

Identification of two new μ -adaptin-related proteins, μ -ARP1 and μ -ARP2

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Received 2 December 1996

Abstract We report the cDNA cloning, primary structure and tissue distribution of two new proteins homologous to μ -adaptins, the medium chains of the clathrin coat adaptor complexes. Both predicted proteins share 60% amino acid sequence identity with each other and 27–31% identity with μ 1-adaptin (ap47) and μ 2-adaptin (ap50). Lower similarity (23–25% identity) is found with two other μ -adaptin-related proteins, p47A/B, and there is similarity over the N-terminal 150 amino acids with the adaptin small chains and δ -COP. The mRNAs of both molecules are expressed in all tissues analyzed, but with different profiles of relative abundance. μ -ARP1 is most abundant in brain, ovary and lung, whereas μ -ARP2 is prominently expressed in testis. These proteins suggest the existence of as yet uncharacterized types of clathrin- or non-clathrin-associated protein coats in cellular membrane traffic, of which they are probably prototype subunits, and provide molecular markers and probes for their characterization.

Key words: Membrane traffic; Membrane vesicle; Clathrin; Coat protein; Endocytosis; Endosome

1. Introduction

The controlled budding of vesicles from the plasmalemma and from intracellular membrane compartments is mediated by protein coats. Clathrin-coated vesicles form from the trans-Golgi network and the plasma membrane, whereas COP-I and COP-II-coated vesicles are involved in membrane traffic between the endoplasmic reticulum and the Golgi complex. The subunit compositions of these protein coats have been elucidated in detail, but there is growing evidence for the existence of other types of protein coats mediating the formation of vesicle populations involved in the trafficking between various membrane compartments (reviewed in [1,2]).

During the formation of clathrin-coated vesicles, a polyhedral cage of the protein, clathrin, assembles around the membrane bud. Interaction between the clathrin cage and intrinsic membrane proteins is mediated by a heterotetrameric adaptor protein (AP) complex. Clathrin-coated vesicles originating from the trans-Golgi network and from the plasma membrane utilize different adaptor complexes, AP1 and AP2, respectively. AP2 is composed of two large subunits, α - and β 2 (β)-adaptin, one medium chain (μ 2-adaptin or ap50) and one small chain (σ 2-adaptin or ap17). AP1 is composed of distinct isoforms of these subunits, γ , β 1 (β'), μ 1 (ap47) and σ 1 (ap19) (reviewed in [3,4]). Characterization of the components of the COP-I coat has revealed that three of its subunits, β -, δ - and ξ -COP, have partial sequence similarity with the β -,

μ - and σ -adaptins, respectively [5–8]. These subunits presumably reflect common mechanistic features of clathrin and COP-I coats which otherwise differ in morphology, subunit composition and function. Two isoforms of α -adaptin (α A and α C) have been known for long [9], and further ‘orphan’ coat subunit homologs were identified more recently by cDNA cloning. One additional, closely related isoform each is known for β 1/ β 2-adaptin (95%/84% amino acid identity [10]) and for clathrin heavy chain (85% identity [11–13]). More distant homologs are β -NAP (neuronal β -adaptin-like protein [14]), which is related to β -adaptins (36% identity over its N-terminal half) and β -COP, and p47A/p47B [15] which are similar to μ -adaptins (27–30% identity) and δ -COP. These proteins may be prototype subunits of as yet uncharacterized protein coats associated with the formation of other vesicle populations.

To identify new proteins involved in neuronal membrane traffic, we have performed an immunoscreening of cDNA expression libraries with antisera directed against synaptic plasma membranes [16]. One of the cDNAs thus identified was found to encode a protein of 29% amino acid sequence identity with μ 1-adaptin (ap47) and 27% identity with μ 2-adaptin (ap50), leading us to name it μ -adaptin-related protein 1 (μ -ARP1). Lower similarity is found with p47A/B, σ -adaptins and δ -COP. Sequence database screening led to the identification of another molecule, μ -ARP2, of 60% amino acid sequence identity with μ -ARP1. The mRNAs of both proteins are expressed in all tissues analyzed but display different profiles of relative abundance. These proteins are probably subunits of novel types of clathrin- or non-clathrin-associated protein coats in cellular membrane traffic, and their cloning provides molecular markers and probes for the characterization of these coats.

2. Materials and methods

Immunoscreening of a chicken brain cDNA expression library in λ gt11 with antisera raised against synaptic plasma membranes was carried out as described [16]. One immunopositive clone (μ -ARP1-2.5) was found to encode a polypeptide similar to the N-terminal region of μ -adaptins. A full-length chicken cDNA (μ -ARP1-2.5b) was isolated by hybridization-rescreening of chicken brain libraries. Sequence database searches with the μ -ARP1 sequence identified, besides other μ -adaptin-related sequences from mammals, nematodes, *Dictyostelium* and yeast, an EST cDNA from human fetal brain (IMAGE consortium [LLNL] cDNA clone 48136 [17]) with ~60% predicted amino acid sequence identity. The cDNA clone was obtained through Research Genetics, Inc., and sequenced. By alignment with μ -ARP1, it lacked 50 codons at its 5' end. The remaining sequence was obtained by PCR from a human brainstem cDNA library in λ gt11 (American Type Culture Collection, No. 37432), employing an insert-specific downstream primer and a vector primer. Sequencing was carried out with the ABI Dye-Terminator cycle-sequencing kit and an ABI 373 sequencer. Northern blot analysis was performed

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by standard procedures at high stringency, employing 10 µg of chicken poly(A)⁺ RNA per lane for µ-ARP1 and 2 µg of human poly(A)⁺ RNA per lane (Clontech multiple tissue Northern blots) for µ-ARP2.

3. Results

The cDNA and predicted amino acid sequences of µ-ARP1 and µ-ARP2 are given in Fig. 1, and an alignment with a selection of other µ-adaptin-related sequences is presented in Fig. 2. It can be seen that µ-ARPs, µ-adaptins and p47A/B are full-length homologs that are of similar size and share identical amino acids, between all of them or between subgroups, along their entire lengths of 420–450 amino acids. σ-Adaptins and δ-COP are shorter or longer, respectively, and align only with the N-terminal 150 amino acids. Similarity between µ-ARP1 and µ-ARP2 is distributed almost homogeneously along their sequences, whereas, when all µ-adaptin-related sequences are compared, blocks of high and low sequence conservation are apparent (Fig. 2).

µ-ARP1/µ-ARP2 (60% identity), µ1-adaptin/µ2-adaptin (40%) and p47A/p47B (84%) constitute pairs that are distinctly more closely related to each other than to the other family members (Table 1). The closest relatives of both µ-ARPs, besides each other, are µ1- and µ2-adaptin (27–31%). Sequence similarity to p47A/B, the two other full-length homologs, is lower (23–25%), whereas µ-adaptins are equally related to µ-ARPs and to p47A/B (27–30%). Similarity of µ-ARPs to the partial homologs, σ-adaptins and δ-COPs, is restricted to the first 150 amino acids (16–27%).

Phylogenetic sequence conservation of these proteins is very high. Mammalian orthologs of various adaptins and p47A/B have 99–100% amino acid identity ([18] and recent database entries), and even the putative orthologs of µ1-adaptin and µ2-adaptin from the nematode *C. elegans* have 74% and 81% identity, respectively, relative to their mammalian counterparts [19]. We assume, therefore, that µ-ARP1 and µ-ARP2 are distinct proteins and that the species difference (chicken vs. human) can account for maximally a few percent of their 40% sequence divergence. This is corroborated by their different tissue specificities of expression (Fig. 3). Both mRNAs are expressed in all tissues tested, but µ-ARP1 expression is highest in brain, lung and ovary whereas µ-ARP2 mRNA is distinctly highest in testis but low in brain and lung. µ-ARP2 mRNA expression in testis is also exceptional in that the larger-sized mRNA of ~2.7 kb predominates only in this tissue.

µ-ARP1

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1  CGGGAAATGGGGTAAAAAAGCGGATTTTGAGCAATTTGCCCTCCCGCATGATCTCCCA
2  HTSD
3  CTCTTCATCTGTCTCCAGGGGACCGCGTGTACAGGAACCTTCGGTGGGATGGC
4  LFI LLS S K G D R L V Y R N F R G D G
5  GCGCATGACGTACAGGACGCTTTTACCGCGTGTCACTCCCTGCGGGGATCAGCG
6  G D D V T D A F Y R A V T S L P G D Q A
121 CCCGTCTTCATGCCCCGGAGGGGCGCACTTCGTCCACGTGCGGACGCGGGGCTCTAC
181 P V F M A R E G R H F V H V R H G G L Y
241 GTGGGGGCCACACACCGTGGACACATCCCGCTTCGTCTGTGGAGTTCCTGAACAGG
45 V G A T T T V D T S P F V H V F L N R
301 TTGGTACGCTGCTGCGGAGCACTGTGGGACTGTAGAGGAGAGGCGTCAAGTGAAT
85 L V T L L R E H C G T L S E K S V S V N
361 GTGGCCCTCGTGCAAGAACTGCTGGAGAGATGTTGACTTCGGAATAGTGCAGACACC
105 V A L V Q E I L L G E M V D F G Y V D T T
421 GCCACCGAAGTGTGCGACGCGACCCACGGGGAACCGAGACACCAAGCCTTCAGC
125 A T E V L R S A T H G E P E T T K A F S
481 CTGCTGACCTCGCTCGCTCGGTGGGCTTGTGGCGCTGAGACGACGACAGAGAGTGGC
145 L L D L R S V G L F G A E T D Q S R V A
541 CCCGGCTCCGTACCAACCGCGGCTGCTGCTCTGCTGCGGACGAGGAGTGGTGGCAGG
165 P G S V T N R P V L P P R G E Q G G R R
601 GAGGTCTTTGTGACGTGCTGGAGCGCTGACCGTGTCTGCTGCTGCGACGCGGACGCC
185 E V F V D V V E R L T V V V I A A N G T P
661 CTGAAGTGGACGTGACGGGGAGCTCGGCTGAAGAGTCTGTGCTGGGGCTTCGAG
205 L K V D V Q G E I A R L K S F V P G A C E
721 CTGCGCATGGGGCTGACGAGGAGTGAAGCTGGGACGAGGAGGACGAGGAGTGGC
225 L R M G L T E E L S V G T E E Q R G Y G
781 CGCTCTCCCGTGGCTGCGCTTCCACAGCTCGTGGAATCTGGAAGATTTGAGCAG
245 R L P L A A V A F H S S V D L E E F E Q
841 GAGCGCTCTGCGGCTCACACCGGGCCGGGAGAGTACGCTGATGCGGTACAGCTG
265 E R V L R V T P G P G E V T L M R Y Q L
901 GCTGAGGAGCTTGTGTCGCGCTGCGCTGCGCTGCGGACGCTGCGGAGTGGAGTGGAGCGG
285 A E D V A V P L P F R L L P S V E W E P
961 GCGGGAGGCTCGCATTCACCTGAAGCTGCGCTGCTGCTGCGGACCAAAACACGCGC
305 A G R L R I H L K L R C D L P P K N H A
1021 ATCAACGCTGCTGCTGACGCTGCGGCTGCGGAGGAGGAGGAGTGGAGTGGAGTGG
325 I N V V L Q L P L P R E A S S L A Q E L
1081 AGCAGCGCCGACACAGACAGAGAGTGAAGGAGGAGGAGGAGTGGAGTGGAGTGGAGTGG
345 S S P E Q T A E L Q G G G R S L R W A I
1141 CCACGCTGCGAGGGGGGGGCGAGTGGGGGGGCTGTCAGGCTGAGTGGCGGCGCCAC
365 P R C Q G G A O L G A G V F R V Q L P P T
1201 CCCCCTGGGGCTGGGGCTGGGGCTGACCTTTCAGCTTTCAGCTTTCAGCTTTCAGCTTTC
385 P P L G L G P A A L T F E L P A L T V S
1261 GGGCTGCGGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG
405 G L R L R W L R L S A C P P G A P P G P P
1321 CAGCGCTGGGTTCGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG
425 D R W V R H L T H S D S Y V V R L *
1381 CCGAACCGGGGCGCTTTTGGGGCAATACAGGAAGTTTGGAGGCTCTGAAAAA

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µ-ARP2

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1  AGGGCGGGGAGGCGGACCTTCGCGCTCTCTGTGCTACTCTCCAGAGCGCCATGATT
2  M I
3  TCCGAATTCATCTGTCTCCAGGGGACCGCGTCACTACAAAGACTTCGCGGGG
4  S Q F F I L S S K G D P L Y K D F R G
121 GACATGGCGGGGATGGCGGAGCTCTTCTACCGGAAGTACGCGGAGTGCAGGA
23 D S G G R D V A E L F R K L T G L P G
181 GACGAGTCCCGGTTGTATGATCACCAGTGGCGCTATTCATTACATCAGACAGC
43 D E S P V V M H H G R H F I H I R H S
241 GGGCTCTATTGGTGGTACAACTTCAGAAAGCTTTCTCCCTTCAGCGCTCTAGAGCTG
63 G L Y L V V T T S E N A V S P F S L L E L
301 CTCTCAGGTTGGCCACCTCTGCGGCTATCTGCGCTCCCTGGGCGAGGAGCAGT
83 L S R L A T L L G D Y C G S L L G E G T I
361 TCCGCAATGTGGCTGTGATACGAATCTCGGATGAAGTGTGGAATATGGCTATGTA
103 S R N V A L V Y E L L D V L D Y G Y V
421 CAGACACATCCAGGAGATGCTGAGGAATTTCTCAGACGGAAGCTGTGGTACGAA
123 Q T T S T E M L R N F I Q T E A V V S K
481 CCGTTCAGCTCTTTGACCTCAGCAGGCTGGCTGTGTTGGGCTGAGACACACAGAC
143 P F S L F D L S S V G L F G A E T Q Q S
541 AAATGGCCCCAGCAGTACGAGGACCGCGCTGCTGCGTCAAGTGTGCTGACAGAGC
163 K V A P S S A A S R C P V S S R S D Q S
601 CAAAAGATGAAGTTTGTGGATGGTGGAGAGATGTCTGATGATGATGATCTAAT
183 Q K N E V F L D V E R L V S L I A S N
661 GGATCCCTGCTGAAGTGGATGTGAGGAGAGATTCGCTGCTGAGAGCTCTCTCTAGC
203 G S L L K V D V Q G E I R L K S F L P S
721 GGCTCTGAGATGCGCTTGGCTGACGGAAGTGTGTGTGGGGAAGTCAAGCTGA
223 G S E M R I G L T E E F C V G K S E L R
781 GGTATGGGCGAGGAATCCGGGTGATGAAGTCTCGTTTACAGCTCTGTGAATCTGGAC
243 G Y G P G I R V D E V S F H S S V N L D
841 GAATTTGAGTCTCATCGAATCCTCGCTTGAACACCTCAGGGCGAGCTGACTGTGATG
263 E F E S H R I L R L Q P P Q G E L T V M
901 CCGTACCAACTCTCCGATGACCTCCCTCAGCGCTCCCTTCCGGCTCTTCCCTCTGTG
283 R Y Q L S D D L P S P L P R L F P S V
961 CAGTGGGACGAGGCTCAGGCGGCTCCAGGTTTATCTAAAGTTGGCATGTGACTGCTC
303 D W D R G S G R L Q V Y L K L R C D L L
1021 TCAAGAGCCAGGCCCTCAATGTCAAGCTGACCTCCCTCTGCTGAGGGGTGGTCA
323 S K S Q A L N V R L P L P R G V V S
1081 CTGCTCGGAGCTGAGCAGCCGACGAGAGGCTGAGCTGAGCAGAGGAGGAGCTTCCG
343 L S R E L S S P E Q K A E L A E G A L R
1141 TGGGACCTGCTCGGGTGAAGAGGCTCTCAACTCTCAGGCTTTTCCAGATGAGCTC
363 W D L P R V Q G S O L S G L F O M D V
1201 CAGGGGCCCCAGGACCTCCAGGCTGAGGCTCTCAGCTCAGGCTCTCTCTGAGGCTG
383 P G P P G P P S H G L S T S A S P L G L
1261 GGGCTGCGAGTCTCTCTTGGAGTCTCCCGGCGACGCTGCTGCTGCTGCTGCTGCTG
403 G P A S L S F E L P R H T C S G L Q V R
1321 TTCTCAGGCTGGCTTCAAGGCTGAGGAGTCCAGCAACCCCAAGTGGTGGCAGC
423 F L R L A F R P C G N A N P H K W V R
1381 CTAAGCCACAGCGCGCTTGTGCTGAGTGTGAGGCTCCCAACAGAGACAGCAGC
443 L S H S D A Y V I R I *
1441 GCCAAGGTGGGATTTGTCCCAAGGAGGACGCTGTTTCTTCCAGCTCTGCTGCTT
1501 GGGACTCTGAATCTGGGAGGAGAGTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG
1561 CAGGCTTTCTGTTGATCTGATGAGGAGGAGTCTGAGTGAATGAGACTTACAAAGCA
1621 AACTTTTATCTGAGAACTGGGTGATACATTTCTAAAGAAAGAGTACATGAAGGAGG
1681 AATCTACAACTCTCTCCGCTTAGCGAGATGCAAAAAA

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Fig. 1. cDNA and predicted amino acid sequences of the µ-adaptin-related proteins, µ-ARP1 from chicken and µ-ARP2 from human. Underlined are an in-frame stop codon in the 5'-untranslated region of µ-ARP1 that closes the reading frame, and a sequence stretch in the 3'-untranslated region of the µ-ARP2 cDNA that is identical in multiple human ESTs and similar in some rodent ESTs. This sequence may be a repetitive genomic element or a cloning artifact. The µ-ARP1 sequence is a composite of clone 2.5 (nt 1–408) and clone 2.5a (nt 22–1439). The µ-ARP2 sequence is a composite of the 5'-terminal PCR product (nt 1–437) and of IMAGE clone 48136 (nt 195–1732) (see Section 2). The sequences have been deposited in the EMBL sequence database under accession numbers Y08386 and Y08387.

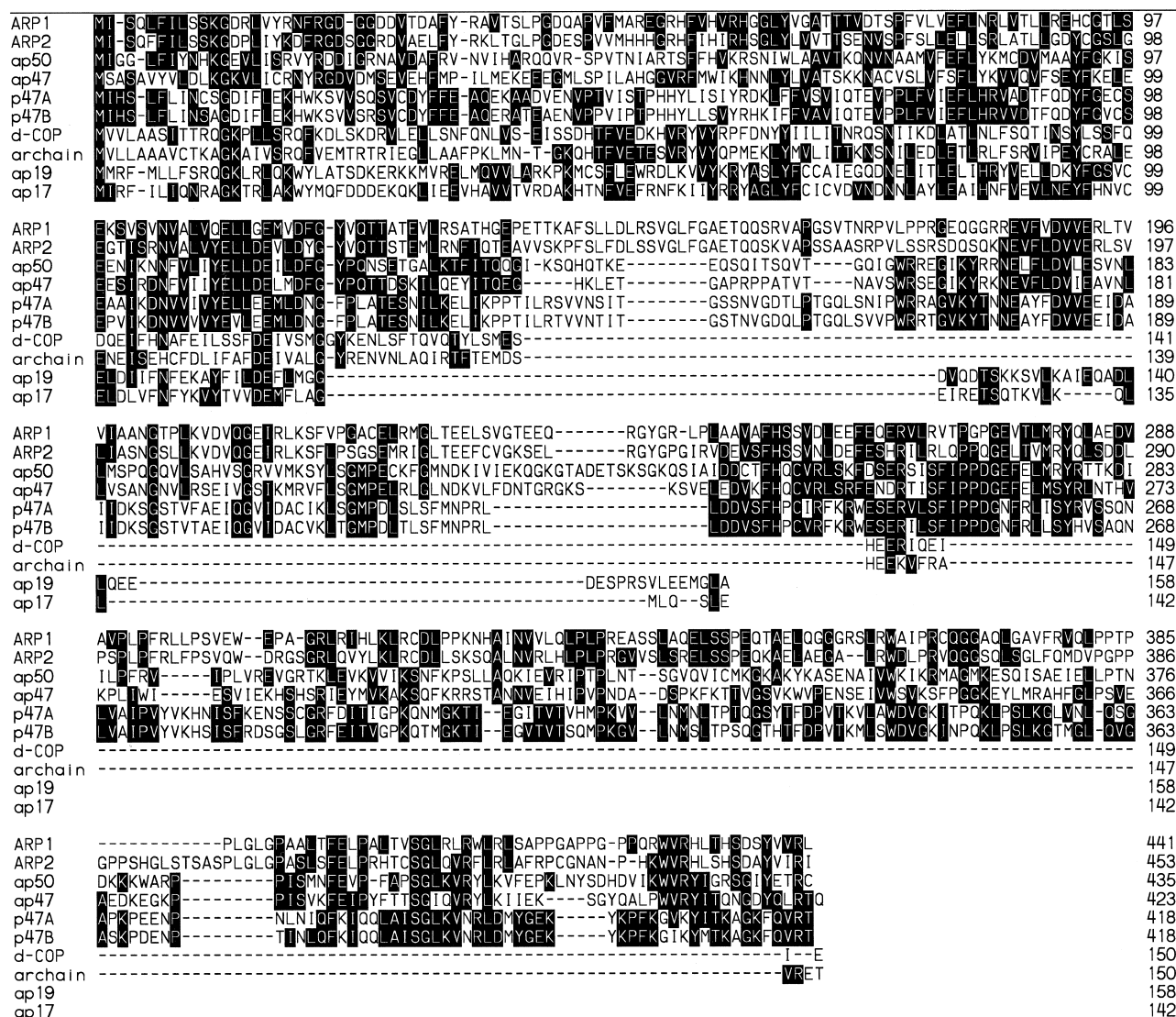


Fig. 2. Sequence alignment of μ-ARPs with μ-adaptins (ap50/47), p47A/B, yeast δ-COP and its full-length human homolog, archain, and σ-adaptins (ap17/19). Consensus residues are shaded. Of δ-COP and archain, only the N-terminal 150 amino acids are included in the comparison to avoid disruption of the alignment by the C-terminal sequence parts that are dissimilar to the other proteins. Alignment was performed with CLUSTAL from the DNASTAR software package. Sequences were taken from the following sources: rat ap50/μ2-adaptin [32], mouse ap47/μ1-adaptin [33], rat p47A and B [15], yeast δ-COP [8], human archain [34], mouse ap19/σ1-adaptin and rat ap17/σ2-adaptin [35].

4. Discussion

μ-ARP1 and μ-ARP2 mRNAs are expressed in all tissues analyzed, though with different relative abundances, indicat-

ing that these proteins serve functions relevant for all cell types. Their sequence similarity to established vesicle coat subunits strongly suggests that they are also components of vesicle coats. Moreover, as they are distinctly more similar to

Table 1
Percent amino acid sequence identity of μ-adaptin-related proteins

	μ-ARP1	μ-ARP2	μ1	μ2	p47A	p47B	δ-COP	archain	σ1
μ-ARP2	60								
μ1 (ap47)	29	31							
μ2 (ap50)	27	31	40						
p47A	23	24	30	27					
p47B	24	25	30	28	84				
δ-COP	16	18	19	21	15	15			
archain	19	22	22	20	18	17	42		
σ1 (ap19)	20	25	21	20	25	25	21	23	
σ2 (ap17)	21	23	19	24	28	29	21	27	46

Sequence comparisons were performed pairwise with the DNASTAR software package (Hein method). For comparisons involving the partial-length homologs (δ-COP, archain, σ1- and σ2-adaptin), only the N-terminal 150 amino acids of all sequences were used.

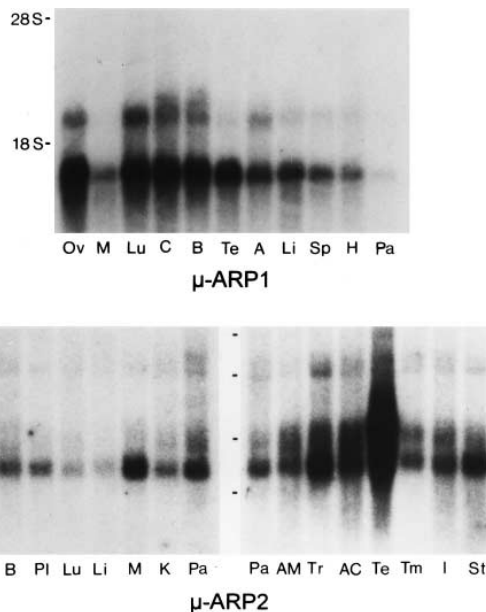


Fig. 3. Tissue distribution of μ -ARP1 and μ -ARP2 mRNA expression. Northern blots were loaded with poly(A)⁺ RNA from the following tissues: Ov, ovary; M, skeletal muscle; Lu, lung; C, cerebellum; B, (fore)brain; Te, testis; A, adrenal gland; AC, adrenal cortex; AM, adrenal medulla; Li, liver; Sp, spleen; H, heart; Pa, pancreas; Pl, placenta; K, kidney; Tr, thyroid; Tm, thymus; I, small intestine; St, stomach.

the μ -adaptins than to δ -COP, it appears quite possible that they are also medium chains of clathrin adaptor complexes. Considering that μ 1-adaptin and μ 2-adaptin each own a complete set of specific isoforms of the other adaptin subunits even though they are more similar to each other (40%) than to μ -ARPs (27–31%), it can be anticipated that μ -ARPs (and perhaps each of them) associate to form coat structures with distinct, novel isoforms or homologs of the other adaptin subunits and perhaps even of clathrin. It remains possible that they are components of non-clathrin coats, but the fact that they are full-length homologs of μ -adaptins but not of δ -COPs relates them more strongly to clathrin coats.

An assignment of mechanistic functions, such as partner protein binding and membrane compartment targeting, to the different subunits of the adaptor complex and their sequence regions is still emerging [20–22]. Recently, evidence has been found for the involvement of μ -adaptins in cargo protein recognition. Formation of clathrin-coated vesicles both at the trans-Golgi network and at the plasma membrane involves the recruitment of specific membrane proteins as cargo and the exclusion of others. Cargo proteins recruited into clathrin-coated vesicles are recognized by specific sequence motifs in their cytoplasmic domains, and one such motif, the tyrosine-based sorting signal, was found to interact with both μ 1- and μ 2-adaptin [23]. It may be meaningful that of all adaptin subunits it is the μ subunit of which the largest number of homologs, six, have been identified to date. Indeed, two new cDNAs encoding one or two additional family members have very recently appeared in the EST database, closely related but not identical to μ 1-adaptin with 77% (amino acids 9–142) and 83% (amino acids 189–302) sequence identity (IMAGE clones 357619 from human fetal heart and 372139

from mouse embryo). Multiple μ -adaptin-related proteins might be instrumental in conferring differential cargo protein selectivity to distinct vesicle populations budding via clathrin-coat- and perhaps also non-clathrin-coat-mediated mechanisms.

Cargo protein specificity, mediated at least in part by a diversity of μ -adaptin-like subunits, could contribute to the selectivity of protein coats for certain membrane compartments. Alternatively, coat proteins with differential cargo selectivity would enable the cell to bud vesicle populations with different protein compositions from the same donor membrane. One attractive site for such a function would be the endosome. Molecular mechanisms mediating the budding, from endosomes, of vesicles with different cargo [24–26] and destined for different target membranes are poorly understood, but evidence indicating the involvement of clathrin- as well as non-clathrin-coat-mediated mechanisms is gathering ([27–31], and references therein). In one study [28], endosome-associated clathrin-coated buds were observed to have distinctly smaller size than plasma-membrane-derived clathrin-coated vesicles and to lack immunoreactivity for both α - and γ -adaptin, suggesting a protein composition that differs from both conventional clathrin coats.

It will be important now to identify the other subunits together with which μ -ARP1 and μ -ARP2 form vesicle coats, to find out to which resident membrane proteins (and sequence motifs) they might bind, and to identify the membrane compartments and vesicle populations with which they are associated.

Acknowledgements: We thank Simone Boldt for her contribution to the isolation of the full-length μ -ARP1 cDNA. This work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie.

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