGC TTT ATT
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Fig. 1. cDNA and deduced protein sequence of human caveolin. A chicken caveolin probe was used to screen a λGT11 library derived from human lung. Several positives were found and the DNA sequence of one is presented.

sequence of one comprising 831 bp is presented here. Southern blotting with the radiolabeled chicken caveolin probe demonstrated that the insert DNA from all three λ clones hybridized very strongly (not shown). The cDNA clone contained a start codon at position 28 and a polyadenylation signal, AATAAA (Fig. 1), at nucleotide 686. A poly(A) tail, however, was not found in this clone. As with the chicken caveolin, an open reading frame encoding a protein with 178 amino acids is observed (Fig. 1).

The sequence of human caveolin shows that it is closely related to chicken caveolin (Fig. 2) both at the nucleic acid (not shown) and protein level (86% amino acid sequence identity). Most of the differences between human and chicken caveolins were in the amino- and carboxyl-ends of the protein with a long central stretch (47–136) containing only a single amino acid difference. Recently the sequence of a 21 kDa vesicle protein from MDCK cells (termed VIP21), thought to be involved in membrane trafficking, was reported [8]. When the se-

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          1        10        20        30        40        50        60
HUMAN     MSGGKYVDSEGHLYTVPIREQGNIYKPNNKAMADELSEKQVYDAHTKEIDLVNRDPKHLND
CHICK     ---T-------F--AA-V-----------M--------A-H-VD----------------
VIP21     ------------------------------E-M-----------------------------

                  70        80        90       100       110       120
HUMAN     DVVKIDFEDVIAEPEGTHSFHGIWKASFTTFTVTKYWFYRLLSALFGIPMALIWGIYFAIL
CHICK     --------------------D-------------------------------------------
VIP21     --------------------D-------------------------------------------

                130       140       150       160       170
HUMAN     SFLHIWAVVPCIKSFLIEIQCTSRVYSIYVHTVCDPLFEAVGKIFSNVRINLQKEI
CHICK     ------------R-Y------I------CI--F-------M--V--SI-ATVR---
VIP21     --------------------I--------------F----------I---M---T
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Fig. 2. Comparison of the amino acid sequences of human and chicken caveolin to the recently described VIP21. The sequence of the human protein is displayed on the top line represented by the single letter code. The chick protein [7] and VIP21 [8] are indicated on the second and third lines where a (–) represents identity with human caveolin and a letter indicates a change to that amino acid.

quence of VIP21 was compared to caveolin it was clearly very closely related. Only 8 differences (out of 178 amino acids) were observed. These sequence differences probably reflect species variability, since VIP was cloned from MDCK (canine) cells compared to the human caveolin protein presented here. It would appear, then, that VIP21 is the canine equivalent of caveolin.

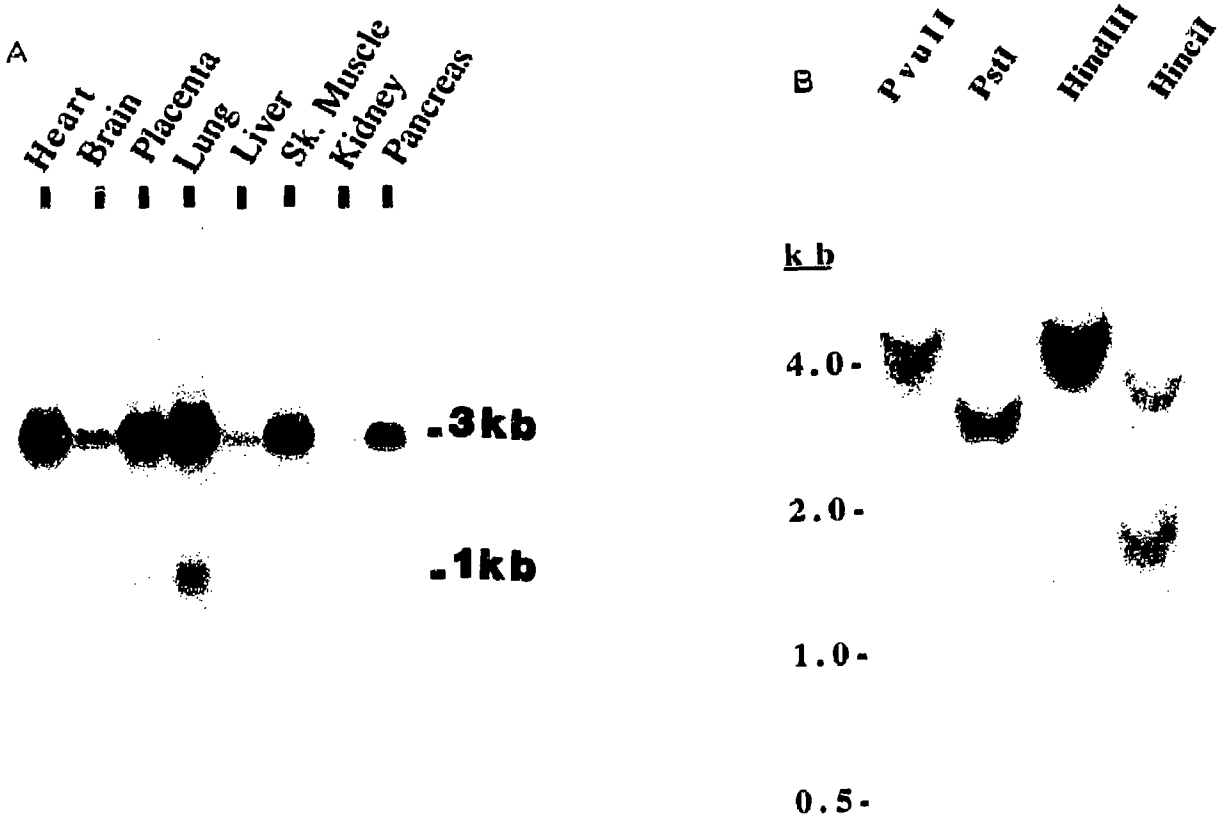

Fig. 3. (A) Distribution of the RNA encoding caveolin in various human tissues. Equal amounts of poly(A)$^+$ RNA derived from the tissues indicated were analyzed by Northern hybridization using the human cDNA probe indicated in Fig. 1. (B) Southern blot of total human genomic DNA cut with the indicated restriction enzymes.

Previous studies using monoclonal antibodies to chicken caveolin have shown that it is expressed at high levels in muscle and lung tissue but is relatively low in liver, brain and kidney [1]. Consistent with this distribution, we find a major 3 kb and a minor 1 kb message abundantly expressed in cardiac and skeletal muscle by Northern blot analysis, but relatively low levels are seen in brain, kidney and liver. The relationship between the two transcripts is currently under investigation.

A single gene encoding caveolin is clearly indicated by Southern blotting (Fig. 3). Both *Hin*dIII and *Pvu*II restriction enzymes gave rise to a single band in Southern blots while *Hin*cII and *Pst*I resulted in 2 bands. The human caveolin cDNA contains an *Hin*cII site at position 183.

4. DISCUSSION

Our previous attempt to clone the cDNA from a cDNA library derived from chicken cells was unsuccessful, thus we resorted to a combination of PCR and genomic DNA cloning [7]. The cloning of human caveolin cDNA from a commercially available cDNA library, by contrast, was straightforward. The resulting clone is clearly the human homolog of chicken caveolin as indicated by the identical length and high degree of sequence identity. Southern blotting indicates that only a single human gene is present and Northern blots result in the same tissue distribution as found previously for chicken caveolin detected with an antibody.

The function of caveolin has not been determined with any degree of certainty. While localization studies assign caveolin to the inner aspect of caveolae membrane invaginations [3] it cannot be concluded that it is the only structural element of these specializations. The assignment of VIP21 [8] as caveolin now allows us to consider other biological roles. VIP21 was found associated with an apical marker, haemagglutinin, in the polarized epithelial cells, MDCK. The authors suggested that VIP21 may be involved in polarized membrane transport. Two lines of evidence, however, would argue against this interpretation. First, while in the most recent study VIP21 was found in association with an apical marker, other studies have noted the same protein in basolateral membranes [9]. Secondly, one would expect the polarized epithelium of the intestine and kidney to contain an elevated level of caveolin if it is required for apical transport, yet only very low levels of caveolin (VIP21) have been found in these tissues in vivo. Thus the MDCK model may not be reflective of the role of caveolin in other situations, such as muscle where it is abundantly expressed [1]. Furthermore it is unclear whether caveolin is a true component of transport vesicles or is a resident plasma membrane protein that can be detected on its way to the membrane [8]. Insolubility of caveolin in the detergent, CHAPS [8], may reflect its disposition in caveolae with other GPI-linked proteins [10,11].

The identification of caveolin as VIP21 and the cloning of human caveolin should now allow rapid progress in the elucidation of the structure and function of caveolae and the dynamics of this membrane compartment.

REFERENCES

[1] Glenney, J.R. (1989) J. Biol. Chem. 264, 20163–20166.
[2] Glenney, J.R. and Zokas, L. (1988) J. Cell Biol. 108, 2401–2408.
[3] Rothberg, K.G., Heuser, J.E., Donzell, W.C., Ying, Y.-S., Glenney, J.R. and Anderson, R.G.W. (1992) Cell 68, 673–682.
[4] Anderson, R.G.W., Kamen, B.A., Rothberg, K.G. and Lacey, S.W. (1992) Science 255, 410–411.
[5] Montesano, R., Roth, J., Robert, A. and Orci, L. (1982) Nature 296, 651–653.
[6] Severs, N.J. (1988) J. Cell. Sci. 90, 341–348.
[7] Glenney, J.R. and Soppet, D. (1992) Proc. Natl. Acad. Sci. USA (in press).
[8] Kurzchalia, T.V., Dupress, P., Parton, R.G., Kellner, R., Virta, H., Lehnert, M. and Simons, K. (1992) J. Cell Biol. 118, 1003–1014.
[9] Wandinger-Ness, A., Bennett, M.K., Antony, C. and Simons, K. (1990) J. Cell Biol. 111, 987–1000.
[10] Brown, D.A. and Rose, J.K. (1992) Cell 68, 533–544.
[11] Lacey, S.W., Sanders, J.M., Rothberg, K.G. and Anderson, R.G.W. (1989) J. Clin. Invest. 84, 715–720.