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Characterization of Yeast Clathrin and Anticlathrin Heavy-Chain Monoclonal Antibodies

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Clathrin-coated vesicles (CVs) were isolated from *Saccharomyces cerevisiae* by using procedures developed by Mueller and Branton [17]. Triskelions were purified from this material by extraction of CVs to release clathrin and by subsequent fractionation on Sepharose CL-4B. Triskelions were composed of ~180,000 M_r heavy chains and a single light-chain type of ~38,000 M_r and were able to undergo self-assembly into polyhedral cages. Trypsin digestion of such reassembled cages showed a peptide pattern very similar to that obtained for mammalian clathrin with two fragments of 125,000 and 110,000 M_r, which represent the major portion of the heavy-chain arm, and a polypeptide of ~43,000 M_r, which is the presumptive terminal domain.

Eight monoclonal antibodies reacting with yeast clathrin heavy chains were produced. All eight bind to the major portion of the heavy-chain arm, and none bind to the terminal domain fragment. Peptide digestion experiments also indicated that at least three major regions on the arm are recognized by these antibodies. These will be useful in further structural and functional studies of clathrin from yeast.

Key words: coated vesicles, clathrin, reassembly, proteolytic digestion, *Saccharomyces cerevisiae*

Clathrin-coated membranes and vesicles are thought to be involved in the selective transfer of membrane and membrane-associated components between different subcellular compartments [1–3]. Coated vesicles (CVs) are important in receptor-mediated endocytosis, which is widely recognized as a major mechanism by which animal cells take up a variety of molecules from the extracellular environment [1,4,5]. Clathrin has also been implicated in membrane recycling [6,7] and in some aspects of vesicular transfer of newly synthesized proteins and membranes that must traverse the secretory apparatus in order to reach their final cellular destinations [2,8,9].

Electron microscopic examination of CVs has revealed a distinctive polygonal surface lattice that surrounds a membrane vesicle [10]. The basic proteinaceous

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subunit of this surface structure is a trimeric molecule or triskelion, which is composed of three clathrin heavy chains of approximately 180,000 M_r and three light chains of 30,000–40,000 M_r [11–13]. The heavy chains are joined to form a vertex with three legs extending radially [13]. The light chains, which are usually of two types in mammalian cells, are bound near the center of the triskelion structure, one per heavy-chain arm [14,15]. Isolated mammalian clathrin trimers have the ability to reassemble spontaneously into polyhedral basketlike structures in vitro [16], and this property of clathrin is thought to be important in driving the formation of coated pits and the pinching off of clathrin CVs within cells [3].

CVs have been isolated from virtually all eukaryotes examined. Recently Mueller and Branton identified CVs in *Saccharomyces cerevisiae* [17]. Their development of a procedure for isolation of CVs from yeast paved the way for further examination of clathrin's function in a simple organism that is highly amenable to molecular and genetic manipulation [18,19]. Here, as the starting point for our investigations of coated vesicle function, we present further evidence that yeast clathrin is structurally and functionally similar to clathrin from mammals. Also, we report on our initial characterization of a set of monoclonal antibodies that bind to the yeast heavy chain.

MATERIALS AND METHODS

Materials

Zymolyase 60,000 was from Kirin Laboratories (Japan); dithiothreitol (DTT) was from Calbiochem—Behring (San Diego, CA); phenylmethyl sulfonyl fluoride (PMSF), RNase, Sephacryl S-1000, Sepharose CL-4B, MES buffer, egg lecithin, TPCK-trypsin, and soybean trypsin inhibitor were purchased from Sigma Chemical Co (St. Louis, MO); Tris base was from Schwartz-Mann (Cleveland, OH) or Sigma; nitrocellulose was from Schleicher and Schuell (Keen, NH); Balb/c mice were from Jackson Laboratories (Bar Harbor, ME); and serum was from Gibco (Grand Island, NY).

Purification of Clathrin

Purification of crude clathrin CVs from the protease-deficient *S. cerevisiae* strain, BJ926 (*α/a trp1/TRP1 his1/HIS1 prc1/prc1 prb1/prb1 pep4/pep4 can1/can1 gal2/gal2*), was based on the method of Mueller and Branton [17]. Cells were grown to late log phase (OD_{600} ~8–9) in 6 liters YEP-sucrose (2% sucrose, 2% peptone, and 1% yeast extract), harvested by centrifugation for 5 min at 1,000 g, washed once with dH_2O, and stored at 4°C overnight in 500 ml 0.1 M Tris, pH 7.6. Cells were repelleted after dilution fourfold with dH_2O, resuspended in 1 liter 10 mM Tris, 10 mM $CaCl_2$, 2 mM DTT, 0.6 mM PMSF, 1.2 M sorbitol, pH 7.5 final, and shaken gently in a rotary shaker for 10–15 min at 30°C. Spheroplasting was initiated by the addition of zymolyase 60,000 to 10–30 μg/ml and cells were shaken at 30°C until a tenfold decrease in OD_{600} of cells diluted into 10% sodium dodecyl sulfate (SDS) was observed. Spheroplasted cells were spun at 500g for 15 min and washed twice at 4°C in 1.2 M sorbitol, 0.1 M MES, pH 6.5, 0.5 mM $MgCl_2$, 1.0 mM EGTA, 0.2 mM DTT, 0.6 mM PMSF. Cells were lysed in 400 ml of buffer A (0.1 M MES, pH 6.5, 0.5 mM $MgCl_2$, 1.0 mM EGTA, 0.2 mM DTT, 0.02% NaN_3, 0.6 mM PMSF) including 0.1 μg/ml RNase (Sigma type IA) by shaking gently for 30 min at 30°C. The lysate was spun at 21,000g for 30 min at 4°C, and the subsequent supernatant was centrifuged at 100,000g for 60 min at 4°C.

The 100,000g pellet was resuspended in 5–8 ml of buffer A by Dounce homogenization and spun 5 min at 10,000g to sediment aggregated material. The milky supernatant was applied to a Sephacryl S-1000 column (1.8 × 110 cm, precoated with egg lecithin phospholipid vesicles) and eluted in buffer A. Fractions containing clathrin CVs were pooled and spun at 100,000g for 60 min.

Pellets were resuspended in 2–3 ml buffer A by Dounce homogenization and extracted by addition of an equal volume of 1.0 M Tris, pH 7.0. After 15 min at room temperature, the extract was spun at 100,000g for 60 min [16]. This supernatant, an enriched triskelion preparation, was stored at 4°C.

The Tris-extracted supernatants of two consecutive clathrin preparations were pooled and concentrated by 70% ammonium sulfate precipitation. The pellet was resuspended in 0.6–1.0 ml 1 M Tris, pH 7.0/buffer A (1:1), spun 1–2 min in a microfuge to sediment insoluble material, loaded onto a Sepharose CL-4B column (0.9 × 57 cm), and eluted in the same buffer at room temperature [16]. The major peak of clathrin was pooled and concentrated with a Centricon-30 miniconcentrator (Amicon, Danvers, MA) to approximately 1.0 ml. This material, which was composed primarily of clathrin heavy and light chains, could be stored at −20°C for long periods of time and was still suitable for reassembly into cages. Yields were approximately 0.5 mg per 100 g cells.

Reassembly Into Clathrin Cages

Purified triskelions (0.2 mg/ml) were dialyzed against 20 mM MES, pH 6.2, 2 mM $CaCl_2$ [12]. Material was sedimented at 100,000g to recover assembled and unassembled clathrin.

Preparation of Monoclonal Antibodies (mABs)

Balb/c mice were immunized intraperitoneally with 20–40 μg of purified clathrin triskelions in Freund's complete adjuvant and boosted 4 wk later with clathrin in incomplete adjuvant. After 12 days mice were bled and sera were tested for anticlathrin antibodies by dot blot analysis (see below). After 6-wk rest the mouse with the highest titer was boosted intravenously and intraperitoneally for 4 consecutive days with 20–40 μg/day of clathrin triskelions. On the fifth day spleen cells were fused to NS-1 mouse myeloma cells by using standard procedures [20]. Cells were plated into ten 96-well plates. Ten days later over 90% of the wells contained hybridoma colonies. Hybridoma supernatants from 5 plates were screened by dot blot analysis. In the initial screen 27 positive wells were identified, 18 were transferred for amplification and tested on immunoblots, and 8 were eventually cloned at least three times by limiting dilution.

Dot Blot and Immunoblot Analysis

Dot blots were performed in 96-well dishes as described by Hawkes et al [21]. Ten to 20 ng of yeast clathrin triskelions were spotted onto 3–4-mm^2 pieces of nitrocellulose and blocked in 10% fetal bovine or horse serum. For immunoblots [22] proteins were separated by SDS polyacrylamide gel electrophoresis (PAGE), transferred to nitrocellulose, and blocked as above. In the case of clathrin triskelions, ~20 μg of material was applied to preparative gels (~12 cm wide). For blots of yeast extracts, 40 μg protein per lane was applied. Blots were incubated overnight at 4°C with primary antibody. Antibody binding was detected by using the Vectastain anti-

mouse ABC kit (Vector Laboratories, Burlingame, CA) with three to four washes for 5–10 min each in phosphate-buffered saline containing 0.05% Tween-20 between incubations.

Trypsin Digestion of Reassembled Cages

Clathrin was reassembled into baskets and resuspended cages (~50 μg protein) were digested with pancreatic TPCK-trypsin essentially as described by Hanspal et al [23] at a weight ratio of ~5:1 clathrin to enzyme. At various times aliquots were removed and proteolysis was stopped with a twofold excess (w/w) of soybean trypsin inhibitor to trypsin. Samples were analyzed on 10% SDS gels and stained with Coomassie blue R. The sample remaining after 180 min of digestion was used for immunoblots.

Electron Microscopy

A drop of material was placed on a 400-mesh Formvar-carbon-coated grid and excess liquid was blotted away. Samples were negatively stained with 1% uranyl acetate, air dried, and examined in a Philips EM300 microscope.

Other Methods

SDS-PAGE was performed as described by Laemmli [24] with 7.5% acrylamide gels, except where indicated otherwise. Protein concentrations were determined by the method of Bradford [25] with the reagent purchased from Biorad Laboratories. (Richmond, CA) Immunoglobulin subclass typing was determined utilizing the Mouse-Ig Subtype Identification Kit from Boehringer Mannheim (Indianapolis, IN). L-α phosphatidylcholine phospholipid vesicles were made from egg yolk L-α lecithin (Sigma V-E) that was lyophilized, resuspended at 10 mg/ml in buffer A, and sonicated for 20 × 30 sec bursts at 60 W on a Branson sonifier. Insoluble material was sedimented by centrifugation for 30 min at 12,000g and 5–8 ml was loaded to the Sephacryl S-1000 column for coating.

RESULTS

Purification of Clathrin

Crude clathrin CVs were isolated from yeast strain BJ926 as described by Mueller and Branton [17] with some minor modifications. We observed that the 280-nm absorption profile from the Sephacryl S-1000 column varied from preparation to preparation, but the CVs eluted consistently as described previously [17]. Examination of the crude CV pool by electron microscopy after negative staining confirmed the presence of cagelike lattice structures (60–70 nm), as well as some contaminating larger and smaller smooth vesicles (not shown) [17].

The CV pool was extracted with 1 M Tris, pH 7.0/buffer A (1:1 v/v) to release clathrin as described by Keen et al [16], and the 100,000g pellets and supernatants were analyzed by SDS-PAGE. The Coomassie blue staining pattern (Fig. 1A) shows that the CV pool was highly enriched for a 180,000 M_r protein, presumably the clathrin heavy chain, which was recovered quantitatively in the Tris-extracted supernatant. Several other components were present, including several minor species of ~100,000–110,000 M_r and a ~90,000 M_r peptide. Another major component of 75,000–80,000 M_r remained associated with the extracted pellet, although some of

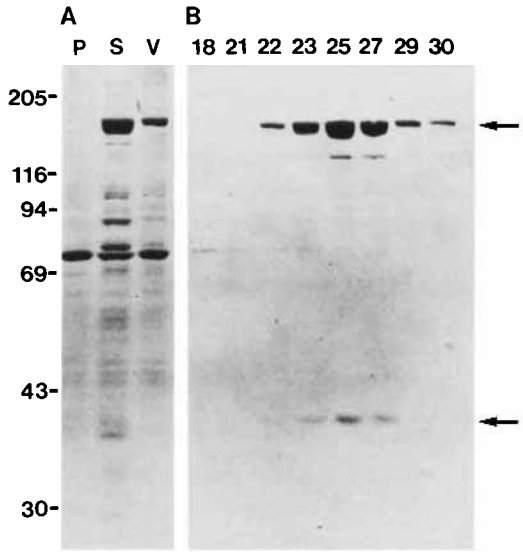

Fig. 1. Coomassie blue staining pattern of clathrin purification fractions analyzed by SDS polyacrylamide gel electrophoresis (SDS-PAGE). **A:** The resuspended coated vesicle pool (V) from the Sephacryl S-1000 column was extracted and spun at 100,000g to generate a Tris-extracted supernatant (S) and pellet (P). **B:** Major clathrin peak fractions from the Sepharose CL-4B column (see Fig. 2); 20 μl from each fraction indicated was applied per lane. The clathrin heavy ($\sim$180,000 M_r) and light ($\sim$38,000 M_r) chains are indicated by arrows. Molecular weight standards from top to bottom are myosin (205 kd), β-galactosidase (116 kd), phosphorylase b (94 kd), bovine serum albumin (69 kd), ovalbumin (43 kd) and carbonic anhydrase (30 kd).

the same or a unique protein of identical molecular weight was found in the supernatant. A portion of this 75,000–80,000-M_r protein was probably contained in residual vesicular material in the soluble fraction (see below).

The Tris-extracted fraction was applied to a Sepharose CL-4B column (Fig. 2). A major UV-absorbing peak of protein (fractions 22–29) eluted with a V_I similar to that observed for mammalian triskelions [16], and this was shown to contain primarily the 180,000-M_r protein and a single light-chain type of 38,000 M_r when examined by SDS-PAGE (Fig. 1B). Some minor bands were also visible and most of these represented minor breakdown products of the heavy chain (see below). Small amounts of the 75,000–80,000 M_r component were carried over from the void volume (fraction 18), which displayed considerable absorbance at 280 nm but contained little protein. This suggests there was residual vesicular material in the load fraction. Comparison on SDS gels confirmed that the yeast heavy chain is slightly larger than the bovine brain molecule [17] and that the yeast light chain is also larger than the two major bovine brain light-chain types (not shown).

Reassembly of Clathrin Triskelions

Evidence that the Sepharose CL-4B–purified material had additional properties similar to those of mammalian clathrin was obtained by reassembly into sedimentable cages. Under the conditions utilized, approximately 50% of the yeast clathrin appeared in the 100,000g pellet, which included both heavy and light chains (not shown). Figure 3 shows examples of reassembled baskets when examined by electron

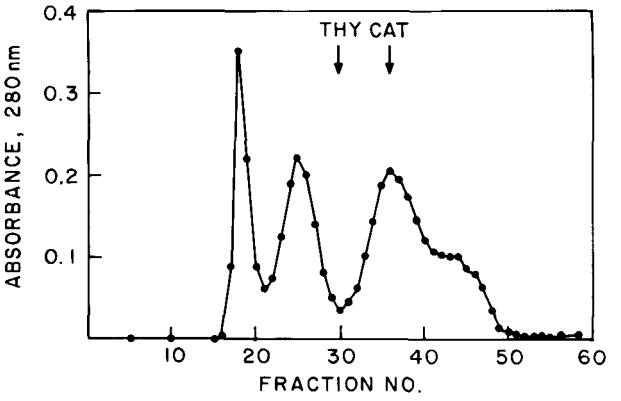

Fig. 2. Sepharose CL-4B gel filtration elution pattern of the Tris-extracted supernatant combined from two coated vesicle preparations. The Tris-extracted supernatant was concentrated and applied to a Sepharose CL-4B column and eluted at 2–3 ml/hr; 0.75-ml fractions were collected. Standards indicated are thyroglobulin (THY, 669 kd) and catalase (CAT, 232 kd).

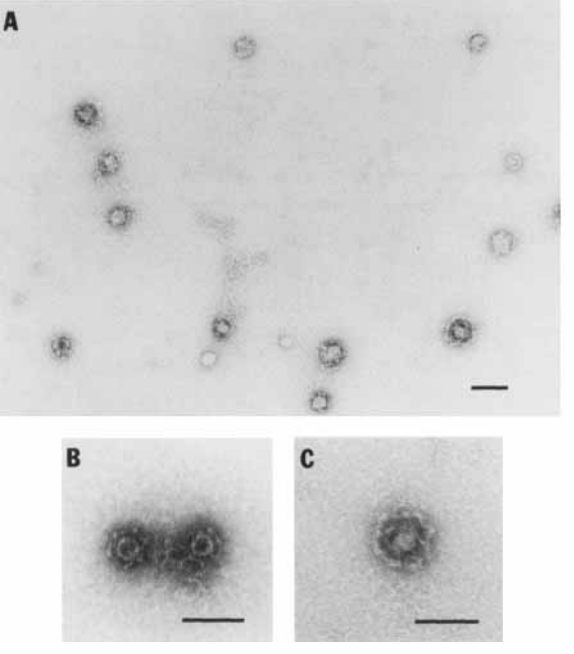

Fig. 3. Electron micrographs of negatively stained reassembled clathrin cages. Scale bars, 100 nm.

microscopy after negative staining. The size distribution was quite broad (60–100 nm), which was similar to the size range observed for bovine clathrin cages reassembled from purified triskelions in the presence of calcium [26]. In addition, as reported previously for yeast CVs, we noticed that cages frequently fell apart on the grids during preparation for electron microscopy [17] (see Fig. 3B).

Monoclonal Antibodies Bind to the Yeast Clathrin Heavy Chain

Eight hybridomas producing mABs to yeast clathrin were cloned. The Ig subtypes of these are indicated in Table I. All eight antibodies bind to the clathrin heavy chain on immunoblots (Fig. 4). The right-hand portion of the panel shows the antibody binding pattern when several of the hybridoma supernatants were tested at lower dilutions. Several unique patterns of minor lower molecular weight peptides, presumably breakdown products of the heavy chain, were revealed with the more concentrated antibodies. This suggested that some of the mABs bind to different epitopes on the heavy chain. Clathrin could also be detected in immunoblots of whole yeast extracts with the mABs; however, none of the mABs crossreacted significantly with bovine brain clathrin (not shown).

Antibody Binding to Peptides From Proteolyzed Clathrin Baskets

Studies of the mAB binding sites on the clathrin heavy-chain arm were initiated by probing immunoblots of trypsin-digested baskets. The left panel of Figure 5 shows the Coomassie blue staining pattern of digests over a 3-hr time period. The digestion pattern shows several similarities to that of bovine clathrin cages [14,23,27,28]. First, the light chain was highly susceptible to the enzyme and was completely degraded. The heavy chain was proteolyzed into two major components—a pair of peptides of 125,000 M_r and 110,000 M_r and a peptide of 43,000–44,000 M_r. In addition, the 110,000-M_r fragment appeared to be the product of further processing of the 125,000-M_r peptide that was generated first, and the 43,000-M_r peptide appeared initially as a slightly larger component of 44,000 M_r. By homology to mammalian clathrin, the 125,000/110,000-M_r pair probably represents the major portion of the extended clathrin heavy-chain arm and the 43,000-M_r species is probably a portion of the globular terminal domain of the triskelion arm. In contrast to mammalian clathrin, no 50,000–60,000 M_r proteolytic precursor of the 43,000-M_r terminal domain was observed, even at the earliest (2–5 min) time points.

Immunoblot analysis of trypsin-digested cages showed that each of the eight antiyeast clathrin mABs binds to the major portion of the heavy chain and not to the 43,000-M_r terminal domain (Fig. 5, right). In all cases, both the 125,000-M_r and the 110,00-M_r peptides reacted strongly. Furthermore, there were three distinct patterns of minor peptide breakdown products that indicate there are at least three different epitopes recognized by the anti-heavy-chain mABs. These have been grouped into three major binding classes—A, B and C—as shown in Table I. Yeast anti-heavy-

TABLE I. Ig Subtypes and Clathrin Binding Classes of Eight Cloned Hybridomas Producing mABs to Yeast Clathrin

Monoclonal AB	Ig subtype	Clathrin binding class
1	IgG_1	B
2	IgA	C
3	IgG_1	C
4	IgG_1	C
5	IgG_{2b}	A
6	IgG_{2a}	A
7	IgA	C
8	IgG_1	C

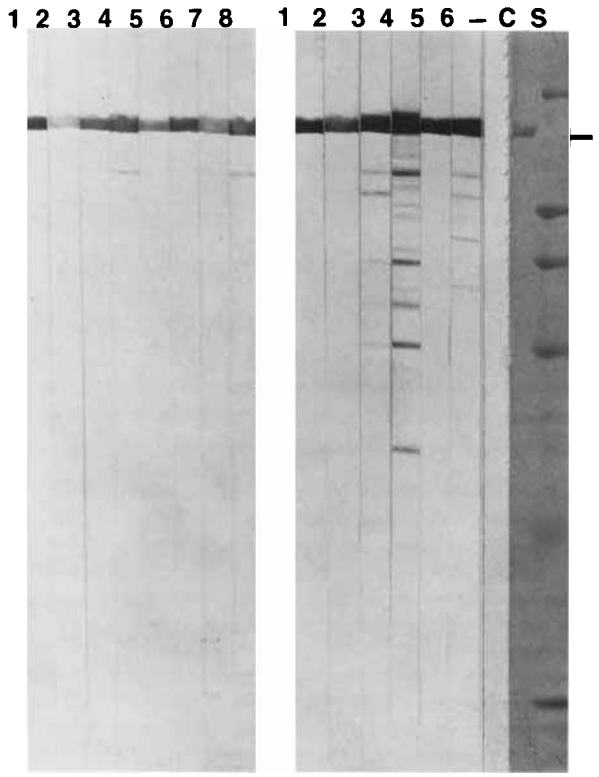

Fig. 4. Pattern of monoclonal antibody (mAB) binding to clathrin triskelions fractionated on SDS gels and transferred to nitrocellulose for immunoblot analysis. **Left:** lanes (1–8), anticlathrin monoclonal antibodies 1–8 tested at 1/100 dilution of hybridoma supernatants. **Right:** lanes (1–6), monoclonal antibodies 1–6 tested at 1/10 dilution of hybridoma supernatants; lane (-), staining in the absence of primary antibody; lanes (C) and (S), amido black staining pattern of clathrin triskelions and molecular weight standards (see Fig. 1), respectively. Arrow indicates heavy chain.

chain mAB-5 and -6 form peptide binding class A, mAB-1 forms a class of its own (class B), and mABs -2, -3, -4, -7, and -8 form class C. Monoclonal antibodies-2 and -7 may be derived from a single B cell clone, since their binding patterns are the same and because isolation of hybridomas secreting mABs of the IgA type is rare. In over 16 fusions and in subtyping 50 hybridomas, this is the first appearance of an IgA mAB (V. Lemmon, unpublished observations). In addition mAB-3 or -4 may form a distinct subgroup in class C, since they bind to different minor breakdown products of the heavy chain (see Fig. 4).

Western blots of *S. aureus* V-8 protease-digested [29] triskelions were also probed with the mABs and confirmed the three major binding classes obtained with trypsin-digested baskets (not shown).

DISCUSSION

Clathrin from the yeast *S. cerevisiae* has been purified and characterized further in order to undertake molecular and biochemical studies, which should further our understanding of the function of this molecule. The procedure we have used to isolate

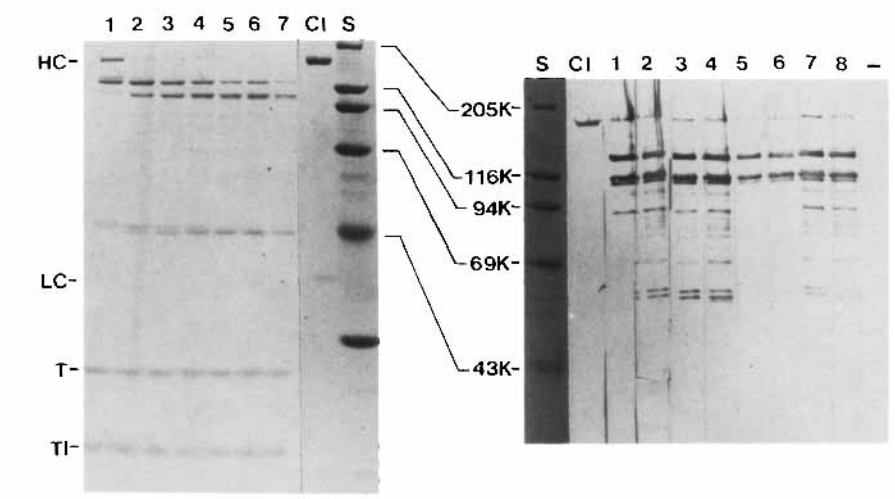

Fig. 5. Peptide pattern of reassembled baskets after digestion with trypsin as analyzed by SDS-PAGE and immunoblots. **Left:** Reassembled clathrin baskets were digested with trypsin over 3 hr with aliquots removed at various times, and reactions were stopped by addition of soybean trypsin inhibitor. Samples were run on SDS gels and stained with Coomassie blue. Lanes 1–7 are 2, 15, 30, 60, 90, 120, and 180 min of proteolysis, respectively. Light chains (LC); heavy chains (HC); trypsin (T); trypsin inhibitor (TI). **Right:** Remaining sample after 3 hr of digestion was analyzed by immunoblot analysis. Lanes 1–8 represent the staining patterns of mABs 1–8; (-) no primary antibody. (Molecular weight standards (S), undigested clathrin triskelions (Cl)).

clathrin triskelions from yeast includes a slightly modified version of the method of Mueller and Branton [17], in combination with the procedures described by Keen et al [16] for release of triskelions from CVs and their subsequent chromatography on a Sepharose column. Similar purification of yeast clathrin triskelions, which are composed of heavy chains of ~180,000 M_r and light chains of ~38,000 M_r, has been obtained by Payne and Schekman [18], who utilized 2 M urea for extraction of crude CVs.

Most mammalian cells contain two major classes of light chains, LCA and LCB, as well as subspecies of each of these classes, which appear to be derived from differential in-frame splicing of mRNA transcripts [30–33]. Yeast have only one light-chain type (this report) [18], and this has an M_r, as determined by SDS-PAGE, that is larger than LCA and LCB from bovine brain, which contains the largest of the mammalian light-chain subspecies. These findings provide considerable interest in determining whether the yeast light chain is more homologous to the LCA or LCB class and in determining whether the apparent larger size of the yeast light chain is due to an additional in-frame insertion in the same region where they occur in the mammalian light-chain subspecies.

In addition to clathrin, several other proteins copurified with the CV fraction. Of particular interest are a group of proteins in the range of 100,000–110,000 M_r, which could be related to the "assembly proteins" that are associated with mammalian CVs [16,26,34,35] and are thought to be important for binding of triskelions to target membranes [36]. Preliminary immunoprecipitation experiments with the eight mABs have shown that these proteins coprecipitate with clathrin from the yeast crude CV

pool, but further evidence for or against a relationship to the assembly polypeptides is required.

In previous reports [17,18], the presence of major copurifying proteins in the range of 50,000–60,000 M_r was also observed in the yeast CV fractions. In contrast, we have found the presence of large amounts of those proteins to be quite variable. In the clathrin preparation shown in Figure 3, very small amounts of polypeptides of this size were present in the CV pool. In other experiments they were a major constituent of this pool, but the majority of the 50,000–60,000 M_r proteins do not appear to immunoprecipitate with yeast CVs (unpublished). In mammalian cells there is a 50,000-M_r assembly protein [16,34,35] that forms a stoichiometric complex with a group of the 100,000–110,000 M_r-assembly peptides [35]. It is possible that the 50,000-M_r assembly protein is present as a minor band in our gels.

Yeast triskelions undergo reassembly into sedimentable cages under conditions that have been used to reassemble mammalian clathrin [12,26]. The assembly, at least by the criterion of sedimentation at 100,000g, was quite efficient, even though relatively low concentrations of protein were used in the dialysis. We feel that most of the sedimentable material was contained in baskets, because we generally did not observe large amounts of amorphous aggregates in electron micrographs of negatively stained cage samples and because the proteolysis of cages was very similar to that of mammalian cages.

The fragmentation pattern of clathrin generated from trypsin treatment of yeast cages showed one major difference when compared to digests of bovine brain baskets [14,23,27,28]. No 50,000–60,000-M_r precursor to the 43,000-M_r terminal domain was observed for yeast digests. The same result was obtained when cages were treated at 37°C with trypsin present at 1% of the clathrin weight as described by Schmid et al [27]. The yeast clathrin heavy chain is slightly larger than the bovine molecule as assessed by SDS-PAGE, and it has been reported that the contour length of the triskelion leg from yeast appears longer than that reported for bovine (~490 Å compared to ~445 Å) [17]. When mammalian cages are digested the terminal domain is released into the soluble fraction and the remainder of the cage remains reasonably intact and can be sedimented [14,23,27,28]. Triskelions can be isolated and the heavy-chain arm is shortened at the terminus. We assume that the digestion pattern for yeast clathrin reflects a similar shortening of the triskelion arm. We suggest that the larger size of the yeast heavy chain might be accounted for by an extension of the flexible connector region between the terminal domain and the rest of the arm. This might allow greater accessibility of the proteolytic enzyme to the junction area of the arm in assembled cages, which could result in direct generation of the 43,000-M_r terminal domain without formation of a 50,000–60,000-M_r intermediate. Alternatively, the terminal domain may have an exposed extension at its end that is cleaved off early, generating the 43,000-M_r peptide. Other possibilities exist for the difference in the proteolytic pattern and the relative sizes of the two molecules, and comparison of the yeast and mammalian heavy-chain protein sequences may help to resolve this issue.

We have produced eight monoclonal antibodies that bind to the 180,000-M_r heavy chain from yeast. Antibodies to mammalian heavy chains initially were difficult to generate in animals, presumably because of conservation of the molecule [37–39]. Therefore, it is not surprising that the antiyeast clathrin mABs do not crossreact with the bovine brain counterpart. However, we have obtained 2 mABs (gift of Greg

Fisher, Carnegie Mellon University) that were made against bovine clathrin; at least one crossreacts with sea urchin clathrin and both bind to yeast heavy chains on immunoblots. This suggests that there are highly conserved domains amongst the heavy chains of all species. Sequencing of the cloned genes from yeast and rat confirm this. We have used the eight antiyeast mABs to clone the gene for the yeast 180,000-M_r protein [19] from a λgt11 expression library [40]. The portion of the yeast gene that we have sequenced encodes a polypeptide that is highly homologous to the same region of the rat brain clathrin heavy chain ($\geq$50% identity, with stretches of 20–30 amino acids of virtually 100% identity) (unpublished; T. Kirchhausen, personal communication).

Each of the eight antiyeast mABs binds to the major arm of the heavy chain and not to the terminal domain. The eight mABS can be grouped into three major classes based on their binding to peptide fragments in trypsin and *S. aureus* V-8 digestion experiments. These classes are ordered to reflect the extent of binding to minor, smaller-molecular-weight trypsin cleavage products of the major heavy-chain arm. Class A mABs bind exclusively to the major 110,000-M_r and 125,000-M_r fragments; class B mABs bind to these major components as well as fragments as small as ~90,000 M_r, while class C mABs bind to many proteolytic fragments down to approximately 60,000 M_r. Efforts are in progress to determine whether these binding patterns correspond to the order of the epitopes recognized by the mABs along the heavy-chain arm.

In conclusion, we have extended the characterization of clathrin from yeast. We have shown that it undergoes self-assembly to form polyhedral cages and that the clathrin in these cages is susceptible to proteolytic fragmentation which generates a pattern that resembles that of mammalian clathrins. We have also generated a battery of monoclonal antibodies that bind to at least three distinct regions on the clathrin heavy-chain arm. These antibodies have already been used in the molecular cloning of the yeast clathrin heavy-chain gene and will enable us to perform further structural and functional studies on this molecule.

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