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Identification of a Putative Yeast Homolog of the Mammalian β Chains of the Clathrin-Associated Protein Complexes

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The clathrin-associated protein complexes are heterotetrameric structures believed to interact with clathrin and with membrane components of mammalian coated pits and coated vesicles. I have identified a yeast homolog of the mammalian β-type large chains, suggesting the existence in yeast cells of clathrin-associated protein complexes. A sequence comparison between the putative yeast β-type chain and its mammalian counterparts shows that their amino-terminal domains are related over their entire length and that their carboxyl-terminal domains diverge completely. This observation is consistent with our earlier proposal (T. Kirchhausen et al., Proc. Natl. Acad. Sci. USA 86:2612–2616, 1989) for the bifunctional-domain organization of the large chains, in which the invariant amino-terminal region interacts with conserved proteins of the coat while the variable carboxyl-terminal domain interacts with different membrane components of coated pits and coated vesicles.

A computer search to uncover proteins related to the β-type large chains of the mammalian clathrin-associated protein (AP) complexes has revealed a DNA sequence that encodes a candidate for a yeast homolog of these polypeptide chains. This yeast DNA sequence is located downstream of the *Saccharomyces cerevisiae* α-agglutinin *AGα1* gene (accession no. M28164) (8). The sequence contains an open reading frame of 2,100 nucleotides, predicting a protein of 700 amino acids and 80,359-Da molecular mass.

AP complexes isolated from calf brains appear to interact in vitro with the cytoplasmic domains of several membrane proteins destined for vesicular traffic (4, 12). The APs are heterotetrameric structures consisting of two large chains of different types (α and β or γ and β′), a medium chain, and a small chain (1, 15; W. Matsui and T. Kirchhausen, Biochemistry, in press). The mammalian β chains, with ~950 amino acids, have two structural and functional domains (6). The amino-terminal domains (~580 amino acids) are highly conserved regions believed to interact with clathrin and with most of the other protein components of the AP complex (2, 5, 6, 15; Matsui and Kirchhausen, in press). The carboxyl-terminal domains (~369 amino acids) are independently folded domains of variable sequence which are postulated to interact with the cytoplasmic domains of membrane proteins (5, 6; Matsui and Kirchhausen, in press).

The sequence alignment between the yeast β-type chain candidate and the two available species of mammalian β chains (6, 14) is consistent with the proposed structural organization of these proteins. As an example, Fig. 1 shows that the sequence alignment of the yeast β-type chain with the rat brain β chain is optimal over the entire amino-terminal domain of the proteins (~580 amino acids), displaying 34% identity (or 58% similarity, allowing for conservative changes) with 14 gaps, most of them less than 3 amino acids in length. Similar results were obtained in a sequence comparison with the related rat brain β′ chain (data not shown). However, the remaining sequence of the yeast protein (~100 amino acids) is unrelated to the carboxyl-terminal domain of either of the mammalian β-type chains. The sequence divergence starts almost precisely at the location of the tryptic cleavage point defining the boundary between the amino- and carboxyl-terminal domains of the mammalian β chain (6). The extent of this sequence divergence does not allow me to establish whether the yeast homolog is more closely related to the mammalian β chain of the AP-2 complex (which is associated with coated structures from the plasma membrane) or to the mammalian β′ chain of the AP-1 complex (associated with coated structures of the Golgi complex). Furthermore, the yeast homolog does not show the preponderance of proline and glycine residues found in the hinge region, between the amino- and carboxyl-terminal domains of the mammalian β chains (6). These results suggest that the putative yeast protein is also divided into two regions, each of a different function. The highly conserved amino-terminal domain may participate in contacts with other conserved proteins present in the coat. Two candidates for such interactions are the yeast clathrin heavy chain (11; S. Lemon, unpublished data) and the recently uncovered yeast homolog of the small chain of the mammalian AP complexes (T. Kirchhausen, A. C. Davies, and S. Frucht, submitted for publication). The second functional region of the yeast homolog of the β-type chains corresponds to the carboxyl-terminal domain. This portion of the polypeptide chain, which is more variable in sequence, may establish contacts with the tails of different classes of proteins presumably located at the cytoplasmic face of the membrane engulfed by the clathrin-coated structures.

I conclude that the clathrin-coated structures of yeasts contain AP complexes. Although direct biochemical confirmation for the actual quaternary composition of the yeast AP complex(es) is still required, it is interesting that at least one group of polypeptide chains with apparent molecular masses in the range of 80,000 to 100,000 Da copurifies with yeast coated vesicles (7, 10). Since this size range includes the predicted molecular mass of the yeast β-type chain homolog described here, I propose that these bands correspond to the large chains of the yeast AP complex. The 80,000-Da species displays the expected stoichiometry with respect to clathrin heavy chains, based on intensities of bands in Coomassie

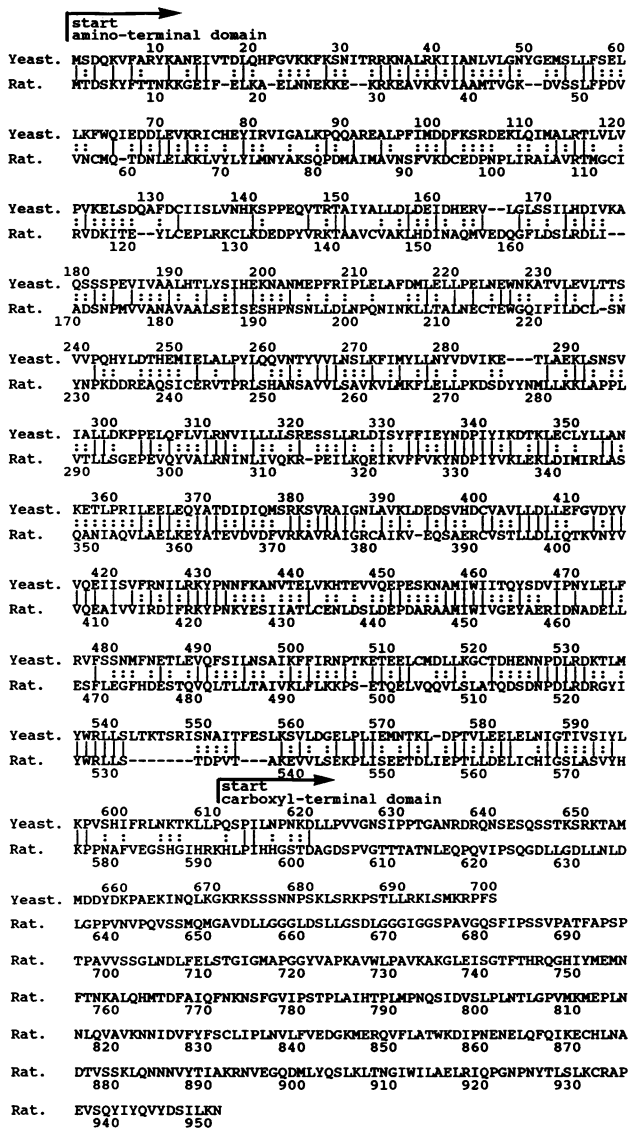

FIG. 1. Sequence comparison of the rat brain β chain and the yeast homolog (single-letter amino acid code). A computer search of the six protein-reading frames of the DNA data bases GenBank (release 63, March 1990) and EMBL (release 22, February 1990) with the program TFASTA (13) identified a DNA sequence in yeasts containing an open reading frame with significant identity to the mammalian β-type chains of AP complexes. This predicted sequence spans nucleotide positions 493 to 2591 of the published DNA sequence (8) and is corrected by insertion of a missing C nucleotide at position 1979. The open reading frame is located downstream from and in the opposite orientation to the α-agglutinin *AGα1* gene (accession no. M2164). The optimal sequence alignment presented here was obtained with the program FASTA (3, 9). Vertical lines indicate identities; colons represent conservative replacements. A polymerase chain reaction performed on yeast genomic DNA over the entire carboxyl-terminal domain and part of the 3′ untranslated region (between nucleotide positions 388 and 862 of the published DNA sequence with the synthetic oligonucleotide primers TK133F [5′-CGT TTT GGA GGA ATT GGA GC] and TK132R [5′-TAT GAT CGC TTA GTT AAG TC]) generated a DNA fragment of the expected size (470 bp; data not shown). This result rules out the possibility that the sequence divergence detected between the yeast and mammalian β-type chains is due to a cloning artifact of the yeast genomic DNA.

blue-stained sodium dodecyl sulfate gels (7, 10). Further evidence that AP complexes are present in yeast cells comes from the discovery of another yeast gene whose predicted protein product displays a very high level of sequence similarity (76%) with the mammalian versions of the small chains of the AP complexes (Kirchhausen et al., submitted).

The GenBank accession number for the sequence discussed in this paper is M38683.

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