Caveolin-1 is similar to the regulatory loop and lipid binding core of the PITPα

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Correction:

The description of the discovered reductase enzyme has been changed to precisely define that it was found in a caveolin-1 co-immunoprecipitate.

Caveolae have a multitude of functions in growth, regulation of signalling and lipid transport, and likely provide a platform to catalyze reactions at the special boundary of plasma membrane and intracellular environment. Lipid binding has been shown for the caveolin-1 protein, it has been found to interact with cholesterol and phosphatidylcholine via its scaffolding region of 20 residues which is proximal to the membrane span of 31 residues. It is found that by a HMM-HMM (Hidden Markov Model) comparison HHpred the top ranking match is PITP (phosphatidylinositol transfer protein) α. Structural overlay by MODELLER demonstrates that a regulatory loop and part of the lipid binding core of the unconventional lipid transfer protein match the full length caveolin-1 protein. Strikingly, part of the caveolin-1 scaffolding region (residues 86-101) corresponds to the structurally invariant part of the lipid binding core of PITP α that interacts with the membrane bilayer.

Remote homology tracking and structure prediction is easily achieved with HHM (Hidden Markov Model) comparison that incorporates the PSIPRED predicted secondary structure score and relates these to actual or predicted DSSP values for structures of database entries (1). The probability of a template to be a true positive is calculated from the distribution of amino acid residues in the alignments using the secondary structure score. Using the secondary structure predicted search with caveolin-1 (2-3) in local alignment mode including global MAP realignment the automated procedure listed PITP α with a P-value of 0.64×10^{-3} as the top entry and with a full length alignment against residues 77-269 of the 271 amino acid protein PITP α. Other alignments were truncated or not top-listed. Residues 119-190 of the regulatory loop of PITP α (4) are approximately aligned with amino acid 29 to 86 of the N-terminal loop of caveolin-1 and can be extended into the lipid-binding helix F that matches the putative membrane span of 31 amino acids of the caveolin-1 (Fig. 1). Strikingly, the scaffolding region of residues 82-101 of caveolin-1 matches the structurally

invariant part of PITP α that serves to contact the membrane and interact with the lipid bilayer. Schouten et al. have determined the stucture of PITP α at 2.0 Å resolution and predict that movement of the C-terminal part and the N-terminal lipid exchange loop of residues 65-83 allows this structurally invariant part of PITP α including Lys202, Trp203, Trp204 and Lys209 to contact the membrane bilayer to bind the lipid to be exchanged (4). Remarkably, these residues are conserved in caveolin-1 (Fig. 1) and exposed to the environment (see Fig. 2c). At least two residues are conserved in the entire family of the caveolin proteins that were included in the alignment with further aromatic residues that could equally contact the bilayer in their vicinity (Fig. 1b). Three out off four are found in all human caveolins with a further aromatic residue Tyr100 inbetween generally found in all aligned caveolins. PITP α is reactive in transfering phosphatidylcholine (PC) and phosphatidylinositol (PI) through the aqueous phase of the cytoplasm, yet a key role that was determined *in vivo* is the presentation of PI to PI specific kinases and phospholipases. This PI specific kinase may thus not have to be released from the membrane, but is presenting the substrate to other interfacial enzymes. Helix F would interact with the phospholipid acyl chains nonselectively mainly by hydrophobic interactions and presentation to lipid modifying enzymes is proposed to include head group binding to the regulatory loop residues 160-190. Unlike other lipid or sterol binding carrier proteins Sec14p and the StAR-related domain and PITP α, caveolin-1 does not contain the lipid exchange loop. In these other proteins they are active in closing the lipid binding site to shield the hydrophobic acyl residues thus caveolins may only be catalyzing lipid reactions at the interfacial region of the membrane bilayer.

The caveolin-1 alignment was used to model its 3 dimensional structure as input to MODELLER with the 1kcm_A structure of PITP α selected as a template (5). MODELLER is fully automatic and yielded one three dimensional structure set that matched one sheet and five helices of the approximate structure (Fig. 1b, 1c) in relatively good quality with the PITP α chain (Fig. 1a). Residues matching Lys202 to Lys209 are fully exposed in caveolin-1 on one side of the molecule,

which would correspond to the caveolin-1 scaffolding domain with side chains pointing to the exterior (Fig. 2c). Also the membrane span (white) is exposed and would serve to contact the bilayer. This suggests that the same region that has been found to bind to cholesterol and phosphatidylcholine in the caveolin-1 scaffolding peptide (6-8) is indeed aligned with the structurally determined part of the lipid interface of PITP α.

Monier et al. have determined (9) that caveolin-1 oligomerization is enhanced by treatment with fatty acyl CoA esters and that acylation of C-terminal cysteines of the protein itself may not be required for this complex of 200 and 400 kDa molecular weight to form. Now fatty acyl CoA esters can be envisioned to shield hydrophobic faces that could be in contact with the exchanged or bound lipid (Helix F) possibly contacting two protomers in the caveolin-1 complex. In PITP α the Cterminal region which is not well aligned with caveolin-1, both structurally and by primary sequence, is a flexible region including a helix likely unwound to allow access of lipid to the lipid binding region which is also involving disordering of C-terminal residues (4).

Our biochemical analysis from rat lung plasmalemmal caveolae isolated from the perfused animal by a biochemical immunoisolation resulted in additional factors that could be assigned to this purified organelle including ESA, ATP synthase and this ATP synthase is not present as mitochondrial contaminant (10-12). And we have predicted it to carry out a key role in ATP release in mechanosignalling with ATP as a paracrine signalling factor. Serendipitously, we also found the microsomal form of NADH cytochrome B5 reductase tightly interacting with caveolin-1 in a high molecular weight complex in a co-immunoprecipitate of caveolin-1 (cf. p. 77 from (10)) from this highly purified rat lung membrane preparation. I would now suggest, that caveolin-1 is coupled to this ternary chain of NADH cytochrome B5 reductase, cytochrome B5, and various fatty acyl CoA desaturases which even act on some phospholipids (Δ 5 desaturase). In support of this role of caveolin-1 it is evident from biochemical reaction studies that the catalytic activities of desaturases can be stimulated by addition of bovine serum albumin. Similar to this activity in regulating

availability of fatty acyl substrates or their removal, caveolin-1 may channel substrates to the ternary chain.

The HHpred (1) and a structural match fully support this function and provide a reasonable prediction of the catalytic activity in lipid transfer or presentation. Yet, it remains to be determined whether other functions are carried out through this modular protein since caveolin-2 e.g. can be matched to RGS8, a regulator of G-proteins, and caveolin-3 to aquaporin-4, a water channel, which is yet only in truncated domains. In agreement with this proposed function, the recently observed lipid differences in caveolin-1 knockout mice included plasma changes of free cholesterol, PC and determined elevated ratios of saturated to unsaturated cholesteryl esters (13). It remains to be seen whether these are a consequence of decreased substrate provision to lipid desaturases.

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Figure 1

(a) The HHpred search (1) was used to search for caveolin-1 homologues in the database. The pdb70_29Nov08 assembly was used. Upon iterative Psi Blasting, the secondary structure enhanced output (PsiPred) listed 10 factors by their PDB structural name (Hit). These included Mus musculus (1), Homo sapiens (2, 3, 8, 10), Escherichia coli (4, 6), Gallus gallus (synthetic domain of residues 123-148; 5), Tityus serrulatus (7) and Silicibacter pomeroyi (9) proteins: Probability of a true type (Prob), an E value (calculated without secondary structure score), the P-value that indicates a probability that in a pairwise comparison a wrong hit will be at least this good, Cols, the number of aligned matching residues, the range of the query match (Query HMM) and template match (template HMM) are indicated.

(b) The alignment generated by the procedure lists the secondary structure propensity (E or H) (SS_pred) with a score (SS_conf). Template DSSP values determined from the 3 D structure by Xray crystallography or NMR (ss_dssp) are indicated where available. The consensus sequence from the query (caveolin-1) or template (1kcm_A) is indicated (Consensus).

Figure 2

1kcm_A structure alignment with the lipid transfer/binding protein. **(a)** The structure of PITP alpha is colored with the central core of the caveolin-1 residues aligned (Fig. 1) (green) from position 22-112 corresponding to residues 112-219 of the phospholipid transfer factor. This was detected in several alignments. The overlaid residues of the true modelled structure of caveolin-1 are shown in **(b)**. **(c)** The fully modelled backbone trace with side chains exposed in the caveolin scaffold stretch (Lys 96, Tyr97, Trp98, Arg101) is shown with the membrane span colored in white. The extended structure is aligned with a second strand, followed by the caveolae scaffold including the conserved residues found in the membrane interacting part, and its membrane penetrating domain.

Figure 1

(a)

No	Hit		Prob	E-value	P-value	Score	SS	Cols	Query HMM	Template HMM
1	1kcm_A	PITP alpha	20.2	12	0.00064	18.9	-0.3	160	1-178	77-269 (270)
2	2jwa_A	ERB-2	19.5	48	0.0025	15.2	3.5	44	73-149	1-44 (44)
3	1bnx_A	BAND 3	7.5	95	0.005	13.4	1.1	34	53-140	1-34 (34)
4	2hiq_A	YDHR	7.3	76	0.004	14.0	0.5	93	53-165	1-113 (113)
5	2amn_A	Cathelicidin	7.1	98	0.0052	13.3	1.0	24	143-166	1-26 (26)
6	1lv7_A	FTSH	6.8	97	0.0052	13.3	0.9	143	1-171	32-257 (257)
7	1c55_A	Butantoxin	6.8	89	0.0047	13.6	0.7	37	128-176	1-40 (40)
8	1bts_A	BAND 3	6.3	1.2E+02	0.0064	12.8	1.2	26	102-129	1-26 (26)
9	3chv_A	DUF849	5.5	1.1E+02	0.006	12.9	0.6	134	1-178	80-237 (284)
10	2j4o_A	TAB1	5.3	1.4E+02	0.0075	12.3	1.0	153	1-178	163-369 (401)

(b)

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Q ss_pred            CCCCCEECCCC--------CEECCCC------CCCCCCCCCCCCCH--HHHHCCCCCCHHHHHCCCCCCCCCCCCCCCCCE
Q ss_conf            99873006776--------2741663------66787669788022--22421011105554005664689886552303
Q caveolin-1         1 MSGGKYVDSEG--------HLYTVPI------REQGNIYKPNNKAM--ADELSEKQVYDAHTKEIDLVNRDPKHLNDDVV   64 (178)
Q Consensus          1 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~r~~~~~~kp~n~~m--~~~~~~~~~~~~~~~~idl~dRDP~~lN~h~l   64 (178)
                       -.++-+++++.        -.||.|-      -.--++|+|+|-+-  +++|++..++.+-+--||..|+||-.-. | .
T Consensus         77 Pk~al~v~EkaWNaYPy~~T~yt~~~~~~~F~i~ieT~h~~d~g~~eNv~~L~~~~l~~ReV~~IDIan~d~v~~~~d-Y  154 (270)
T 1kcm_A            77 PEGALNIHEKAWNAYPYCRTVITNEYMKEDFLIKIETWHKPDLGTQENVHKLEPEAWKHVEAIYIDIADRSQVLSK-D-Y  154 (270)
T ss_dssp               CTTTTEEEEEEEEETTEEEEEEEETTTGGGEEEEEEEEEESSSSCCSSTTCCCHHHHTTCEEEEECTTCGGGSCGG-G-C
T ss_pred               CCCCEEEEEEEECCCCEEEEEEECCCCCCEEEEEEEEEECCCCCCCCCCCCCHHHHHCEEEEECCCCCCCCCCCC-C-C
T ss_conf               975228887520578704889976777870489998887279878066668696785063479842314666775 6-7-8


Q ss_pred            EECHHHHCCCC--CCCCCCCEEE------------EEEEEEEEEE-EEE--HHHHHHHHHHHHHHHHHHHHHHHHHHHH
Q ss_conf            30678860688--7776865279------------9640899974-101--29999999999999999999999987543
Q caveolin-1        65 KIDFEDVIAEP--EGTHSFHGIW------------KASFTTFTVT-KYW--FYRLLSALFGIPMALIWGIYFAILSFLHI  127 (178)
Q Consensus         65 KV~FeDViAEP--~~gthSfd~VW------------~~S~~~F~~s-K~~~~~YriLS~llaiPlA~~~Gi~FA~LSf~hI  127 (178)
                       +-..+-..-..  -|---.+.=|            -|+|++.++. ++|  .-|+=+.+-...-.++.-.-=.++.-+-=
T Consensus       155 ~~eeDp~~f~S~kTgRGPL~~~W~~e~~~~~~~P~McaYKlv~v~f~~wGlq~~vE~fI~~~~r~~~~~~HRq~fcW~De  234 (270)
T 1kcm_A          155 KAEEDPAKFKSVKTGRGPLGPNWKQELVNQKDCPYMCAYKLVTVKFKWWGLQNKVENFIHKQEKRLFTNFHRQLFCWLDK  234 (270)
T ss_dssp               CGGGCTTTCCCTTTCCCCCCTTHHHHHTTSTTCCCEEEEEEEEEEECCSSCHHHHHHHHHHHHHHHHHHHHHHHTHHH
T ss_pred               CCCCCCEEEEEEECCCCCCCCCCCCCCCCCCCCCCEEEEEEEEEEEEECCCCHHHHHHHHHHHHHHHHHHHHHEEECHH
T ss_conf               865586058886538898897741001348988889999999996410146727899998666899999887642410 02


Q ss_pred            HHHHHHHHHHEEEHHHHHHHHHHHHHHHHHHHHHHHHHCCEEEEEECCC
Q ss_conf            33332274713418999999999999999899999999857608986156 9
Q caveolin-1       128 WAVVPCIKSFLIEIQCTSRVYSIYVHTVCDPLFEAVGKIFSNVRINLQKEI  178 (178)
Q Consensus        128 W~v~Pcik~~~I~l~~~~iw~~~v~~~~dP~f~SvG~~fS~Irv~~~ke~  178 (178)
                       |.        -|.+.-++.+=.-.-        +.+.+....=-++..+--
T Consensus        235 W~--------gltmedIR~~E~etq----------~~L~~~~~~~g~~~~  269 (270)
T 1kcm_A           235 WV--------DLTMDDIRRMEEETK--------RQLDEMRQKDPVKGMTAD  269 (270)
T ss_dssp               HT--------TCCHHHHHHHHHHH--------HCCCC-------------
T ss_pred               HC--------CCCHHHHHHHHHHH--------HHHHHHHHCCCCCCCCCC
T ss_conf               12--------88889999999999--------99999716732553257
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Figure 2