Yeast Clathrin Has a Distinctive Light Chain That Is Important for Cell Growth

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Abstract. The structure and physiologic role of clathrin light chain has been explored by purification of the protein from *Saccharomyces cerevisiae*, molecular cloning of the gene, and disruption of the chromosomal locus. The single light chain protein from yeast shares many physical properties with the mammalian light chains, in spite of considerable sequence divergence. Within the limited amino acid sequence identity between yeast and mammalian light chains (18% overall), three regions are notable. The carboxy termini of yeast light chain and mammalian light chain LC_b are 39% homologous. Yeast light chain contains an amino-terminal region 45% homologous to a domain that is completely conserved among mammalian light chains. Lastly, a possible homolog of the tissue-specific insert of LC_b is detected in the yeast gene. Disruption of the yeast gene (*CLC1*) leads to a slow-growth phenotype similar to that seen in strains that lack clathrin heavy chain. However, light chain gene deletion is not lethal to a strain that cannot sustain a heavy chain gene disruption. Light chain–deficient strains frequently give rise to variants that grow more rapidly but do not express an immunologically related light chain species. These properties suggest that clathrin light chain serves an important role in cell growth that can be compensated in light chain deficient cells.

CLATHRIN is a major coat-forming protein that encloses vesicles and forms cell surface patches involved in membrane traffic within eukaryotic cells (for review see reference 7). The clathrin coat can be disassembled by treatment with urea into units referred to as triskelions, which consist of three heavy (180-kD) and three light (23–27-kD) chains (26, 58). The heavy chains form three kinked arms, with one light chain noncovalently associated with each heavy chain in the region between the triskelion vertex and elbow (27, 57). Mammalian cells contain two classes of light chains, LC_a and LC_b, which are distributed randomly among isolated triskelions (27). Molecular cloning and sequence analysis of the LC_a and LC_b genes from cow, rat, and human show that the genes are distinct but related (23, 24, 29). Each class contains at least two tissue-specific subtypes. Light chains expressed in brain contain all sequences found in light chains from other tissues plus an additional brain-specific insert that arises from alternative splicing of the mRNA.

The function of clathrin light chain is not known, nor is it clear how cells exploit the differences in the various light chain forms. Speculation has focused on a role for light chain in the assembly or disassembly of clathrin coats. Isolated triskelions assemble spontaneously into cage structures similar to the clathrin lattice enclosing coated vesicles. The heavy chain is responsible for forming the connections in these cages as limited proteolysis with trypsin or elastase removes light chain without disassembling the cage structure (26, 52, 60). Trypsin-treated triskelions lacking light chain and a portion of the distal heavy chain arm assemble into regular cages (52). However, elastase-treated triskelions, which contain intact heavy chain, assemble irregularly (26, 52). The difference in cage-forming ability between elastase- and trypsin-treated triskelions suggests that light chain is required to position the outer heavy chain arm properly during cage assembly. Indeed, the direction of curvature of the outer heavy chain arm is randomized after treatment with elastase (52). Furthermore, regions of heavy chain involved in light chain binding and mediating heavy chain–heavy chain contacts in assembled cages are located close together on the triskelion arm (4). Thus, light chain may modulate assembly or influence the geometry of the heavy chain in assembling cages, while not being absolutely required for the fundamental cage structure.

Light chains may function in disassembly of the coat. During receptor-mediated endocytosis, clathrin coats dissociate shortly after vesicles bud from the plasma membrane. In a purified system, coated vesicles and cages are disassembled by a 70-kD heat shock cognate protein, also referred to as uncoating ATPase (6, 9). Disassembly requires that cages contain light chains (51).

The physiologic role of clathrin heavy chain has been ex-

plored by deletion of the single heavy chain gene in *Saccharomyces cerevisiae* (34, 36, 46–49). Most strains survive but grow slowly when the heavy chain gene is deleted (48). Although most avenues of protein transport are not disrupted in heavy chain deficient strains, the pathway of propheromone processing is incapacitated because of a defect in localization of a processing enzyme that normally resides in the Golgi apparatus (47).

To test the role that light chain performs in vivo, we purified yeast clathrin light chain, which allowed us to isolate the light chain gene and disrupt the corresponding chromosomal locus. The results presented here suggest that light chain, like heavy chain, is important in normal cell growth.

Materials and Methods

Strains, Plasmids, and General Methods

Yeast and bacterial strains used in this study are listed in Table I. The high copy number (2μ-based) plasmid YEp352 (20) was used to overproduce yeast clathrin light chain. pUC118 and 119 (59) were used in sequencing and subcloning. pCS19 contains the 1.7-kb Bam HI fragment containing the *HIS3* gene (54) and was kindly provided by Colin Stirling, (Division of Biochemistry and Molecular Biology, University of California, Berkeley, CA). pchc-Δ10 (48) was used to delete the *CHC1* gene and was kindly provided by Greg Payne, Department of Biological Chemistry, University of California at Los Angeles).

The absorbance of dilute cell suspensions (OD_{600}) was measured in a 1-cm cuvette at 600 nm in a Zeiss PMQII spectrophotometer. Cell counts were performed using a hemacytometer (Fisher Scientific Co., Pittsburgh, PA).

Standard recombinant DNA techniques such as restriction digestion, Southern and Northern hybridization analyses, bacterial transformation, and plasmid isolation were performed essentially as described in Maniatis et al. (39) or Ausubel et al. (2).

Antibody against yeast phosphoglycerate kinase was kindly provided by Jeremy Thorner, Division of Biochemistry and Molecular Biology, University of California at Berkeley.

Buffers and Media

Buffer A consists of 100 mM 2(*N*-morpholino)ethanesulfonic acid, titrated to pH 6.5 with KOH, 0.5 mM $MgCl_2$, 1 mM EGTA, 0.2 mM DTT, 0.02% NaN_3. Buffer C consists of 20 mM Tris-HCl, pH 7.5, 50 mM NaCl, 1 mM EDTA, 0.2 mM DTT, 0.02% NaN_3. Denhardt's solution (50×) and SSC (20×) were prepared as described in Maniatis et al. (39). SSPE (20×) is 3.6 M NaCl, 0.2 M $NaH_2PO_4 \cdot H_2O$, 20 mM EDTA, adjusted to pH 7.4 with NaOH.

YPD medium consists of 1% Bacto-Yeast extract, 2% Bacto-Peptone (Difco Laboratories, Inc., Detroit, MI), 2–3% glucose. Wickerham's minimal medium with 2% glucose was used for growing yeast strains under selective conditions (61). NZCM medium consists of 1% NZ amine (casein, bovine, acid hydrolysate; Calbiochem-Behring Corp., La Jolla, CA), 86 mM NaCl, 25 mM $MgCl_2$, 0.1% casamino acids (Difco Laboratories, Inc.). Acetate spore plates consists of 0.1 M potassium acetate, 0.25% Bacto-Yeast Extract, 2% Bacto-Agar (Difco Laboratories, Inc.), 0.1% glucose. Solid media contained 2% Bacto-Agar.

Purification and Analysis of Coated Vesicle Components

Yeast coated vesicles were prepared from yeast strain BJ926 or X2180 as described previously. Clathrin triskelions were stripped from the vesicles using 2 M urea (46).

To denature heat-sensitive vesicle proteins, clathrin-coated vesicles (in buffer A and 0.6 mM PMSF) or triskelions (in buffer C and 0.6 mM PMSF) were heated in boiling water for 5 min. Aggregated proteins were sedimented at 133,000 *g* for 25 min in an airfuge (Beckman Instruments, Inc., Palo Alto, CA). The supernatant and pellet fractions were analyzed by SDS-PAGE and stained with Coomassie blue R-250.

Two-dimensional gel electrophoresis was performed on the supernatant fraction as in O'Farrell (44) except for the indicated changes in the first (isoelectric focusing) dimension. The ampholines mixture was composed of one part pH 3.5–10 to 1.35 parts each pH 4–6, 6–8, and 7–9 ampholines (LKB Instruments Inc., Bromma, Sweden). Bottom gel buffer was 30.5 mM

Table I. Strains Used in This Study

Strain	Genotype	Source or reference
Yeast		
X2180-1B	*MATα gal2 mal mel SUC2 CUP1*	YGSC*
SEY2108	*MATα leu2-3, 112 ura3-52 suc2Δ prc1::LEU2*	S. Emr‡
BJ926	*MATα/MATα his1/HIS1, trp1/TRP1 prc1-126/prc1-126 pep4-3/pep4-3 prb1-1122/prb1-1122 can1/can1 gal2/gal2*	E. Jones§
BJ743	*MATα/MATα leu2/leu2 ura3-52/ura3-52 his1/HIS1 ade6/ADE6 SCD1^L/SCD1^L*	Reference 34
LSY91	BJ743 with *clc1Δ::URA3/CLC1*	This study
LYS92	*MATα/MATα ura3-52/ura3-52 leu2-3, 112/leu2-3,112 his3-Δ200/his3-Δ200 trp1-Δ901/trp1-Δ901 suc2-Δ9/suc2-Δ9 lys2-801/LYS2 ade2-101/ADE2*	This study
LYS93	LYS92 with *clc1::HIS3/CLC1*	This study
LYS94	LYS93 with *chc1::LEU2/CHC1*	This study
Bacteria		
LEY392	*F$^-$hsdR514(r_k^-,m_k^-) supE44 supF58 lacY1* or Δ(*lac IZY*)*6 galK2 galT22 metB1 trpR55*λ^-	L. Pillus‖, reference 39

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H_2SO_4 and top buffer was 40 mM NaOH, 10 mM $Ca(OH)_2$. Gels were analyzed by silver staining (1).

To perform the TCA/neutralization experiment, coated vesicles or triskelions were precipitated in 10% TCA on ice for 10 min. Precipitated proteins were sedimented at 12,000 *g*, 4°C for 10 min. Buffer (50 mM Tris-HCl, pH 7.5, 2 mM EDTA) was added to the pellet fraction and the pH adjusted to 7.5 with NaOH if necessary. After incubation at 37°C for 20 min followed by vigorous mixing, precipitated proteins were separated by centrifugation at 12,000 *g* for 5 min. Supernatant and pellet fractions were analyzed by SDS-PAGE followed by staining with Coomassie blue.

Clathrin light chain was purified from coated vesicles using the TCA/neutralization regimen described above. To prepare clathrin light chain from a total yeast extract, the yeast strain BJ926 was grown at 30°C in 12 liters of YPD to OD_{600} 25–30. Yeast cells were harvested by centrifugation and washed three times in distilled water. Cells (150 g) were lysed at 4°C in 300 ml 0.5 M Tris-HCl, pH 7.4, 0.5 mM $MgCl_2$, 0.5 mM DTT, 1 mM PMSF in a Bead-Beater (Biospec Products, Bartlesville, OK). The lysate was cleared by centrifugation at 48,900 *g* for 30 min and solid ammonium sulfate was added to 20% saturation (4°C) to the supernatant. The precipitate that formed was removed by centrifugation at 27,000 *g* for 30 min and ammonium sulfate was added to 30% saturation to the supernatant. The resulting precipitate was collected as above, resuspended in 20 mM Tris-HCl, pH 7.4, 7 mM β-mercaptoethanol, 1 mM PMSF, and dialyzed against 20 mM Tris-HCl, pH 7.4, 7 mM β-mercaptoethanol. Dialyzed protein was heated 10 min in a boiling water bath and denatured proteins removed by centrifugation at 95,800 *g* for 30 min. Soluble material was heated and centrifuged as before to remove residual heat-denatured proteins, and the supernatant fraction was adjusted to pH 5.5 with citric acid and further centrifuged at 12,000 *g* for 10 min. This soluble fraction was loaded onto a 17 × 1-cm DEAE-Sepharose CL-6B (Pharmacia Fine Chemicals, Piscataway, NJ) column equilibrated with 50 mM sodium citrate, pH 5.5, 0.5 mM DTT. The column was washed with 50 mM sodium citrate, pH 5.5, 25 mM NaCl, 0.5 mM DTT, and developed with a 260-ml 25–140-mM NaCl gradient in 50 mM sodium citrate, pH 5.5, 0.5 mM DTT. 5-ml fractions were collected at 27 ml/h. The heating/DEAE regimen yielded ~1 μg of light chain per 400 OD_{600} units of cells. Protein concentration was determined by the Bradford assay (5).

Preparation of Antibody

Clathrin light chain isolated from coated vesicles was further purified by preparative SDS-PAGE. The protein was visualized with Coomassie blue, the band excised, and the gel slice homogenized by centrifugation through a wire mesh (30). Clathrin light chain was extracted from the homogenized gel slice as described (46).

Approximately 10 μg of light chain protein in PBS (20 mM sodium phosphate, pH 7.4, 0.15 M NaCl) was emulsified in Freund's complete adjuvant and injected subcutaneously into each of two mice. The mice received a booster injection of light chain after 4 wk, with subsequent boosts (six total) at 3–4-wk intervals. For each boost an average of 4 μg of gel-purified light chain per mouse was resuspended in PBS and emulsified in Freund's incomplete adjuvant.

The antibody was affinity purified using light chain protein (purified from yeast extracts) bound to nitrocellulose filters (63).

Protein Fragmentation and Peptide Sequence Analysis

Protein purified by the heating/DEAE method was precipitated in 25% TCA for 1 h on ice, sedimented at 12,000 *g* for 15 min, washed in cold acetone, and air dried. The precipitate was resuspended in 25 mM NH_4HCO_3, pH 7.5. The protein was then precipitated with 25% TCA for 1 h on ice, sedimented at 12,000 *g* for 15 min, washed in cold acetone two times, and dried in a Speed-Vac (Savant Instruments, Inc., Hicksville, NY) until the odor of acetone was absent from the pellet. The protein was dissolved in 6 M guanidine-HCl, 0.2 M *N*-ethylmorpholine acetate, pH 8.5, and 3 mM EDTA for treatment with β-mercaptoethanol and 4-vinylpyridine to reduce disulfide bonds and protect cysteine residues, respectively (13). Lysine residues were modified with citraconic anhydride to limit subsequent digestion with trypsin to the COOH-terminal side of arginine residues (41). After exhaustive dialysis into 50 mM *N*-ethylmorpholine acetate, pH 8.5 buffer, an amino acid analysis was performed to confirm the amount of protein present after the precipitation, chemical modification, and dialysis steps (3). After lyophilization of the buffer, trypsin digestion was performed in 2 M urea, 0.1 M Tris-HCl, pH 8.2, and 1 mM calcium chloride for 16 h at 37°C using 5% TPCK-trypsin by weight. After decitraconylation of lysine residues (titration to pH 2 with 25% trifluoroacetic acid) for 3 h at room temperature, peptides were resolved by reverse-phase HPLC using a standard bore C4 column and an acetonitrile gradient (0–65% [vol/vol] in 0.1% TFA) developed over 2 h. Peptide peaks were subjected to Edman degradation using a protein sequencer (model 470A; Applied Biosystems, Inc., Foster City, CA) with an on-line 120A PTH-Analyzer for identification of the amino acid residues. All runs were performed using the standard 03RPTH program supplied by the manufacturer.

Cloning of *CLC1*

A codon bias table (16) was used to design a nondegenerate 30-base "guessmer" oligonucleotide (5'CTTGAATTCGTCICCIAAIATTTCAGCTTC) that could hybridize to DNA encoding the peptide sequence obtained by microsequence analysis (EAEILGDEFK) (33). Inosines (I) were used in positions where a clear bias was not present (40). The oligonucleotide was designed to complement the coding strand so that it could hybridize to both DNA and mRNA. The oligonucleotide was purified by gel electrophoresis, as described by Ausubel et al. (2).

To identify the yeast clathrin light chain gene, a λgt·wes yeast genomic library (kindly provided by Lorraine Pillus, Division of Biochemistry and Molecular Biology, University of California, Berkeley, CA) was screened. This library, constructed by Lorraine Pillus and Joe Heilig (Division of Genetics, University of California, Berkeley, CA), consists of 4–17-kb genomic Eco RI fragments from the yeast strain FSY1981 (a/α, *his4/HIS4*, *lys2/LYS2*, *ura3/ura3*) in λgt·wes (Bethesda Research Laboratories, Gaithersburg, MD) phage arms. The library was grown on bacterial strain LE392 in top agarose (0.7%) on NZCM plates. DNA from phage plaques was transferred to nitrocellulose filters and denatured by treatment with base. Filters were probed with the synthetic oligonucleotide labeled with ^{32}P by T4 polynucleotide kinase. Hybridizations were performed in 4× SSPE, 2× Denhardt's solution, 100 μg/ml calf thymus DNA, 0.2% NP-40 at 37°C for at least 12 h. Filters were washed with 1× SSC, 0.1% SDS, twice at room temperature, and once at 42°C for 30 min each wash.

Plating and purification of phage, and preparation of phage DNA, were performed essentially as in Maniatis et al. (39), with minor modifications. Phage were grouped into classes by restriction mapping and hybridization. Of ~80,000 phage screened, one clone of the class encoding *CLC1* and at least eight clones of another class, which were not further characterized, were obtained.

Overproduction of Clathrin Light Chain Protein

The 3.6-kb Xba I to Sph I fragment of *CLC1* was subcloned into the multicopy yeast vector YEp352 and introduced into SEY2108 by spheroplast transformation (21). Transformants were grown in Wickerham's minimal medium to an OD_{600} of ~0.4. Cells were washed in distilled water, resuspended at 44 OD_{600}/ml in Laemmli sample buffer (31), and lysed by vigorous vortexing with glass beads. The lysates were heated immediately, resolved on SDS-PAGE, and analyzed by immunoblotting (56) with affinity-purified clathrin light chain antibodies. Immunoreactive bands were visualized by incubating the blot with goat anti-mouse IgG antibodies coupled to alkaline phosphatase (Bio-Rad Laboratories, Richmond, CA) and developing band color as per manufacturer's instructions.

DNA Sequencing

To determine the DNA sequence of *CLC1*, the 3.6-kb Xba I to Sph I fragment was cloned in both orientations into the Hinc II site of pUC119. A deletion series was generated by unidirectional digestion of the inserts with exonuclease III (Bethesda Research Laboratories) (18). Single-stranded template was generated as described (59). Sequencing was performed by the dideoxynucleotide chain termination method using Sequenase (United States Biochemical Corp., Cleveland, OH) as per manufacturer's instructions. Sequence data was analyzed with Intelligenetics (Mountain View, CA) and University of Wisconsin sequence analysis programs (11).

Gene Disruptions

A null allele of *CLC1* was generated in vitro by ligating sequencing deletions that contained regions upstream or downstream of the *CLC1* gene in pUC119. The upstream flanking region used contained sequence from the Xba I site to ~nucleotide −1. The downstream region started within the *CLC1* coding sequence ~40 nucleotides upstream of the stop codon and extended to the Sph I site. Because of a difficulty in reading bases close to

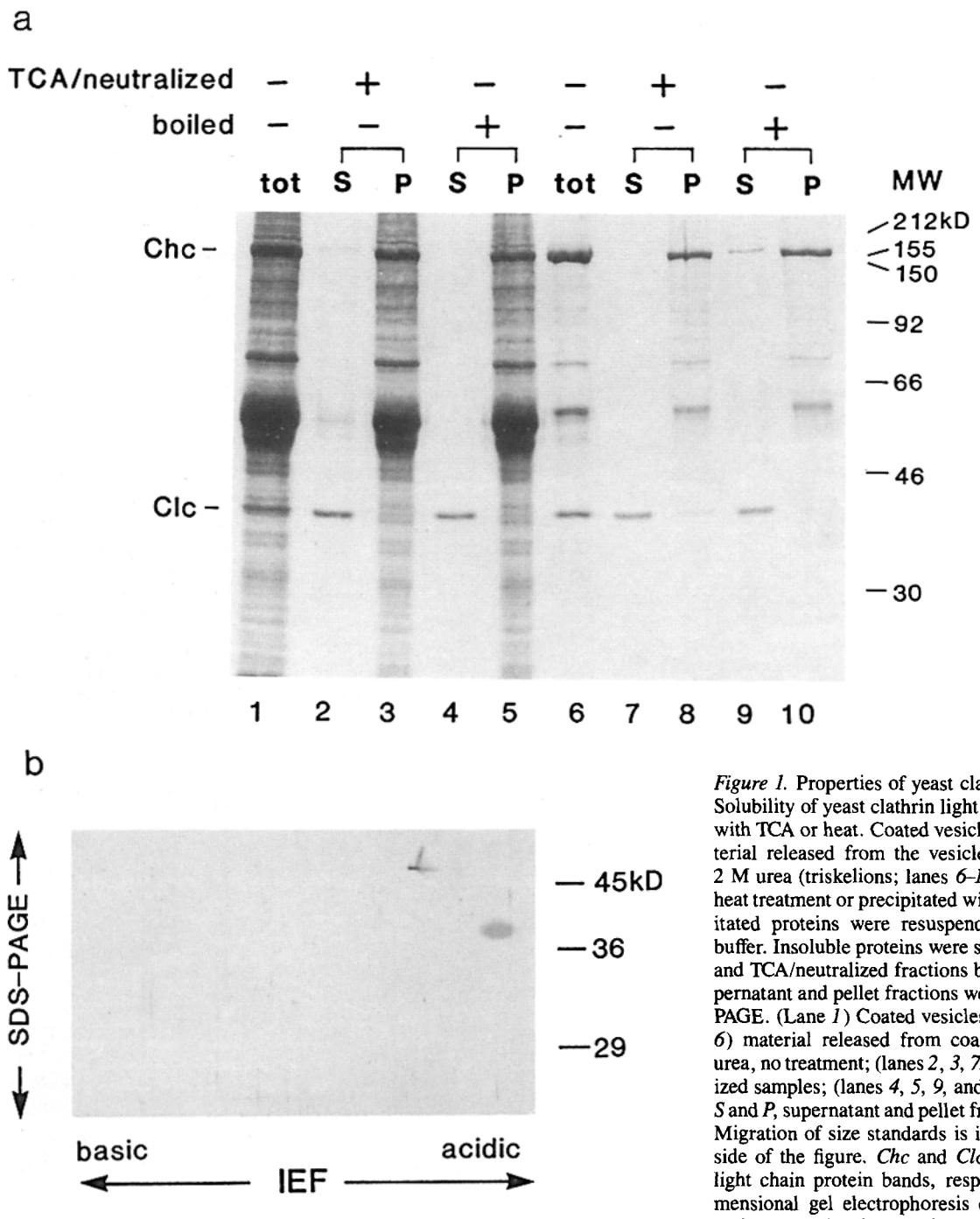

Figure 1. Properties of yeast clathrin light chain. (*a*) Solubility of yeast clathrin light chain after treatment with TCA or heat. Coated vesicles (lanes *1–5*) or material released from the vesicles by treatment with 2 M urea (triskelions; lanes *6–10*) were subjected to heat treatment or precipitated with TCA. TCA-precipitated proteins were resuspended in a neutral pH buffer. Insoluble proteins were separated from heated and TCA/neutralized fractions by centrifugation. Supernatant and pellet fractions were analyzed by SDS-PAGE. (Lane *1*) Coated vesicles, no treatment; (lane *6*) material released from coated vesicles by 2 M urea, no treatment; (lanes *2*, *3*, *7*, and *8*) TCA/neutralized samples; (lanes *4*, *5*, *9*, and *10*) heated samples. *S* and *P*, supernatant and pellet fractions, respectively. Migration of size standards is indicated on the right side of the figure. *Chc* and *Clc*, clathrin heavy and light chain protein bands, respectively. (*b*) Two-dimensional gel electrophoresis of heat-stable coated vesicle protein. Heat-stable material from coated vesicles was analyzed by two-dimensional gel electrophoresis followed by silver staining. The horizontal dimension was isoelectric focusing (*IEF*), the vertical dimension SDS-PAGE. Only 25% of the gel is shown; other regions contained no coated vesicle proteins. The positions of size standards are indicated on the right-hand side of the figure, and the direction of the pH gradient in the isoelectric focusing dimension is indicated at the bottom. The right-hand side is the acidic pole of the gel.

the primer in sequencing reactions, the exact endpoints of the deletions were not determined. The 1.7-kb Bam HI fragment from pCS19, carrying the selectable marker gene *HIS3*, was inserted between the two flanking sequences after subcloning into another vector to create ends with appropriate restriction sites. This construct (pclcl-Δ2; see Fig. 3) was linearized with Pvu II (cuts in vector sequence a short distance from the ends of the insert) and Eco RV (cuts in flanking sequence downstream of *CLC1*) and the fragment containing *HIS3* and the *CLC1* flanking sequences was purified by electrophoresis on an agarose gel. Diploid yeast cells (LSY92, and BJ743) were transformed with pclcl-Δ2 by the lithium acetate method (22), and transformants selected on minimal medium-lacking histidine. Transformants were induced to sporulate on acetate spore plates and dissected into tetrads. The construct pchcl-Δ10 (47) was used to replace one allele of the clathrin heavy chain gene with the *LEU2* marker gene in one of the diploid LSY93 *clc1Δ/CLC1* transformants.

Immunoblot of clc1Δ Deletion Mutants

To verify that *clc1Δ* mutants lacked the light chain protein, *clc1Δ* and *CLC1* strains were grown in YPD to an OD_{600} of 2 to 6. Cells were washed in

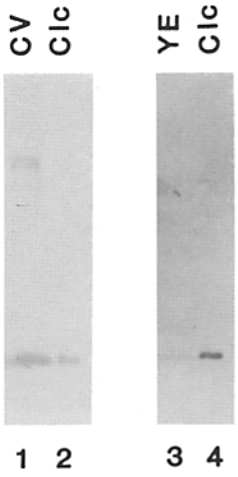

Figure 2. Affinity-purified clathrin light chain antibodies specifically detect light chain in coated vesicles and total yeast extracts. (Lane *1*) Yeast coated vesicles (*CV*); (lane *2*) yeast clathrin light chain (*Clc*) purified from coated vesicles using the TCA/neutralization protocol; (lane *3*) total yeast extract (*YE*); (lane *4*) light chain purified from yeast extract using the heating/DEAE protocol.

distilled water, resuspended in 50 mM Tris-HCl, pH 7.3, with 1 mM PMSF, 1 mM EDTA, 10 μM leupeptin, and 10 μM pepstatin added to reduce proteolysis, and were broken in a Bead-Beater. Samples were removed, added to Laemmli sample buffer (31), and heated to 95°C ("total extract" fractions). The remainder of the lysates were heated for 5 min in a boiling water bath and centrifuged at 60,000 *g* for at least 30 min (supernatant fraction is "boiled extract"). Samples were analyzed by SDS-PAGE and immunoblotting as described above in the overproduction experiment.

Results

Yeast Light Chain Shares Unusual Physical Characteristics of Mammalian Light Chains

Mueller and Branton (43) first noted the presence of candidates for clathrin light chains in partially purified coated vesicle preparations from yeast. The identities of 190- and 38-kD (36 kD in reference 46) coated vesicle proteins as yeast clathrin heavy chain and light chain, respectively, were confirmed by their ability to be released from vesicles in characteristic triskelion complexes after treatment with urea (46). Like mammalian clathrin, yeast triskelions could be reassembled into empty clathrin cages in vitro (35).

Mammalian clathrin light chains remain soluble after heat treatment and are readily resolubilized in neutral pH buffer after acid precipitation (37). These properties were explored with yeast coated vesicles which were either heated or precipitated with TCA and then resuspended in neutral buffer (Fig. 1 *a*, lanes *1–5*). Denatured proteins were sedimented and the supernatant and pellet fractions subjected to SDS-PAGE. Either heating or TCA/neutralization treatment caused most proteins to become insoluble (lanes *3* and *5*, as compared to total, lane *1*). Only the 38-kD protein was soluble after each treatment (lanes *2* and *4*). This protein was identical to that cofractionating with triskelions; the 38-kD protein released from coated vesicles by treatment with urea (lane *6*) displayed the same solubility properties as the protein from coated vesicles (lanes *6–10*).

Mammalian cells contain two classes of clathrin light chains encoded by related but distinct genes (23, 24, 29). Although yeast light chain appeared as only one species in SDS-PAGE, it was possible that two forms migrated coincidentally. Two-dimensional gel electrophoresis of purified light chain demonstrated a single light chain species (Fig. 1 *b*), suggesting that yeast cells, unlike mammalian cells, express only one light chain.

Mammalian light chains bind calmodulin-agarose in a Ca^{2+}-dependent fashion (38). Yeast light chain also bound calmodulin-agarose in the presence of Ca^{2+}, eluting upon addition of EGTA (data not shown), and bound Ca^{2+} directly (Näthke, I., and F. Brodsky, Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, CA, personal communication), like its mammalian counterpart (42).

Purification of Yeast Light Chain

TCA/neutralization treatment of coated vesicles was used to purify small amounts (~5–10 μg per preparation) of yeast light chain in order to raise a polyclonal antiserum. The antiserum allowed detection of light chain in crude homogenates, thereby facilitating development of a procedure for purifying large amounts of light chain directly from a total yeast extract. In the new procedure (heating/DEAE), light chain was enriched by ammonium sulfate fractionation of total yeast extract followed by heat treatment. The resulting soluble fraction was chromatographed on DEAE-Sepharose at low pH.

The specificity of the affinity-purified antibodies for yeast clathrin light chain in crude and purified fractions is shown in Fig. 2. Initial and final fractions from each purification were analyzed by immunoblotting with yeast light chain antiserum. The affinity-purified antibodies specifically recognized the 38-kD light chain in coated vesicles and a yeast extract (lanes *1* and *3*, respectively) and reacted with the same protein purified from coated vesicles by the TCA/neutralization method and from a yeast extract by the heating/DEAE procedure (lanes *2* and *4*, respectively). The higher molecular weight species present in the coated vesicle sample were probably aggregates of light chain protein that formed during storage of the sample.

Yeast Clathrin Light Chain Gene

A portion of the light chain protein was sequenced in order to design a probe for the gene. Tryptic fragments of protein purified by the heating/DEAE method were subjected to protein microsequence analysis. One peptide, EAEILGDEFK, contained six amino acids encoded by relatively nondegenerate codons. A unique DNA oligonucleotide complementary to a sequence capable of encoding this fragment was synthesized, with the codon selection based on yeast codon bias (16). The oligonucleotide hybridized to two species in low stringency Southern analysis of yeast genomic DNA and a single 1-kb species in Northern analysis of yeast poly(A)$^+$ RNA (data not shown). Hybridization of the oligonucleotide to a yeast genomic library identified two classes of clones, one of which hybridized to a poly(A)$^+$ RNA of ~1 kb. We pursued this clone as the message was of an appropriate size to encode a 38-kD protein.

A restriction map of the DNA insert that hybridized to a 1-kb yeast mRNA is shown in Fig. 3. Production of light chain was examined in cells transformed with a multicopy plasmid bearing the 3.6-kb Xba I to Sph I fragment or the vector alone. Clathrin light chain, detected by immunoblotting, was overproduced in strains carrying this insert, while

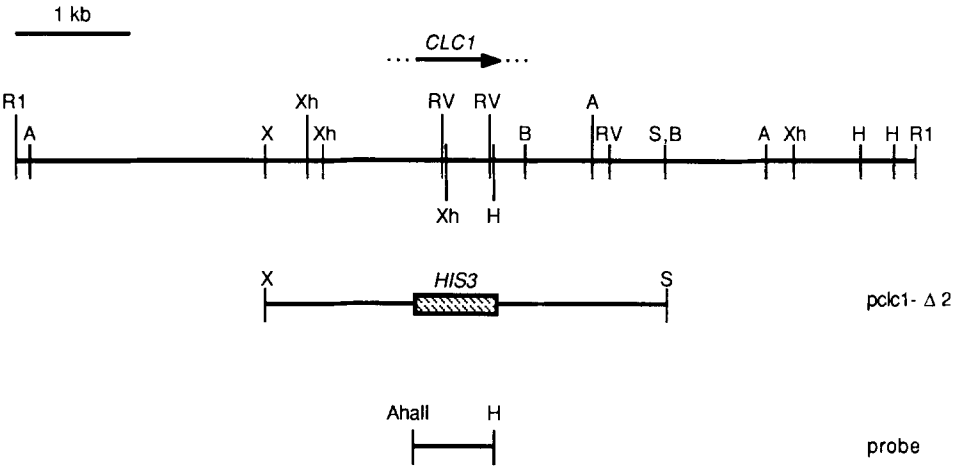

Figure 3. Restriction map of the *CLC1* region. The map of the original clone containing *CLC1* is given. The endpoints of the *CLC1* transcript (*arrow*) are approximate and the arrow indicates the direction of transcription. The construct, pclc1-Δ2, used to generate the light chain null mutants is shown beneath the map. The *HIS3* insert is not drawn to scale. The probe used in Northern and low stringency Southern analysis is shown. The Aha II cuts ~30 bp upstream of the *CLC1* translation start site. *A*, Asp718; *B*, Bam HI; *RI*, Eco RI; *RV*, Eco RV; *H*, Hind III, *S*, Sph I; *X*, Xba I; *Xh*, Xho I.

vector alone produced normal levels of the protein (Fig. 4). Whereas transformants bearing the Xba I to Sph I insert overexpressed a form of light chain that exactly comigrated with purified light chain, cells transformed with a smaller subclone (Xba I to Hind III) expressed a slightly faster migrating form (data not shown). These data suggested that clathrin light chain is encoded in the direction from the Xba I site towards the Hind III site, and that the Hind III site lies near the 3′ end of the light chain coding sequence.

It was unlikely that this clone encoded an immunoreactive contaminant protein present during microsequence analysis. The light chain protein used to generate antiserum and that used in microsequence analysis were prepared by distinct purification regimens. Furthermore, another antibody, generated against a β-galactosidase hybrid protein derived from this clone, recognized a 38-kD heat-stable coated vesicle protein. This immunoreactive protein was released from coated vesicles by treatment with urea, and cofractionated with heavy chain in the position of free triskelions during chromatography on Sepharose CL-4B (data not shown). Thus, we assert that the insert contains the authentic clathrin light chain gene (*CLC1*).

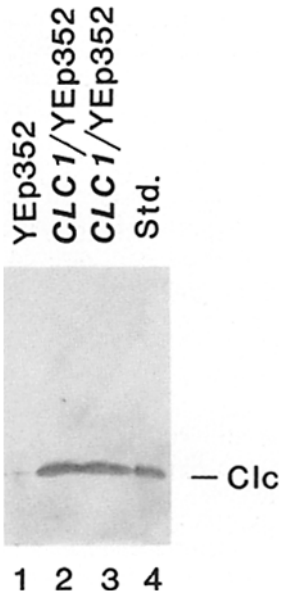

Figure 4. Multiple copies of the *CLC1* gene lead to overproduction of yeast clathrin light chain. Yeast were transformed with the multicopy vector YEp352, with or without the *CLC1* gene as insert. Extracts from the transformants were analyzed by immunoblotting with affinity-purified light chain antibody. Extract from 0.5 OD_{600} units of cells was loaded in lanes *1–3*. (Lane *1*) Extract from yeast transformed with YEp-352 alone; (lanes *2* and *3*) extract from yeast transformed with YEp-352 carrying *CLC1*; (lane *4*) purified clathrin light chain.

DNA sequence analysis of *CLC1* revealed an open reading frame capable of encoding a protein of 26.5 kD (Fig. 5). Thus, the predicted molecular mass of yeast light chain deduced from the DNA sequence is at variance with that determined by SDS-PAGE of the purified protein (26.5 kD predicted; 38 kD measured). Similar discrepancies have been noted before for the measured and predicted molecular masses of mammalian clathrin light chains (23, 24, 29). The deduced amino acid sequence included the peptide sequenced from the purified light chain protein (located at amino acids 31–40, marked by arrowheads in Fig. 5). The predicted isoelectric point of the protein encoded by *CLC1* was 4.2, consistent with light chain's migration near the acidic pole in two-dimensional gel electrophoresis (see Fig. 1 *b*).

The nucleotide sequence contained features typical of expressed open reading frames. First, potential transcription initiation signals (TATTA) were located 190 and 240 bp upstream of the putative translation start codon (55). Second, the bases in the open reading frame, but not in the surrounding sequences, were constrained in a manner typical of coding sequences as evaluated by Testcode analysis (11, 12). In addition, the Aha *II* to Hind *III* fragment (probe, see Fig. 3), which closely parallels the open reading frame, hybridized to a 1-kb mRNA (data not shown).

Low stringency Southern hybridization analysis of yeast genomic DNA using the probe shown in Fig. 3 revealed that *CLC1* was a single-copy gene with no close homologs (data not shown). This result is in agreement with the detection of a single light chain species in isolated coated vesicles (see Fig. 1).

Light Chain Sequence Homologies

Although the amino acid compositions of the yeast and mammalian light chains were similar, there was little sequence identity. The low identity of the yeast and mammalian light chains made alignment of their sequences subjective. An alignment of the yeast sequence with that of human brain light chain LC_b that places their amino termini in register is

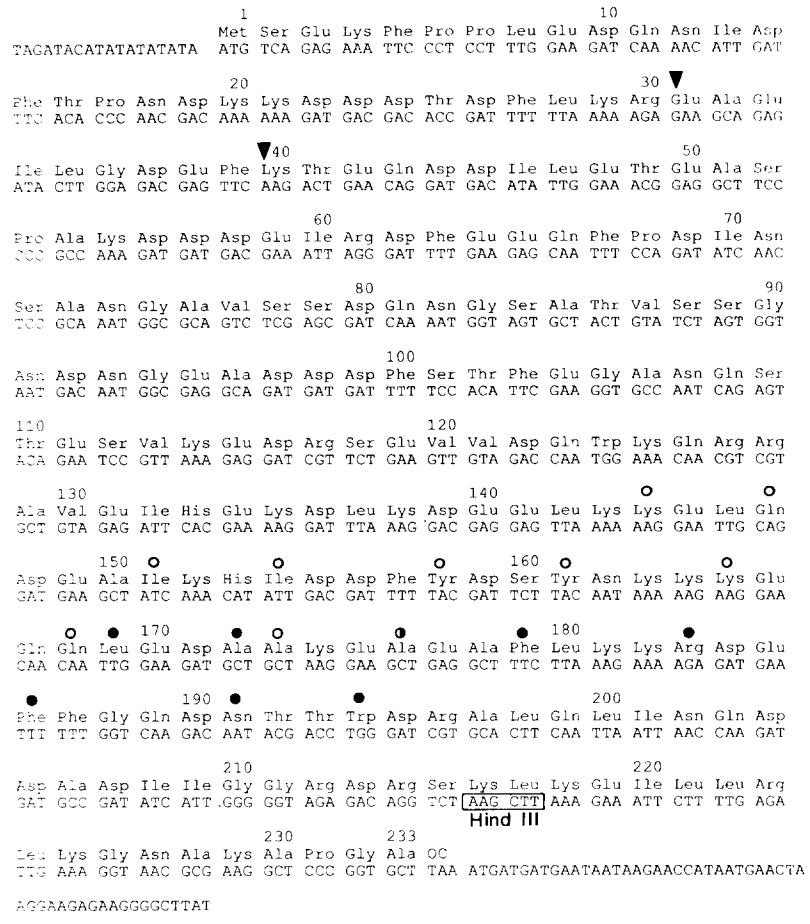

Figure 5. Nucleotide sequence of *CLCl* and predicted amino acid sequence of the light chain protein. Numbers indicate amino acid number. The sequence identified by protein microsequencing is bounded by arrowheads. Dots overlie the first and fourth positions of the first (○,◑ and second (●,◑) sets of heptad repeats. Each set of repeats contains five repeat motifs and one skip residue. The Hind III site used in making the probe pictured in Fig. 3 is indicated. These sequence data are available from EMBL/Gen Bank/DDB under accession number X52272.

shown in Fig. 6. The overall homology between yeast light chain and mammalian brain LC_b in this alignment was 26% (18% identity); however, the last 57 amino acids were 39% homologous (Fig. 6). Another short region (located at positions 28–49 in Fig. 6 *a*) was 45% homologous to an amino-terminal conserved region of mammalian light chains. This region (shown in box, Fig. 6 *a*) is completely conserved between both classes of light chains in all species previously examined (cow, human, and rat) and therefore may be especially important in light chain function (23). The yeast light chain sequence lacks the amino-terminal serines that are phosphorylated in LC_b (19), and the cysteines that form intrachain disulfide bonds in mammalian light chains (45).

A domain from residues 193–213 in the yeast sequence (positions 218–238 in Fig. 6) is 55% homologous to the brain-specific insert of bovine and human LC_b (Fig. 6 *b*). The function of this insert in mammalian light chains is unknown. As its name indicates, this region arises from tissue-specific splicing of LC_b mRNA in mammalian brain tissue. Expression of this sequence in yeast is not controlled by splicing, as no consensus splicing elements were identified in the *CLCl* sequence (32). The region of brain-specific insert homology in the yeast sequence (underlined in Fig. 6 *a*) is displaced ~40 amino acids toward the carboxy terminus relative to the mammalian insert (shown boxed in Fig. 6 *a*).

The central region of mammalian light chains, thought to mediate their binding to heavy chain (8), contains heptad repeats (29) and is similar to intermediate filaments (24). Heptad repeats, a pattern of seven amino acids, abcdefg, in which a and d are hydrophobic, are characteristic of intermediate filaments and other proteins that form coiled-coil structures (53). The repeats in mammalian light chains are somewhat irregular in that lysine or arginine is sometimes found in the first position and consecutive repeats may be interrupted by a "skip" residue (29). Two short overlapping regions of five heptads each were found in the mid-region of the yeast sequence (dots in Fig. 5 overlie the first and fourth positions). These regions are half the length of those found in mammalian light chains. The region from amino acids 106–187 is devoid of helix-breaking proline and glycine residues which occur regularly in other parts of the protein, and is predicted to be capable of forming an α-helix by Chou and Fasman (10) structural analysis. A screen of the National Biomedical Research Foundation Protein Identification Resource sequence database with the yeast light chain sequence using the FASTP sequence search program (37) detected similarity to coiled-coil proteins in the mid-region of the protein (typically amino acids 130–175), although most of these similarities were weak. The most striking similarity was to the rod region of myosin heavy chain from *Acanthamoeba castellanii* (17). Limited identity (20%) covered nearly the entire light chain protein (amino acids 1–221); the greatest density of identical residues occurred in the putative clathrin heavy chain binding region (37% identity over amino acids 130–172).

a

```
          1        10        20        30        40        50        60        70
YCLC      MSEKFPPLEDQNIDFTPNDKKDDD**TDFL*KREAEILGDEFKTEQDDILETEASPAKDDDEIRDFEEQP
          : . :           :    ..:    ::   : :: : :           :  :          :
HLCB      MADDFGFFSSSESGA*PEAA*EEDPAAAFLAQQESETAGIENDEGFGAPAGSHAASAQPGPTSGAGSEDM
HLCA   MAELDPFGAPAGAPGGPALGNGVAGAG----------------------A-AILDGGAPGPQPH-EPP-G PDAV
                                  CONSERVED  REGION

            80        90       100       110       120       130       140       150
YCLC      PD*INSANG*AVSSDQNGSATVSSGNDNG*EADDDFSTFEGANQSTESVKEDRSEVVDQ*WKQRRAVEIHEKDLKD
             .:           : : .       : .     :         . .  : : .: :. .   .. :   .
HLCB      GTTVNGDVFQEANGPADGYAAIAQADRLTQEPESIRKWREEQRKRLQEL*DAASKVTEQEWREKAKKDLEEWNQRQ
HLCA      DGVM--EYY--S---T-S----S-V---QS----------ME--EA- --N-RKQ-A--K---I-E----YA--

                 160       170       180       190       200       210       220
YCLC      EELKKELQDEAIKHIDDFYDSYNKKKEQQLEDAA            KEAEAFLKKRDEFFGQDNTTWDRALQLINQ
           :              ::                              :::.:   :      : :..  ::
HLCB      SEQVEKNKINNRIADKAFYQ****QPDADIIGYV             ASEEAFVKESKE**ETPGTEWEKVAQLCDF
HLCA      D--LQ-T-A---V--E---K     --F--V----TNINHPCYSLEC-A-----NDID-  SS------R--R----
                          BRAIN-SPECIFIC  INSERT

          230       240       250
YCLC      DDADIIGGRDRSKLKEILLRLKGNAKAPGA.
                 .: :.:. .:  ::
HLCB      NPKSSKQCKDVSRLRSVLMSLKQTPLSR**.
HLCA      -------A-----M----I----A--VH  .
```

b

```
YCLC   218   TWDRALQLINQDDADIIGRD   238
               :.:    .: ::::::
HLCB   165   IADKAFY**QQPDADIIGYV   186
```

Figure 6. Alignment of yeast and human clathrin light chain amino acid sequences. (*a*) Alignment of the full yeast (*YCLC*) and human brain LC_b (*HLCB*) sequences. Identical amino acids between YCLC and HLCB are indicated by pairs of dots (:). Single dots (.) indicate chemically similar amino acids. Gaps introduced to maximize the YCLC/HLCB alignment are indicated with asterisks (*). The sequence of human brain LC_a (*HLCA*) is shown to indicate the relatedness of LC_a and LC_b. Only those amino acids in HLCA that differ from HLCB are shown; identities between HLCA and HLCB are indicated with dashes (−). As HLCA is larger than HLCB, spaces have been introduced into the YCLC and HCLB sequences where necessary. The amino-terminal conserved region and brain-specific inserts of HCLB and HCLA are boxed. A possible homolog of the brain-specific insert is underlined in the YCLC sequence. (*b*) Alignment of human LC_b brain-specific insert with a similar sequence from yeast light chain. Numbers indicate the position of these sequences in *a*.

Clathrin Light Chain Is Important in Normal Cell Growth

A yeast strain deficient in clathrin light chain (*clcl*Δ) was generated by one-step gene disruption (50). Most of the *CLCl* coding sequence (93%: from ~nucleotide −1 to ~44 nucleotides upstream of the stop codon) was replaced by the selectable marker gene *HIS3* (pclcl-Δ2; Fig. 3). A fragment containing *CLCl* flanking sequences and the *HIS3* gene was introduced into His⁻ (*his3/his3*) diploid yeast by transformation. Southern hybridization analysis of genomic DNA from the transformed cells confirmed that one copy of *CLCl* was replaced by the *clcl*-Δ2 allele (data not shown). The diploid transformants (LSY93; *CLCl/clcl*Δ) were induced to

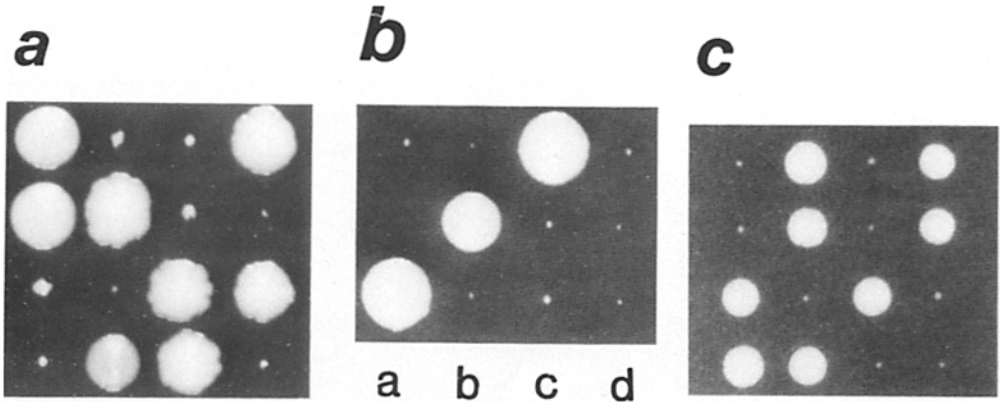

Figure 7. Yeast lacking clathrin light chain are viable but grow slowly. (*a–c*) Tetrads derived from sporulation of a diploid heterozygous for a *CLCl* gene disruption (*CLCl/clcl*Δ) are displayed horizontally. (*a*) Tetrads derived from LSY93. One allele of the *CLCl* gene was replaced by *HIS3*. Small colonies scored His⁺; large colonies were His⁻. (*b*) Tetrads derived from LSY94, a diploid with one allele of *CHCl* replaced by the *LEU2* gene and one allele of *CLCl* replaced by *HIS3*. Colonies in row 1 (from top) position d, row 2 position a (not visible in photo), and row 3 position c were His⁺Leu⁺, indicating that they contained neither clathrin light nor heavy chain. All other small colonies were either His⁺ or Leu⁺. (*c*) Tetrads derived from LSY91, an $SCD1^L/SCD1^L$ strain. One allele of *CLCl* was disrupted as before except *URA3* was used in place of *HIS3* in the deletion construct. Small colonies were Ura⁺.

Table II. Segregation Analysis of the Clathrin Light Chain Null Allele in LSY93

Type of tetrad		Number of tetrads of each type	Number of tetrads tested for histidine prototrophy*	Ratio of histidine prototrophs to auxotrophs	
Ratio of viable to inviable spores	Ratio of large to small colonies			Large colonies	Small colonies
4:0	2:2	30	24	0:48	48:0
3:1	2:1	10	10	0:20	10:0
	1:2	4	4	0:4	8:0
2:2	1:1	1	1	0:1	1:0
1:3	1:0	1	1	0:1	–
	Totals	46	40	0:74	67:0

* Some colonies were too small to be tested for histidine prototrophy. Tetrads containing such colonies were not used in this analysis.

sporulate and dissected into tetrads (Fig. 7 *a*). In most tetrads, two large and two small colonies of haploid cells were obtained (Table II). In every case, small colonies grew on medium lacking histidine, indicating that they carried the allele of *CLC1* replaced by *HIS3* (Table II, column *6*). The slow-growth phenotype of *clc1*Δ strains was complemented by the *CLC1* gene on a plasmid (data not shown).

The growth rates of *clc1*Δ strains were unstable; during propagation of the original segregants, faster growing variants often arose. The growth rates of cells in one tetrad that possessed one *clc1*Δ segregants of the faster growing and one of the slower growing class were measured. The slower growing mutant strain (Fig. 8, curve *C*) had a doubling time about two times slower than the wild-type segregants (Fig. 8, curve *A*). A decrease in growth rate of similar magnitude was observed in the clathrin heavy chain mutant strains, which grow two to three times more slowly than wild-type strains (46). Surprisingly, the faster growing *clc1*Δ mutant grew at nearly wild-type rates. As all of the *chc1*Δ colonies on the original dissection plate were much smaller than wild-type colonies (Fig. 7 *a*), some high probability secondary event(s) in the *clc1*Δ strain selected for faster growing cells. In support of this theory, papillations were evident on some of the small colonies on the original dissection plate (Fig. 7 *a*). Variations in growth rate have also been observed in clathrin heavy chain mutants and appear to depend somewhat on strain background (36).

Both faster and slower growing *clc1*Δ mutant cells were swollen and grew in small clumps. The difference in cell size between wild-type and mutant strains was reflected in a difference in the number of cells per OD_{600} unit, with the wild-type strains having about twice as many cells as the mutant strains in one OD_{600} unit (wild type, 1.2×10^7 cells/OD_{600}; mutants, 6.3×10^6 cells/OD_{600}).

Immunoblotting of extracts of faster-growing *clc1*Δ cells confirmed that light chain was absent. Samples derived from an equivalent number of wild-type or mutant cells were compared and light chain was detected in total extracts of wild-type cells only (Fig. 9 *a*, lanes *1* and *2*). Likewise, preparation of a heat-stable fraction, which substantially enriched light chain from crude extracts of wild-type cells (compare lane *3* with lane *1*), failed to uncover light chain in a lysate of *clc1*Δ cells (lane *4*). The absence of an immunoreactive species in *clc1*Δ cells confirmed that our affinity-purified antibody recognized a single yeast protein encoded by *CLC1*; thus *CLC1* encodes clathrin light chain, not merely a contaminant recognized by the light chain antiserum. Another protein, phosphoglycerate kinase, was detected in both wild-type and mutant extracts (Fig. 9 *b*). Coomassie blue staining of a gel identical to that analyzed by immunoblotting showed no significant differences in protein composition between wild-type and mutant extracts (data not shown).

Light Chain/Heavy Chain Double Deletion Mutants Are Viable

To obtain mutants lacking both clathrin heavy and light chains, we disrupted one allele of the clathrin heavy chain gene, *CHC1*, with a *LEU2* selectable marker in a diploid heterozygous for a *CLC1* deletion. The resulting diploid, LSY94 (*CHC1/chc1*Δ *CLC1/clc1*Δ), was induced to sporulate and dissected into tetrads (Fig. 7 *b*). Two genes segregating in-

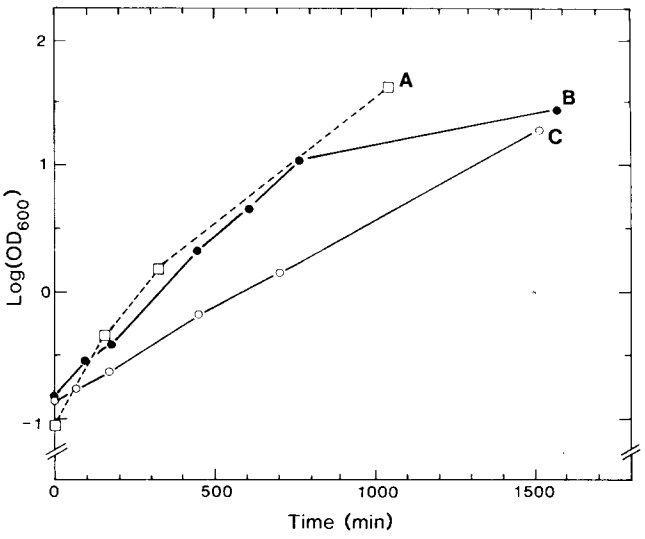

Figure 8. Growth rates of wild-type and clathrin light chain deficient cells. Growth rates of cells from each of four colonies in a tetrad derived from LSY93 were analyzed. Cells were grown in liquid medium (YPD) at 30°C with agitation, and cell density was monitored by direct count in a hemacytometer and by measurement of OD_{600} after mild sonication to disperse clumps. The number of cells per OD_{600} was virtually constant for each strain over the course of the experiment. The change in OD_{600} over time is shown for one wild-type and two mutant strains, as the growth rates of the two wild-type strains were virtually identical. (Curve *A*) Wild-type strain; (curves *B* and *C*) mutant strains.

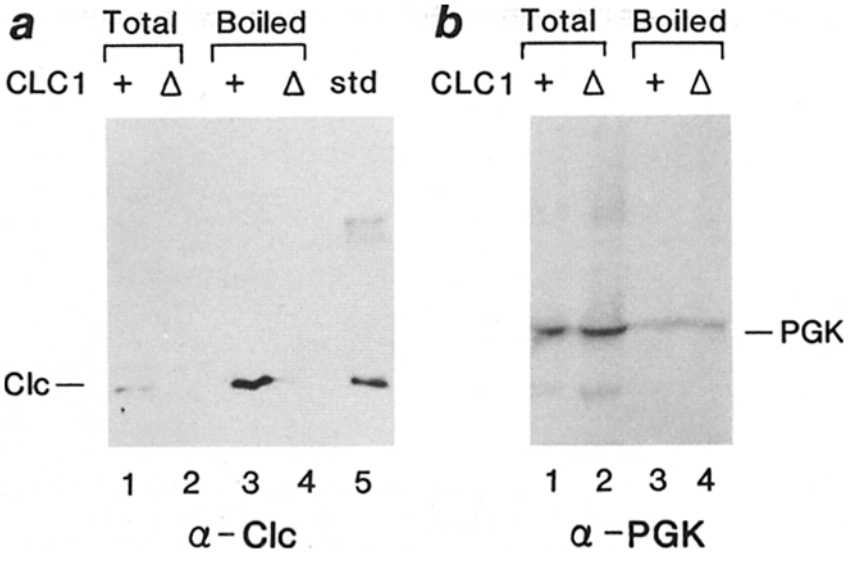

Figure 9. Mutant *clc1*Δ cells lack the clathrin light chain protein. Haploid cells were obtained by sporulation of LSY93. Extracts from a His^+ (*clc1*Δ) and a His^- (*CLC1*) colony were prepared by glass bead lysis. Total extracts were heated in a boiling water bath and then centrifuged, leaving the heat-stable material in the supernatant. Samples were analyzed by SDS-PAGE and immunoblotting. (Lanes *1–2*) Total extracts from *CLC1* and *clc1*Δ cells, respectively; (lanes *3–4*) heat-stable components from *CLC1* and *clc1*Δ extracts (+, wild-type extracts; Δ, *clc1*Δ extracts). Material from an equal number of cells (as judged by OD_{600}) was loaded in each lane (total extracts: 9.6×10^6 cell equivalents; heated extracts, 2.7×10^7 cell equivalents). The factors used to convert OD_{600} measurements to cell numbers were derived in a separate experiment in which known volumes of cells were counted in a hemacytometer. (*a*) Immunoblot probed with affinity-purified light chain antibody. (*b*) Identically prepared immunoblot probed with yeast phosphoglycerate kinase (*PGK*) antiserum (diluted 1:10,000).

dependently should yield tetrads of three classes in a ratio of 1:1:4: tetrads with two *clc1*Δ spores and two *chc1*Δ spores, tetrads with two *chc1*Δ *clc1*Δ spores and two wild-type spores, and tetrads with one each of wild-type, *clc1*Δ, *chc1*Δ, and double mutant spores. If the two genes are linked, one of the first two classes should be rare. The data in Table III show that most tetrads contained four viable spores. Among tetrads with four viable spores, the distribution of the three classes indicated that *CLC1* and *CHC1* were unlinked.

Several His^+Leu^+ colonies were recovered from the dissection plate, indicating that the *chc1*Δ *clc1*Δ double mutants were viable. If the double mutants were no less viable than the single mutants, among all spores, one quarter should be wild type, one quarter double mutants, and one quarter each of the single mutants. Table IV shows that double mutant colonies were numerous, but somewhat less frequent than expected. The growth characteristics of the double mutants were not distinct from either of the single mutants.

Light Chain and Heavy Chain Gene Disruption Phenotypes Are Not Equivalent

Lemmon and Jones identified a strain that was inviable when *CHC1* was disrupted (34). The inviability of the *chc1*Δ strains was traced to a single locus, $SCD1^L$ (it has not been rigorously determined whether the lethal allele of *SCD1* is dominant or recessive; therefore we refer to the alleles as $SCD1^V$ [*chc1*Δ strains are viable] and $SCD1^L$ [lethal to *chc1*Δ strains]). To determine whether light chain gene disruptions were also lethal in this strain, one allele of *CLC1* was deleted as before, except *URA3* was used in place of *HIS3* to mark the deletion. In contrast to the *chc1*Δ mutants, when one allele of the *CLC1* gene was disrupted in this strain and the diploid induced to sporulate, both *CLC1* and *clc1*Δ spores gave rise to viable colonies (see Fig. 7 *c*). In the eight tetrads scored, each tetrad contained two small and two large colonies. All 16 small colonies were Ura^+, indicating that they carried the *CLC1* deletion. In confirmation of the Lemmon and Jones observation (34), we failed to obtain viable *chc1*Δ segregants upon sporulation of $SCD1^L/SCD1^L$ diploids heterozygous for a clathrin heavy chain deficiency. Thus, *CLC1* and *CHC1* deletion phenotypes were not entirely equivalent.

Discussion

The chemical properties of yeast and mammalian clathrin light chains are strikingly similar. Both are acidic, heat-stable proteins that migrate aberrantly in SDS-PAGE, and bind Ca^{2+} and calmodulin. The similar physical properties of yeast and mammalian light chains may result from evolutionary conservation of their structures.

In contrast, the sequences of yeast and mammalian light chains have diverged. While yeast and rat clathrin heavy chains display ~50% identity (Lemmon, S. (School of Medicine, Department of Molecular Biology and Microbiology, Case Western Reserve University, Cleveland, OH), personal communication), the light chains are conserved only one third as much (18%). This finding may have been anticipated by the polymorphism of light chains within a species. Mammals contain two classes of light chains, LC_a and LC_b,

Table III. Analysis of Tetrads from LSY94 (CLC1/clc1Δ, CHC1/chc1Δ)

Ratio viable to inviable spores	Ratio large to small colonies		Number of tetrads
4:0	0:4		5
	1:3		11
	2:2		3
		Subtotal	19
3:1	0:3		3
	1:2		4
		Subtotal	7
2:2	0:2		3
	1:1		1
		Subtotal	4
		Total	30

Table IV. Recovery of chc1Δ clc1Δ Colonies from Sporulation of LSY94

Type of spore	Number of spores
His⁻ Leu⁻ (*CLC1 CHC1*)	22
His⁺ Leu⁻ (*clc1Δ CHC1*)	30
His⁻ Leu⁺ (*CLC1 chc1Δ*)	25
His⁺ Leu⁺ (*clc1Δ chc1Δ*)	17
Inviable	15
Unscored*	11
Total	120

* Some colonies were too small to be scored.

which are 60% homologous and which are, in addition, expressed in tissue-specific forms. In contrast, the heavy chain appears to be a unique species in both rat (28) and yeast (46). Taken together, these results suggest that light chain can tolerate more amino acid sequence degeneracy than can heavy chain. Although the various light chains may be adapted for different functions, they are equivalent in at least one function: binding to heavy chain (62). Other proteins, such as lysozyme, can vary greatly in sequence between species, yet retain a similar structure and function (15). One stringent test of the similarity of yeast and mammalian light chains would be to attempt to complement *clc1Δ* mutant phenotypes by expressing a mammalian light chain in yeast.

When compared to the mammalian LC_a and LC_b, the yeast light chain sequence does not resemble one class more than the other. Aside from the homology to the brain-specific insert in LC_b, the yeast light chain defines an independent class. Light chain sequence information from additional species is required to determine whether there are other light chains resembling the yeast protein or if other novel classes exist.

Homology between yeast and mammalian light chains is significant in an amino-terminal region, at the carboxy terminus, and in a brain-specific insert region. The amino termini of LC_a and LC_b, with the exception of the perfectly conserved region (Fig. 7 *a*, positions 28–49), are distinct. Similarly, the amino-terminal sequences of yeast and mammalian light chains are most homologous in this 28–49 region (see Fig. 7). The mid-region of light chain, thought to bind to clathrin heavy chain, is not particularly well conserved between yeast and mammals. Nevertheless, the association of yeast heavy chain with light chain is, as observed with mammalian triskelions, stable to 2 M urea (46). In the mammalian proteins this region contains heptad repeats and bears some similarity to intermediate filament proteins. The yeast protein contains a less extensive series of heptad repeats and weak similarity to coiled-coil proteins. However, yeast and mammalian light chains are not similar to the same regions of the coiled-coil proteins, suggesting that the similarities indicate a common structural motif rather than evolutionary relatedness. The lack of helix-breaking residues and predicted α-helical structure of the mid-regions in both yeast and mammalian light chains is consistent with the hypothesis that a coiled structure may mediate light chain–heavy chain association (8, 24, 29).

The existence of a possible homolog of the brain-specific insert in yeast light chain is intriguing. The homology to the insert is not colinear with the other homologies we detected, and its significance is difficult to assess because of the short length of the brain-specific insert (18 amino acids). It may be that the location of the insert has shifted during evolution relative to the rest of the protein. Alternatively, the apparent homology may arise from the similar amino acid composition of the two proteins. Because there are no splicing signals in *CLC1*, the expression of this region is not limited to specialized light chains in yeast. If light chain in a unicellular eukaryote may be said to serve a basic function, the existence of this region in yeast would imply that the light chains expressed in mammalian brains may be adapted to perform this basic function, rather than a novel, specialized one.

Brodsky (7) has proposed that the brain-specific insert may serve to adapt coated vesicles to a configuration best suited for recycling membrane after the release of neurotransmitter at a nerve synapse. Perhaps a major function of clathrin in yeast is membrane recycling. Studies on the role of clathrin in the intracellular retention of the Golgi membrane protein, Kex2p, are consistent with this proposal. Kex2p, a resident secretory pathway enzyme involved in processing α-factor mating pheromone (14, 25), is mislocalized to the cell surface in yeast cells lacking clathrin heavy chain, resulting in secretion of unprocessed α-factor. Clathrin light chain mutants also fail to process α-factor (Payne, G. (Department of Biological Chemistry, UCLA School of Medicine, Los Angeles, CA), personal communication) (47). Kex2p may normally be tethered intracellularly by a clathrin patch or retrieved from the cell surface via a clathrin-dependent mechanism. Further experiments should distinguish between the various models for the mechanism of Kex2p retention in the cell and determine whether alteration of the light chain in its "brain-specific" sequence affects retention.

Light chain deletion mutant cells grow poorly, suggesting that this protein plays as important a role in coated vesicle function as the heavy chain. Like heavy chain deletion mutants, *clc1Δ* cells exhibit variable growth rates when propagated. Such variation might arise by increasing the copy number of some gene(s) that serve to ameliorate the growth of cells lacking clathrin. Clathrin heavy chain mutants often contain multiple nuclei (34, 36); perhaps polyploidy arising from multiple nuclei is responsible for the variation in growth rates of *clc1Δ* cells.

The similar slow-growth phenotype of mutants lacking clathrin heavy chain, light chain, or both subunits, suggests that light chain may be required for efficient heavy chain expression or function. Cages lacking light chain can be formed in vitro (52, 62); however, such a structure may not be stable in vivo because of some cellular mechanism requiring complete triskelions for coat assembly or maintenance. Similarly, although light chains are not necessary to maintain heavy chain trimers in vitro, the absence of light chain may affect heavy chain oligomerization. Differences in the severity of light chain and heavy chain deletion phenotypes in an *SCD1*^{L} strain could indicate some residual amount of heavy chain activity in light chain deletion strains.

Alternatively, coats lacking light chain may be unusually stable, thus interfering with the normal cycle of coated vesicle assembly–disassembly. Clathrin light chain is required for the enzymatic disassembly of coated vesicle coats by the uncoating ATPase protein in vitro (51). Stable recruitment of clathrin heavy chain to the membrane-bound pool in *clcl*Δ cells would provide in vivo evidence for a light chain–dependent uncoating function. Furthermore, if the poor growth phenotype of *clcl*Δ cells is due to unusually stable coats, *CHCl* and *CLCl* gene disruption phenotypes could affect cellular processes in distinct ways, as one lesion would cause the vesicles to be without coats and the other would yield vesicles that could not be uncoated. In this case the heavy chain gene deletion phenotypes should be epistatic to (mask) light chain gene deletion phenotypes in double mutant cells. Further characterization of the single and double deletion mutants should clarify the relationship of their phenotypes.

The poor growth of *clcl*Δ cells could be caused by the absence of a crucial interaction between light chain and some other (nonclathrin) cellular component(s). This model predicts that some of the phenotypes in a heavy chain deletion strain may actually be a result of light chain mislocalization. Studies of the expression, oligomerization state, and localization of clathrin heavy chain in *clcl*Δ cells will begin to address the nature of the growth defect in light chain deficient cells.

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