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Identification of a major protein on the cytosolic face of caveolae

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Abstract

Cav-p60, a specific and ubiquitous caveolar protein, was immunoprecipitated from solubilized rat adipocyte plasma membranes and identified as similar to a GeneBank entry annotated mouse polymerase transcript release factor (PTRF) by MALDI-TOF and MS-MS of major fragments. Cloning and virtual translation of the corresponding rat adipocyte cDNA sequence revealed 98.7% identity with mouse PTRF. In vitro translation of this sequence produced a protein, which was recognized by antibodies to both cav-p60 and PTRF. EM gold labeling studies showed that a rabbit antiserum against murine PTRF immunolabeled caveolae specifically in adipocytes from both mouse and rat. In view of the reported function of the protein, which is exerted in the cell nucleus, its subcellular localization was investigated. We found that the protein could be purified by differential solubilization of a plasma membrane fraction followed by SDS-PAGE, and that the protein was as abundant as caveolin in this fraction. We were unable to detect the protein in cell nuclei by subcellular fractionation or fluorescence microscopy. The results show that in a large number of cell types, PTRF is essentially located to caveolae, and that each caveola harbors many copies of the protein. Consequently, we suggest the name Cavin for this protein.

Keywords: Rat adipocyte; Caveolin; cav-p60; Electron microscopy; Polymerase and Transcript Release Factor; Mass spectrometry

1. Introduction

The elusive biological significance of plasmalemmal caveolae has been the inspiration for many recent studies. Unfortunately, the intriguing morphology and distinct tissue distribution of caveolae have given no immediate indication of their function. The studies have therefore focused on establishing their specific content of proteins and lipids. In an early stage of this line of work, the caveolin proteins were discovered as resident in caveolae and obligatory for their presence [1–4]. Multiple information on the association of caveolins with signaling proteins derives from the study of detergent insoluble membrane fractions (rafts), but the mere presence of proteins in this fraction does not prove that the proteins interact in caveolae in the living cell [5]. Other approaches are based on schemes of fractionation of cell homogenates along with electron microscopic assessment of subcellular localization [6,7]. Results solely based on cell fractionation suffer from the inborn uncertainty of whether a preparation is purely caveolar or in addition contains adjacent membrane domains. At present, the most direct evidence for the localization of a membrane protein to caveolae or to the surrounding part of the membrane is obtained by immunoelectron microscopy on the intact plasma membrane [7,8]. With this direct approach, we have recently demonstrated the presence of a previously undetected protein, cav-p60, on the cytosolic face of caveolae utilizing a monoclonal antibody (2F11) as reported [9]. In continuation, we have shown that cav-p60 immunoreactivity is a general caveolar marker in a broad range of cell types, and that cav-p60's localization in caveolae is independent of the prevailing caveolin isoform [10]. The present work describes the identification of cav-p60 by immunopurification, peptide sequencing, cDNA cloning and in vitro translation. Surprisingly, cav-p60 was identical to the previously described *polymerase 1* and *transcript release factor* (PTRF) protein [11] which has also been named BBP for

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BFCOL1 (binding factor of a type-1 collagen promoter) *binding protein* [12]. The identity of cav-p60 and PTRF/BBP was further ascertained by comparison of immunomicroscopical labeling patterns of 2F11 and a polyclonal antibody to PTRF in a variety of rat tissues.

Our results are in line with the recent finding that PTRF resides on the trypsin accessible face of a vesicular adipocyte membrane preparation enriched in caveolin [13]. PTRF was further shown to co-localize with caveolin in human adipocyte plasma membranes [13] utilizing confocal microscopy on a membrane preparation we developed for the study of the membrane localization of cav-p60 by electron microscopy [9]. Since the designation cav-p60 now appears misleading for a protein made up of 392 amino acid residues, we will use the term *cavin* for cav-p60, PTRF or BBP in the following to emphasize its specific localization.

2. Materials and methods

2.1. Immunopurification of cav-p60/cavin

Fat cells were isolated from epididymal fat pads of male Wistar rats by collagenase digestion as previously described [8]. Fat cells were homogenized in the presence of phenylmethylsulphonylfluoride (PMSF) and a plasma membrane-enriched fraction was prepared by differential and gradient ultracentrifugation according to [14]. The pelleted plasma membrane fraction (approximately 200 μl) was solubilized in 1 ml of Tris-buffered saline (TBS), pH 7.4 containing 1% w/v sodium dodecyl sulphate at room temperature and centrifuged at 100,000×*g* for 1 h. The supernatant was diluted to 10 ml with TBS containing octylglycoside 80 mmol/l, and loaded slowly (20–25 min) on a 1-ml HiTrap protein G column (Amersham Pharmacia Biotech) preloaded with 100 μg of the mouse monoclonal antibody 2F11. Upon washing with buffer containing octylglycoside 75 mmol/l, antibody and complexes with antigen were eluted with TBS containing 2% SDS and collected in 400 μl fractions. Samples of load, run-through (including chase with 5 ml of washing buffer) and SDS-eluate fractions were run on non-reducing 10% polyacrylamide gels in the presence of SDS. The load sample was diluted 1.5-fold with washing buffer to allow comparison with the chased run through fraction. Parallel gels, one with application of 10 μl samples for immunoblotting with 2F11 and one with application of 100 μl samples for staining with colloidal Coomassie blue from Pierce, were run. Upon identification by means of the parallel immunoblot, labeled with 2F11 and developed in the ECL system from Amersham, protein bands in lanes with high protein load were excised and saved. The remains of this gel was immunoblotted in order to verify that the excised band corresponded to the immunoreactive protein (not shown). The gel piece was digested with trypsin and the released peptides were analyzed by mass spectrometry.

2.2. Protein identification by mass spectrometry

The protein was identified as described previously [15]. In short, the relevant band was excised from the polyacrylamide gel, reduced, alkylated using iodoacetamide, and digested by trypsin. The resulting fragments were extracted, purified using C18 ZipTip (Millipore) and measured by MALDI-TOF mass spectrometry on a Biflex instrument (Bruker, Bremen). An aliquot of 2 μl of the purified peptide mixture was introduced into a Q-Tof-2 tandem mass spectrometer (Micromass, Manchester, UK) using the nanospray interface and analyzed in MS mode as well as in MSMS mode for a number of fragments to obtain sequence information.

2.3. Cloning, sequencing and in vitro translation of rat cavin

Using the Marathon cDNA amplification kit from Clontech with primers designed from the published mouse PTRF cDNA sequence [11], 5′ and 3′ RACE reactions were performed on an anchor ligated rat cDNA library purchased from Clontech, and full-length cDNA obtained by amplification of mixed, overlapping 5′ and 3′ products with primers corresponding to the anchors. The full-length rat cDNA was ligated into the TOPO-TA plasmid from Invitrogen, cloned and sequenced in an ABI PRISM 310 sequencer. The sequence was found identical in all positions to the predicted rat PTRF sequence accessible in GenBank (gi|27689506).

Using the TNT T7 Quick Coupled Transcription/Translation System kit from Promega, transcription and translation directed by the TOPO-TA plasmid with an insert of nucleotides 1 to 903 of the rat cavin cDNA sequence was performed. The reaction product was analyzed by immunoblotting with the antibody 2F11. A plasmid with the same sequence in the reverse orientation was used as negative control.

2.4. Differential solubilization of adipocyte plasma membranes

Adipocytes in a 50% (vol/vol) suspension in TBS containing 1% bovine albumin were diluted 20 fold in ice cold, Tris-buffered, isotonic sucrose and disrupted by suction through a 20 gauge, 5 mm long cannula with a pressure gradient of approximately 1 bar. A plasma membrane-enriched fraction was prepared from this homogenate by differential ultracentrifugation according to the previously described method [14]. The plasma membrane fraction was pelleted and extracted at 4 °C with 0.5% Triton X-100 in TBS. The amounts of protein in the plasma membrane fraction, extract and non-solubilized residue were determined with the DC protein assay from Bio-Rad (cat. no. 500-0113). Samples of the fractions were separated by SDS-PAGE and the gel stained with either colloidal Coomassie blue (GELCODE Blue Stain from Pierce, cat. no. 24590) or classical Coomassie blue as detailed in, e.g., [16]. Prominent bands excised from the lane loaded with non-solubilized residue were identified by MALDI-TOF. By comparison of band patterns in lanes loaded with fractions obtained as described above it was checked that the performance of the colloidal Coomassie stain was similar to that of the classical Coomassie stain.

2.5. Preparation of plasma membrane and nuclear fractions

Isolated fat cells were disrupted in an isotonic sucrose-containing Tris buffer by gentle homogenization in a Potter Elvehjelm homogenizer with a Teflon pestle (25 μm clearance). A plasma membrane and a nuclear fraction were obtained by differential and gradient ultracentrifugations according to [14]. The purity of the nuclear fraction was checked by phase contrast microscopy. Fractions were analyzed by SDS-PAGE and immunoblotting, using the monoclonal antibodies 2F11 and 6B6 towards cavin and caveolin-1, respectively [9]. After stripping, blots were digested with alkaline phosphatase (bovine intestinal alkaline phosphatase from Sigma, cat no. 5521) at 37 °C for 30 min and reprobed with 2F11. The effectiveness of phosphatase treatment was confirmed by the disappearance of numerous bands apparent after staining of parallel blots with an antibody to phosphoserine epitopes.

2.6. Specimens for immunogold-electron microscopy

Plasma membrane sheets with the cytosolic face exposed were prepared by adsorption of adipocytes to a polylysine coated carbon layer, deposited on a formvar film carried on an EM grid, followed by rupture of the cells by a jet of buffer, as described earlier [8]. The adherent sheets of plasma membrane were briefly fixed with 0.2% phosphate-buffered glutaraldehyde for 20 to 120 s and blocked with bovine albumin. Indirect immunolabeling was done using the monoclonal mouse 2F11 or a polyclonal rabbit antibody towards murine PTRF kindly donated by I. Grummt, Heidelberg (17) as primary antibodies, and 8 nm gold particles conjugated with affinity purified goat anti-mouse IgG (DAKO, cat. no. Z420) or porcine anti-rabbit IgG (DAKO, cat. no. Z196), respectively. The labeled specimens were negatively stained with sodium silicotungstate.

2.7. Specimens for fluorescence microscopy

Male Wistar rats were anesthetized with pentobarbital and fixed by perfusion with 70% ethanol through a cannula inserted in the aorta through

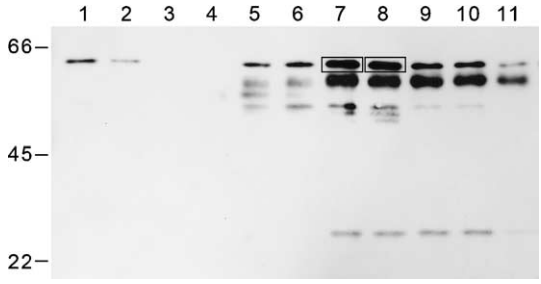

Fig. 1. Immunoblot of fractions from immunopurification of cavin from solubilized adipocyte plasma membranes with 2F11 as primary antibody. Solubilized adipocyte plasma membranes before (lane 1) and after (lane 2) immunosorption on a protein G column loaded with mab 2F11. Lane 3 is column wash, 4 to 11 are 10-μl aliquots of 400 μl fractions collected during elution with Tris buffer containing 2% SDS. From a parallel gel loaded with 100-μl aliquots gel pieces located as the framed bands were excised and used for tryptic digestion and mass spectrometry.

an incision in the left ventricle. Tissue samples (tongue, liver and epididymal fat pad) were excised and placed in 96% ethanol, dehydrated further in absolute ethanol, passed through xylene and finally infiltrated and embedded in paraffin. De-paraffinized sections were blocked with 10% goat serum in TBS for 30 min and incubated overnight at 4 °C with primary antibodies. Primary antibodies were monoclonal 2F11 [9] or rabbit anti-PTRF [11]. Secondary antibodies were goat anti-mouse 115-095-146 from Jackson Immunoresearch and porcine anti-rabbit Z205 from DAKO, both labeled with fluoresceine.

3. Results

3.1. Immunopurification of cavin

Preliminary experiments showed that the immunoreactive protein in a plasma membrane-enriched, subcellular fraction from isolated rat fat cells was only partially soluble in non-ionic detergents, but that it could be completely solubilized in 1% SDS. Immunoreactivity of the protein was, however, reversibly lost in the presence of 1% SDS. Therefore, a plasma membrane-enriched fraction was solubilized with 1% SDS, diluted ten fold in 80 mM octylglycoside and run through a protein G column loaded with 2F11. Samples of load, run-through and SDS-eluate fractions were run on non-reducing 10% polyacrylamide gels in the presence of SDS. Parallel gels, one with application of 10 μl sample for immunoblotting with 2F11 and one with application of 100 μl samples for staining with Coomassie blue, were run. Protein bands in lanes with high protein load were identified by means of the parallel immunoblot (see Fig. 1), excised and saved. The remains of this gel was immunoblotted in order to verify that the excised band corresponded to the immunoreactive protein (not shown). The immunoreactive protein is referred to as *cavin* in the following. The piece of gel containing cavin was digested with trypsin and the digest analyzed by MALDI-TOF. The spectrum of tryptic peptide fragments was compared to the patterns predicted from the proteins in the SWISS-protein database, and the best match was confirmed by MS–MS sequencing of three fragments. This procedure identified cavin as PTRF, cf. Fig. 2. The band identified by labeling with 2F11 could also be labeled with a commercial monoclonal antibody raised against a peptide representing amino acid residues 157–272 of PTRF (BD Biosciences cat. no. 611258, results not shown).

3.2. Cloning and sequencing of rat cavin

The rat cDNA corresponding to mouse PTRF was PCR-amplified from an anchor ligated cDNA library from rat fat cells using primers derived from the mouse PTRF sequence. Upon cloning into a plasmid vector the rat cDNA sequence was determined in both directions and found identical to the rat PTRF sequence which had meanwhile become accessible in GenBank. The deduced amino acid sequence of rat PTRF/Cavin was 98.7% identical to the corresponding mouse

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  rat   1 MEDVTLHIVE RPYSGYPDAS SEGPEPTPGE ARATEEPSGT GSDELIKSDQ VNGVLVLSLL
mouse     ---------- -----F---- -------Q-- ---------- ---------- ----------
  man     ---P-Y---- --LP-----    -----SSAG -Q-A-----A --E------- ----------

  rat  61 DKIIGAVDQI QLTQAQLEER QAEMEGAVQS IQGELSKLGK AHATTSNTVS KLLEKVRKVS
mouse     ---------- ---------- ---------- ---------- ---------- ----------
  man     ---------- ---------- ---------- ---------- ---------- ----------

  rat 121 VNVKTVRGSL ERQAGQIKKL EVNEAELLRR RNFKVMIYQD EVKLPAKLSV SKSLKESEAL
mouse     ---------- ---------- ---------- ---------- ---------- ----------
  man     ---------- ---------- ---------- ---------- ---------I ----------

  rat 181 PEKEGDELGE GERPEEDAAA IELSSDEAVE VEEVIEESRA ERIKRSGLRR VDDFKKAFSK
mouse     ---------- -----D-T-- ---------- ---------- ---------- ----------
  man     -----E---------------- L--------- ---------- ---------- ----------

  rat 241 EKMEKTKVRT RENLEKTRLK TKENLEKTRH TLEKRMNKLG TRLVPVERRE KLKTSRDKLR
mouse     ---------- ---------- ---------- ---------- ---------- ----------
  man     ---------- ---------- ---------- ---------- -----a---- ----------

  rat 301 KSFTPDHVVY ARSKTAVYKV PPFTFHVKKI REGEVEVLKA TEMVEVGPDD DEVGAERGEA
mouse     ---------- ---------- ---------- ---------- --------E- ----------
  man     ---------- ---------- ---------- ---Q------ -------A-- --G-------

  rat 361 TDLLRGSSPD VHTLLEITEE SDAVLVDKSD SD 392
mouse     ---------- ---------- ---------- -- 392
  man     G--R------ --A------- ---------- -- 390
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Fig. 2. Aligned sequences of rat, mouse and human PTRF/cavin. The 3 partial sequences determined by MS–MS are shown in italics or bold and these sequences gave the primary identification of cavin. Other aa residues were determined by conceptual translation of the cDNA sequence.

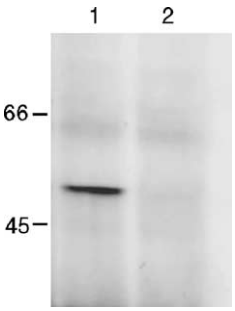

Fig. 3. Immunoblot of an in vitro translation reaction directed by the truncated rat cavin sequence corresponding to nucleotides 1–903 (lane 1) and of the reaction directed by the corresponding reverse sequence (lane 2). The primary antibody was 2F11.

sequence, as revealed by the Homologene feature accessible through the homepage of The National Center for Biological Information. Fig. 2 shows the alignment of the rat PTRF/Cavin sequence to the corresponding mouse and human sequences. It appears from the alignment that the only non-conservative differences between the rat and mouse sequences were located in positions 28 and 198. As the monoclonal antibody 2F11 is rat specific, its epitope can be assumed to be located near to one of these positions. Furthermore, the fact that 2F11 also does not cross-react with the human protein (data not shown) and that the human sequence is identical to the rat sequence in the region near position 198 indicates that the epitope for 2F11 cannot be located there and must consequently be located near position 28.

3.3. In vitro translation of truncated rat cavin

Fig. 3 shows a Western blot of the translation products from a combined in vitro transcription and translation reaction directed by nucleotides 1–903 of the rat cavin cDNA sequence. The immunolabeled band appears with an apparent Mw of about 50 kDa, which could be expected from the apparent size of 60 kDa of the full translation product, cf. Discussion. The band is absent in the lane representing the product of a control reaction directed by the reverse rat cavin cDNA sequence.

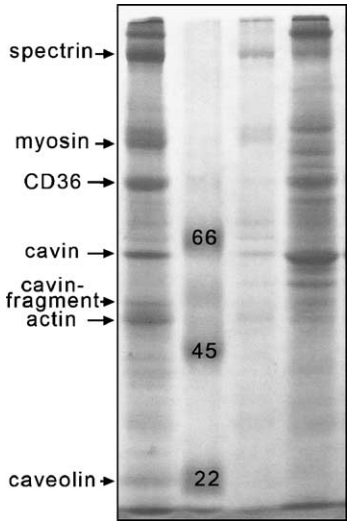

Fig. 4. Coomassie blue stained SDS-PAGE of differentially solubilized adipocyte plasma membranes. Left lane: Fifteen μg of protein from the Triton X-100 insoluble fraction were applied; the indicated bands were identified by mass spectrometry. Thirty-five μg of protein from the Triton X-100 soluble fraction were applied in the lane right of the MW standards, and in the rightmost lane 50 μg of protein from the unfractionated plasma membrane.

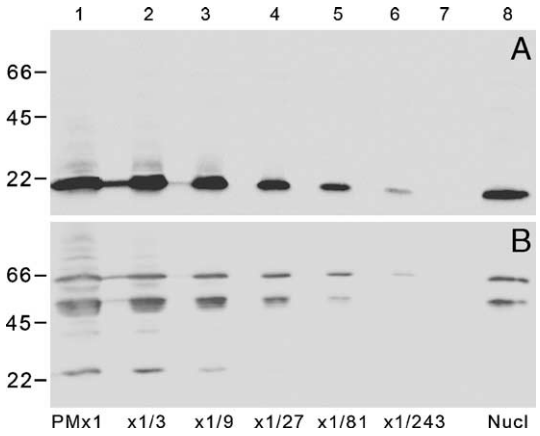

Fig. 5. Immunoblots of SDS-PAGE gels for assessment of recovery of caveolin and cavin in fractions enriched in plasma membrane and nuclei. The primary antibody against caveolin (blot A) was 6B6, and that against cavin was 2F11 (blot B). Lane 1 was loaded with 50 μg of plasma membrane fraction protein and lanes 2–7 with a 3-fold dilution series of this fraction as indicated. Lane 8 was loaded with 4 μg of nuclear fraction protein.

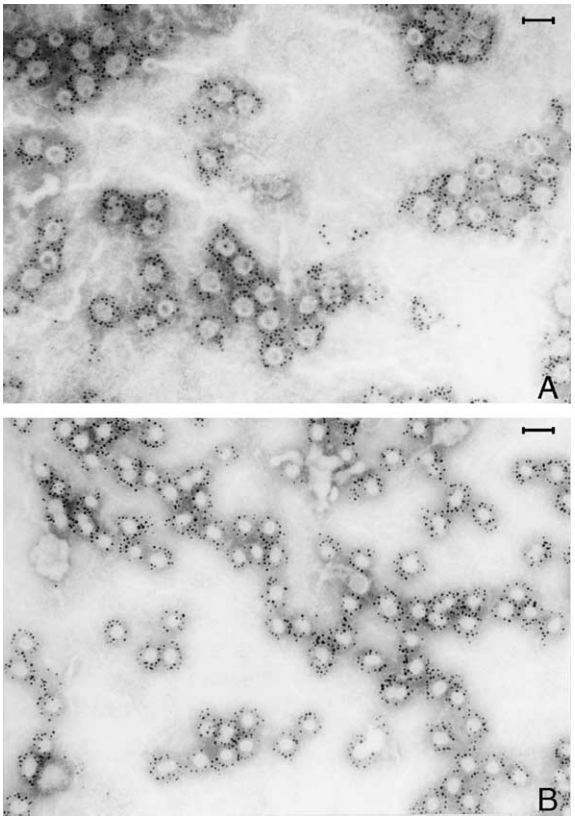

Fig. 6. Electron micrograph of immunogold labeled adipocyte plasma membrane sheets. (A) Labeling with 2F11 as primary antibody. (B) Labeling with polyclonal rabbit antibody against PTRF. The labeling pattern is the same and restricted to caveolae.

3.4. Representation of cavin in the plasma membrane fraction

Exploiting the poor solubility of cavin in non-ionic detergents, its recovery among the Triton X-100 insoluble proteins from the plasma membrane fraction was investigated. Fig. 4 shows that the main part of cavin is insoluble in Triton X-100, and that the band pattern of the insoluble fraction is different from that of the plasma membranes. Also, caveolin is highly enriched in the insoluble fraction. Coomassie blue staining of the SDS-gel shows that the recovery of the protein is about the same as the recovery of caveolin. Excision and subsequent MALDI TOF and MS–MS analysis indicated that the bands were not contaminated with other proteins.

3.5. Recovery in a nuclear fraction

Fat cell nuclei were prepared according to [14]. The purity of the fraction was checked by phase contrast microscopy, and no contaminating membrane vesicles or cell debris were observed. The recovery of caveolin and cavin in the nuclear fraction was compared by immunoblotting, see Fig. 5. Judged from the intensity of immunolabeling of bands and dilution factors, the recovery of cavin in the nuclear fraction did not exceed that of caveolin and was about 3% of the recovery in the plasma membrane fraction. Reprobing of the blot after digestion with alkaline phosphatase did not alter the relative recovery of immunoreactivity showing that the reactivity of the antibodies was independent of phosphorylation of the cavin/PTRF protein.

Immunogold labeling of fat cell plasma membranes sheets labeled with the monoclonal antibody 2F11 as primary antibody produced a distinct caveolar localization of the gold particles (Fig. 6, panel a). An identical pattern of labeling was obtained using a rabbit antiserum against PTRF as primary antibody (panel b). Caveolae in corresponding specimens of mouse fat cell membranes were readily labeled with the rabbit antiserum against PTRF, but could not be labeled with 2F11 (data not shown). Thus, 2F11 is rat specific.

3.6. Fluorescence microscopy of rat tissues

To study the cellular localization of cavin in different cell types, sections of perfusion fixed rat tongue, adipose tissue and liver were labeled with antibodies towards murine PTRF or the monoclonal antibody 2F11 to rat cavin as primary antibodies followed by the appropriate fluorescent secondary antibodies. Nuclei were located by examination of the specimens in phase contrast. With the limited resolution of this method, individual caveolae cannot be identified, but cells rich in caveolae as, e.g., striated and smooth muscle cells exhibit a distinct, seemingly

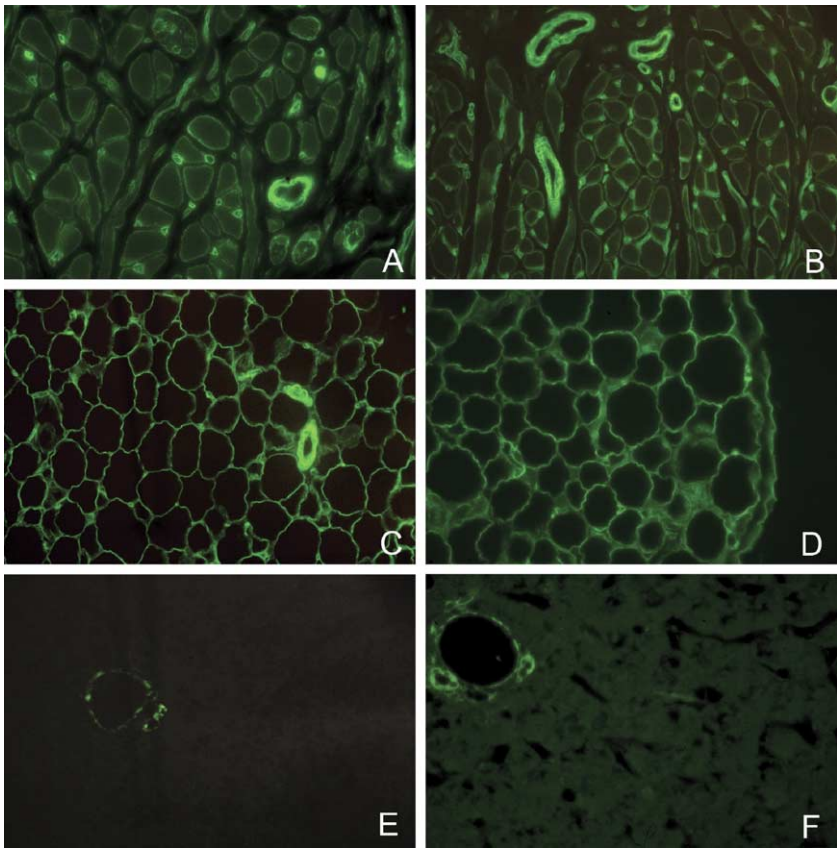

Fig. 7. Indirect immunofluorescence micrographs of rat tongue (A, B), fat (C, D) and liver (E, F). Frames A, C and E were labeled with 2F11 as primary antibody and frames B, D and F were labeled with rabbit polyclonal antibody against PTRF. The labeling pattern is the same with the two antibodies. Skeletal muscle of tongue exhibits labeling of the sarcolemma, capillaries and vascular smooth muscle. In adipose tissue, the cytoplasmic rim of fat cells and vasculature are labeled. In liver hepatocytes are unlabeled, only the vasculature of portal spaces is labeled.

continuous plasmalemmal labeling with both antibodies, see Fig. 7. Adipocytes and endothelial cells were heavily labeled corresponding to the cell outline, but the thinness of their cytoplasm do not allow distinction between plasmalemmal and cytoplasmic labeling. Liver cells were devoid of labeling, but endothelial cells in portal and hepatic veins and in some sinusoids were labeled as were endothelial cells in other tissues. Cell nuclei were unlabeled in all tissues studied.

4. Discussion

The present results identify the sequence of an abundant caveolar protein, cavin/cav-p60. The primary identification was achieved by purification of the rat homologue of the protein from rat fat cell plasma membranes by means of a specific monoclonal antibody and subsequent mass spectrometric analysis of the purified protein. Furthermore, the protein was purified from rat fat cell plasma membranes by differential detergent solubilization and SDS-gel electrophoresis and again identified by mass spectrometric analysis. Surprisingly, the same protein has previously been named PTRF for Polymerase I and Transcript Release Factor due to its ability to induce dissociation of paused ternary transcription complexes in various in vitro assays [11]. The present identification was confirmed by the demonstration of specific labeling of the in vitro translated protein by the monoclonal antibody to cavin, and by the finding that a rabbit antibody as well as a commercial monoclonal antibody raised against the homologous, recombinant mouse protein, PTRF [16], labeled the purified rat cavin in Western blots. The electrophoretic mobility of the PTRF/cavin protein of 392 amino acid residues is reduced, since it appears as a protein of about 60 kDa, cf. [9] and, e.g., Fig. 4 in ref. [17]. Reduced electrophoretic mobility can be due to protein glycosylation, but the post-translational modifications reported for PTRF/cavin [13] are serine phosphorylations and N-terminal acetylation, which are not likely to reduce electrophoretic mobility. Further, we have observed that full-length, recombinant cavin produced in *E. coli* has the same mobility as that of cavin isolated from rat adipocytes (unpublished data). The reduced mobility might reflect a special behavior of the protein towards SDS micelles.

The rabbit antibody to PTRF also labeled caveolae specifically in immunogold electron microscopy and produced a pattern fully compatible with caveolar labeling in fluorescence microscopy. Our present findings corroborate and extend a recent report that the labeling by a commercial antibody to PTRF colocalized with caveolin labeling in human adipocyte plasma membranes by confocal fluorescence microscopy [13]. We have previously found that cavin is specifically located to caveolae at the ultrastructural level [9], and to the plasma membrane at the cellular level [10], and that cavin is consistently expressed in parallel with caveolin, irrespectively of the isoform of the latter [10,19].

At the level of light microscopy, the labeling for cavin is confined to the plasma membrane, and no labeling of nuclei was demonstrable in any of the cell types examined. In a nuclear fraction from mature adipocytes the relative recovery of cavin was equal to the recovery of caveolin, which was only about 3% of the recovery in the plasma membrane fraction. Based on the heavy and specific labeling of the cytosolic face of caveolae which can be achieved with antibodies towards cavin, we have previously argued that the protein was likely to be a structural component of caveolae due to its presence in many copies [9]. The present finding that both cavin and caveolin are recovered in the detergent insoluble fraction from adipocyte plasma membranes supports this notion. The relative abundance of the two proteins in this fraction is, however, difficult to estimate because caveolin has been reported not to stain with Coomassie blue [18]. In our hands, caveolin did stain with Coomassie blue, as judged from the fact that mass spectrometric analysis of the excised band did not indicate the presence of other proteins. The discrepancy with regard to staining might possibly be due to differences in prior preparative steps.

PTRF was originally identified in a two-hybrid screen for molecules interacting with TTF-1 (transcription termination factor 1) and found to facilitate the dissociation of ternary complexes of pre-rRNA, Pol1 and TTF-1 [11,17]. Later, PTRF has also been reported to interact with the binding factor of a type 1 collagen promoter zinc-finger transcription factor and assigned a role as a regulator of proá2(I) collagen proximal promoter activity and thereby also to be involved in polymerase II reactions [12]. Lately, caveolae have been identified as a site of targeting and post-translational modification by proteolysis of PTRF and other PEST-domain containing proteins, and it was reported that the proteolytic fragments of cavin/PTRF could be dissociated from a caveolar preparation in contrast with the full-length protein [13]. The implication of PTRF in nuclear transcriptional and regulatory functions is intriguing. We found that only a minor fraction of the adipocyte full-length cavin was recovered in the nuclear fraction and that this was fully explicable by plasma membrane contamination. This finding is in line with the finding of Naboulaich et al. [13] of minor quantities of full-length PTRF in the nuclear fraction, but does not preclude the possibility that fragments of cavin/PTRF lacking epitopes for the antibodies could have regulatory functions in the cell nucleus.

However, our observation that caveolae, cavin and caveolin are absent in liver parenchymal cells is difficult to reconcile with a fundamental transcriptional regulatory function of cavin/PTRF. Lack of expression of cavin/PTRF mRNA in liver cells has earlier been reported [12].

Our present and earlier [9] results show that cavin is a major caveolar resident, with a representation similar to that of caveolin. Cavin and caveolin were shown to colocalize in caveolae at the ultrastructural level [9]. In relation to our previous suggestion of cavin as a structural protein of caveolae [9], it is noteworthy that cavin lacks membrane spanning domains and a caveolin binding motif to account for its affinity to caveolae. A sequence homology search on the web site http://www.ncbi.nlm.nih.gov/ showed that cavin has a 20% sequence identity and 44% sequence similarity in the region from aa residues 60–338 with myosin heavy chain, and that there were 47% and 63% identity between two stretches of

cavin (aa residue nos. 76–153 and 276–296, respectively) and a phosphatidylserine binding protein isolated from blood platelets [20]. As the caveolin scaffolding domain has recently been shown to induce the formation of lipid domains enriched in phosphatidylserine [21], it could be suggested that an affinity for phosphatidylserine promotes the association of cavin to the caveolar structure.

Acknowledgments

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