Clathrin light chains are calcium-binding proteins (Mooibroek, M. J., Michiel, D. F., and Wang, J. H. (1987) J. Biol. Chem. 262, 25-28) and clathrin assembly can be modulated by calcium in vitro. Thus, intracellular calcium may play a regulatory role in the function of clathrin-coated vesicles. The structural basis for calcium's influence on clathrin-mediated processes has been defined using recombinant deletion mutants and isolated fragments of the light chains. A single calcium-binding site, formed by residues 85-96, is present in both mammalian light chains (LC, and Lc) and in the single yeast light chain. This sequence has structural similarity to the calcium-binding E/F-hand loops of calmodulin and related proteins. In mammalian light chains, the calcium-binding sequence is flanked by domains that regulate clathrin assembly and disassembly.

Clathrin-coated vesicles mediate transport of receptors between the cell membrane and intracellular compartments (1). The polyhedral clathrin coat is formed by polymerization of triskelion-shaped clathrin molecules composed of three 180-kDa heavy chains and three light chains (25-27 kDa). Cytoplasmic domains of receptors are trapped in the clathrin network by association with adaptor proteins that coassemble with clathrin (2) and interact with receptor tails (3,4). Assembly of the clathrin coat can be reproduced in vitro in the absence of adaptor proteins, receptors, or membranes. This in vitro polymerization occurs spontaneously below pH 7.0 and requires calcium but not magnesium in low ionic strength buffers (5). Mooibroek et al. (6) have shown that the light chains are the calcium-binding components of the clathrin triskelion and that each light chain contributes a single calcium-binding site with a dissociation constant of 25-50 μM. These findings implicate the light chains as the calcium-sensitive elements in clathrin assembly.

We have now localized the calcium-binding site of mammalian clathrin light chains to a linear segment of 12 residues in the amino-terminal half of the polypeptide. A related sequence is found in the single light chain of yeast, which is also shown to bind calcium. These results provide further evidence that the clathrin light chains consist of a linear array of different functional domains. From the amino terminus, they are comprised of a phosphorylation site (7), a binding site for the uncoating protein,1 a heavy chain binding region that spans the middle third of the molecule (8), and a region near the C-terminus that is exposed to the cytoplasm and contains tissue-specific insertion sequences (9). The calcium-binding site of clathrin light chains maps between the uncoating domain and the heavy chain-binding domain. The uncoating domain is the target sequence for the 70-kDa heat shock cognate protein (hsc70) that depolymerizes clathrin-coated vesicles (10), whereas the region of heavy chain-light chain interaction has been shown to participate in assembly of clathrin triskelions (11). Thus, calcium binds a region of the clathrin light chains which could readily contribute to the regulation of clathrin assembly and disassembly.

Although, several calcium-binding proteins with important regulatory functions have been identified (12), the function of most calcium-binding proteins is not known. Additionally, the structural basis for calcium binding has been characterized for only a few of them (13, 14). One structure is the "EF-hand" loop sequence (15) which binds calcium to induce conformational changes that alter the affinity of the calcium-binding protein for its substrate (16). In the EF hand, calcium ions are chelated by residues in a 12-amino acid loop that is flanked by α-helices on both sides. The prototype example is located between the E and F helices of parvalbumin and can be modeled using the human hand. Other types of calcium-binding sites have been mapped to cysteine-rich regions of proteins (17) or to sequences that contain γ-carboxy glutamic acid residues (18). The sequences of the calcium-binding sites of clathrin light chains appear to be modified versions of the EF hand-binding motif.

** Experimental Procedures

Purification of Clathrin Light Chains—A membrane fraction containing coated vesicles was isolated from bovine tissues (19) and coat proteins stripped off with approximately 25 ml of extraction buffer (0.75 M Tris, 25 mM EGTA, 0.12 mM MgCl₂, 0.02% NaN₃, pH 6.2) per 300 g of tissue for 1 h, 4 °C. The stripped membranes were removed by centrifugation at 125,000 g, and the supernatant boiled for 10 min to isolate clathrin light chains (20). After removing the precipitated proteins by centrifugation at 135,000 × g, the supernatant was dialyzed extensively against 10 mM Tris, 0.2 mM EGTA, 0.02% NaN₃, pH 7.5, and loaded onto a DE52 column (21). The clathrin light chains were eluted with a 40-300 mM NaCl gradient with Lc eluting before Lc. Yeast clathrin light chain was a gift from L. Silveira and R. Schekman.

Generation of Light Chain Mutants—A full-length cDNA clone of bovine brain Lc (22) was inserted into a Neo-SacI cloning site in

1 DeLuca-Flaherty, C., McKay, D. B., Parham, P., and Hill, B. L. (1990) Cell 62, 875-887.

2 The abbreviations used are: MES, 4-morpholineethanesulfonic acid; EGTA, [ethylenebis(oxyethylenenitrilo)tetraacetic acid; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.
the bacterial expression vector pOTsNca12 (23). This placed the initiation codon in frame to result in the translation of full-length LCb without any additional amino acids at either the N or C terminus. Light chain mutants Δ26-56 was made by removing the internal BamHI fragment which eliminates amino acids P26-G56 and inserts an R residue between D25 and S57 of the wild-type protein. Deletion mutant Δ22-96 was constructed by removing the Nael-Clal fragment which eliminates residues G22-D86. The mutant without the C-terminal third of the molecule, Δ163-243, was generated by deleting the HindIII-SacI fragment and by creating an in-frame doublet of stop codons after amino acid A162 with synthetic oligos to result in LCb truncated at amino acid A162.

Wild-type proteins and mutants Δ26-56 and Δ163-243 were expressed in Escherichia coli strain N5151 by heat induction. Mutant Δ22-96 was expressed in E. coli strain AR120 with naldixic acid. For heat induction cultures were grown at 30 °C to OD550 = 0.8-1.0 and the temperature raised rapidly to 42 °C by addition of 1/5 volume of media at 65 °C. Incubation at induction temperature was for 0.5-1.0 h before the cells were harvested. For naldixic acid induction, cultures were grown at 37 °C to OD550 = 0.8. Naldixic acid was added to 60 mg/ml and incubation continued at 37 °C for 2.5 h before cells were harvested.

Recombinant light chain proteins were purified from cell extracts by affinity chromatography using anti-LCb monoclonal antibody X16 (9) coupled to CNBr-activated Sepharose (Pharmacia LKB Biotechnology Inc.). Cell extracts were generated by boiling cells that had been lysed in 50 mM Tris, pH 8.0, 12% sucrose, 70 mM EDTA, 0.05% Triton X-100, 0.17 mg/ml lysozyme, and 10% saturated ammonium sulfate for wild-type light chain, mutant Δ26-56, and mutant Δ22-96 or 50 mM Tris, pH 8.0, 12% sucrose, 50 mM EDTA, and 0.8% Triton X-100 for mutant Δ163-243.

CNBr Digests of Clathrin Light Chains—A solution of purified light chains was desalted into water using an EconoPac 10DG column (Bio-Rad). The protein was lyophilized and 50-100 μl of 70% formic acid saturated with CNBr was added to 50-200 μg of protein. After incubation for 14 h at room temperature, the sample was diluted 4-fold with water and excess CNBr removed by desalting into water on EconoPac 10DG columns. The fractions containing protein were lyophilized prior to further analysis.

45Ca Binding and Antibody Binding to Immobilized Proteins—Calcium binding to light chains and other proteins was detected according to the method of Maruyama et al. (24). Proteins were either transferred by electrophoresis to nylon-supported nitrocellulose membranes after separation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (25) or blotted onto Zeta-probe nylon membranes (Bio-Rad) using a dot-blot apparatus. The membrane was washed in 10 mM imidazole, pH 6.8, 3 mM MgCl2, 100 mM KC1 for 1 h and then incubated with 45Ca (1 μCi/ml) for 10 min in the same buffer. The blots were washed with 100 ml of 50% ethanol in three to four washes and exposed to x-ray film after drying at room temperature overnight. Calmodulin (Behring Diagnostics) was used as positive control and ovalbumin (Sigma) as a negative control for 45Ca binding.

Monoclonal antibody binding after SDS-PAGE and transfer to nitrocellulose was detected with goat-anti-mouse Ig conjugated to horseradish peroxidase (Bio-Rad) (11) using 4-chloro-1-naphthol as substrate (26).

Antibody Inhibition of Calcium Binding by Light Chains—Light chains were incubated with equimolar amounts of purified monoclonal antibodies. The mixture (40 μg of total protein) was blotted onto nylon membranes in a dot-blot apparatus and probed with 45Ca as described above. The data was evaluated by scanning the autoradiograph of the blot at 595 nm in a DU-64 Beckman spectrophotometer.

RESULTS

Clathrin light chains bind 45Ca after separation by SDS-PAGE and transfer to nitrocellulose (6). This assay, done in the presence of 3 mM MgCl2, was used to test the calcium-binding properties of different forms of clathrin light chains, deletion mutants, and fragments of clathrin light chains. This strategy allowed progressive elimination of protein sequences from involvement in calcium binding and localization of the calcium-binding site.

Calcium Binding by Light Chains and Deletion Mutants—Mammalian tissues express two classes of clathrin light chain, LCa and LCb, which are the products of different genes (22, 27). Distinctive splicing patterns of LCa and LCb mRNA in neuronal cells contribute to additional light chain heterogeneity (28). In neuronal cells, LCa and LCb both contain additional domains of either 18 or 30 amino acids, inserted in the sequences of the non-neuronal light chains at homologous positions near the carboxyl terminus. These different forms of clathrin light chains were tested for calcium binding (Fig. 1). Clathrin light chains from bovine brain bound 45Ca, confirming the results reported by Mooibroek et al. (6). In addition, 45Ca binding to adrenal gland light chains demonstrated that calcium binding is a shared property of clathrin light chains from neuronal and non-neuronal tissue and that the inserted sequences are not involved in formation of the calcium-binding site. Yeast clathrin light chain, which has limited primary sequence homology with mammalian light chains (29), also bound 45Ca (Fig. 1), suggesting that calcium binding is a generally conserved property of clathrin light chains.

cDNAs encoding three mutant light chains were constructed from cDNA for bovine brain LCb with deletions encompassing residues 26-56 (mutant Δ26-56), 163-243 (mutant Δ163-243), and 22-96 (mutant Δ22-96). The mutant proteins were expressed in E. coli, purified, and tested for 45Ca binding (Fig. 1). Mutant Δ26-56 and mutant Δ163-243 bound calcium whereas mutant Δ22-96 did not. These results showed that residues 26-56 and 163-243 are not involved in calcium binding and that the site involves sequences between residues 56-96 and/or 22-25.

Calcium-binding Site Mapping with CNBr Fragments—Further definition of the calcium-binding site was achieved by analysis of the calcium-binding properties of cyanogen bromide (CNBr)-fragments prepared from bovine brain LCb (Fig. 2). CNBr digestion produces three fragments, CNBr1+2 (residues 71-233/243), CNBr1 (residues 71-125), and CNBr2 (residues 127-233/243) (9). CNBr1+2 and CNBr2 are generated with and without cleavage at the C-terminal methionine at position 284. The CNBr fragments were identified by their reactivity with different anti-light chain monoclonal antibodies (Fig. 2). In a CNBr digest of LCb, the CNBr1+2 fragment bound 45Ca but the CNBr2 fragment did not, although both were present in equal amounts. This indicated that the calcium-binding site is located between residues 71 and 126. CNBr1 was not present in large enough quantities to test for calcium binding directly and was found to be highly susceptible to degradation in subsequent digests.

Analysis of the calcium-binding properties of the LCb CNBr fragments and of the LCa deletion mutants, reveals that the calcium-binding site is located between residues 71 and 96. This result was derived by progressive elimination of sequence segments from involvement in calcium binding (Fig. 3A). Calcium binding by the deletion mutants rules out residues 1-21, 26-56, 97-243, and 163-243 and implicates residues 22-25 or 56-96. Since CNBr1+2 binds 45Ca, residues 1-70 are ruled out, narrowing down the site to residues 71-96. Residues 127-234 are also ruled out by lack of 45Ca binding by CNBr2, again showing that the neuron-specific insertion sequences are not involved in calcium binding. These results also eliminate residues 23-44, a region of the molecule that, on the basis of its conservation and sequence, might have been predicted to bind calcium. This sequence (EEDPAAAFLAQKQSFVAGIRND) is identical in LCa and LCb from three different species (30) and comprises one of the two regions of homology with the yeast sequence (29). Acidic residues are commonly associated with calcium chelation and this region contains seven acidic and no basic amino acid residues. How-
Fig. 1. Calcium binding to light chains and related proteins. Proteins were incubated with $^{45}$Ca after transfer to nylon-supported nitrocellulose by electrophoresis, following SDS-PAGE. $^{45}$Ca binding was detected by autoradiography (panel A). Blots with equivalent protein samples were stained with Amido Black (panel B). The lanes were loaded with the following samples: bovine adrenal LCAb, 20 µg (a); ovalbumin, 15 µg (b); calmodulin, 1 µg (c); yeast light chain, 25 µg (d); bovine brain LCAb, 20 µg (e); LCAb mutant Δ26-56, 20 µg (f); recombinant LCAb, 20 µg (g); bovine brain LCAc, 15 µg (h, panel A) and 2 µg (h, panel B); LCAc mutant Δ22-96, 20 µg (i, panel A) and 2 µg (i, panel B); LCAc, mutant Δ163-243, 20 µg (j, panel A) and 1.5 µg (j, panel B); bovine brain LCAb, 20 µg (k, panel A) and 1.5 µg (k, panel B). Migrations of molecular mass markers (kilodaltons) are indicated at the left of panels A and B. Arrowheads to the left of lanes g, i, and k mark the positions of mutants Δ26-56, Δ22-96, and Δ163-243, respectively. The lanes numbered to the right of lanes a, c, and e mark the positions of cellular (upper) and non-cellular (lower) forms of bovine LCAb.

However, since mutant Δ26-56 and fragment CNBr1+2(71-233) bound $^{45}$Ca, this region is not part of the calcium-binding site.

These experiments also mapped the sequences responsible for the anomalous migration of the clathrin light chains in SDS-PAGE. It is well documented that on the basis of electrophoretic mobility, they appear 6-9 kDa larger than their predicted molecular weights. The anomalous migration of clathrin light chains was first reported by Black et al. (1991). The anomalous migration of clathrin light chains is due to the presence of a positively charged amino acid residue (arginine) in the calcium-binding loop of the light chain. This arginine residue is not present in the heavy chain of clathrin, which has a negatively charged amino acid residue (aspartic acid) in the calcium-binding loop of the heavy chain. These results, in combination with the results reported here, further localize the effect to residues 22-25. This sequence is "GEED" in LCAb and "AEED" in LCAc. The high local concentration of negatively charged residues could reduce local SDS binding and thereby alter mobility in SDS-PAGE. Alternatively, strong ionic interactions...
FIG. 4. Models of the calcium-binding loop sequence of clathrin light chains. Residues 85–96 from LCa, LCb, and yeast light chain (Fig. 3C) were modeled by alignment with the known structure of the calcium-binding loop in the second EF-hand of carp parvalbumin as a template. Models were derived using the program INSIGHT: A, carp parvalbumin (residues 90–101) based on the determined structure (53); B, LCa (residues 85–96), C, LCb (residues 85–96) with the position of the calcium ion adjusted to optimize the distances from chelating residues; D, overlay of the carp parvalbumin loop (purple/pink) with the predicted calcium binding loop of LCa (blue); E, yeast light chain (residues 85–96); F, overlay of carp parvalbumin loop (pink) with yeast light chain (yellow). The amino terminus of each loop is at the bottom of the model, behind, or to the right of the calcium ion which is shown in green. The C-terminal amino acid appears at the top right of the modeled loop. The distances (Å) between the chelating residues and the calcium ion are indicated in the loop for carp parvalbumin (a) and LCa (b).

between the negatively charged cluster and another portion of the molecule might cause formation of a nonlinear species that produces the effect. The recombinant light chains made in E. coli show the same mobility properties as the mammalian wild-type proteins. This further substantiates that the anomalous mobility is due to the encoded primary structure rather than post-translational modification.

Structural Similarity to the EF-hand Calcium-chelating Loop—The calcium-binding region of clathrin light chains (residues 71–96) was compared with calcium-binding se-
which cannot chelate calcium directly. A water molecule is binding loop of carp parvalbumin, position 9 is a glycine contain oxygen and/or nitrogen atoms. In many EF-hand sequences used to bind calcium (39). For example, in the second calcium-parvalbumin loop.

Refrains of other proteins using the “Bestfit” sequence comparison program (31, 32). These included several EF-hand sequences as well as cysteine-rich calcium-binding sequences from the low density lipoprotein receptor and protein C (33), the γ-carboxy glutamate sequences from human prothrombin (34) and matrix GLA protein (35). Additional calcium-binding sequences were from cell adhesion molecules (36), lipocortins (37), and calcium-dependent cytoskeletal proteins (38). Structural similarities were found between the 12-amino acid calcium-chelating loops in four EF-hand sequences and residues 85–96 of both LC, and LCb (Fig. 3C). A search of the complete yeast light chain sequence identified a similar loop, at a corresponding location, as the only candidate for a calcium-binding sequence. To determine if the loop sequences identified in light chains are compatible with the structural motif characteristic of EF-hands, they were modeled to fit the known structure of the second calcium-binding loop in carp parvalbumin (residues 90–101) (Fig. 4A), using the program INSIGHT. Calcium-binding properties of the clathrin light chain loops were clearly apparent (Figure 4, D–F). When the backbone atoms in clathrin light chain residues 85–96 were aligned using the structure of the parvalbumin loop as a template, the amino acid side chains could be oriented to accommodate a calcium ion at a position similar to that found in the parvalbumin loop. Optimization of the distance between the bound calcium ion and the chelating residues in the modeled structures required adjusting the calcium ion position by less than 0.5 Å from its corresponding location in the parvalbumin loop.

In EF-hand structures, the residues that have potential for chelating calcium are the first, third, fifth, seventh, ninth, and twelfth residues within the loop of the helix-turn-helix motif. Chelating residues are usually negatively charged or contain oxygen and/or nitrogen atoms. In many EF-hand structures, only 4 or 5 of the potential chelating residues are used to bind calcium (39). For example, in the second calcium-binding loop of carp parvalbumin, position 9 is a glycine which cannot chelate calcium directly. A water molecule is associated with this portion of the loop and chelates the calcium ion. Mammalian LC, and LCb can chelate calcium with 4 residues: D87, Y89, A91 (carbonyl from backbone), and D96 (Fig. 4). Yeast light chain has 5 calcium-chelating residues, D85, N87, S89, T91 (carbonyl from backbone), and N96. The distances between the chelating atoms and the calcium ion were 2–3 Å in all models of the clathrin light chains, distances similar to those found in the crystal structures of known calcium-binding sites.

Antibodies to the Amino-terminal Portion of the Heavy Chain Binding Region Reduce Calcium Binding—The calcium-binding site in clathrin light chains is next to the heavy chain binding region which spans the middle portion of the light chain molecule (Fig. 3B). The heavy chain binding region has been proposed to form a long α-helix with a nonpolar face that could interact with a complementary α-helix in the clathrin heavy chain (30, 40). This helix could act as the C-terminal helix in the helix-loop-helix arrangement of the proposed EF-hand. Antibodies which bind to this helix near the predicted EF-hand loop caused a reduction in calcium binding by purified LC, light chain (Fig. 5). Antibodies to other sites on LC, (mapped in Fig. 3B) had no effect on calcium binding. Calcium binding by LCb was also reduced by antibodies to the amino-terminal portion of the heavy chain binding region (X43, X49, and X50), whereas antibodies specific for the brain insertion sequence (LCB.3 and LCB.72) had no effect (data not shown). These results support mapping of the calcium-binding site to the predicted loop adjacent to the amino terminus of the heavy chain binding region.

DISCUSSION

The calcium-binding site of clathrin light chains was localized to residues 71–96 by calcium-binding studies of deletion mutants and of light chain fragments. A comparison with sequences from other calcium-binding proteins indicated that clathrin light chain residues 85–96 have similarity to the calcium-chelating loop in the classical EF-hand calcium binding motif. Calcium-binding properties of residues 85–96 were revealed when this sequence from LC, and LCb, and yeast clathrin light chain was modeled to fit the structure of the second EF-hand loop from parvalbumin. In the known structures of calcium-binding proteins, EF-hand loops are generally flanked by α-helices on either side. Secondary structure predictions for the clathrin light chains (41, 42) indicate that the 85–96 loop could be part of a helix-loop-helix motif. This is particularly clear for the predicted structure of the yeast light chain. In all three types of clathrin light chains (LC, LCb, and yeasts), the C-terminal helix flanking the calcium-binding loop coincides with the heavy chain binding region (8). This arrangement is similar to the second EF-hand loop in calmodulin where the calcium-chelating loop is flanked by a long α helix that interacts with the target enzymes of calmodulin (43). In the clathrin triskelion, the helical region of heavy-light chain binding has been shown to participate in triskelion-triskelion interactions during assembly of the clathrin coat (11). Calcium influences triskelion assembly (5) and may do so by binding adjacent to this helical region of triskelion interaction. This effect on clathrin polymerization is specific to calcium and not mediated by magnesium, correlating with the specificity of the binding site localized in this study.

Most proteins with EF-hand sequences, have a higher affinity (Kd = 0.1–5 μM) for calcium than clathrin light chains. However, some calcium-binding proteins with sequences homologous to EF-hands such as annexins (37) and flagellar calcium-binding protein (44) have calcium affinities similar to light chains, in the range of 25–50 μM (14). The low calcium...
affinity in these proteins is due to fewer chelating residues and the presence of amino acids in the EF-hand structure that chelate calcium less efficiently than those in calmodulin and other classical calcium-binding proteins. Modeling suggests this may also be true for clathrin light chains, which are predicted to have 4 or 5 chelating residues, compared with the 5 to 6 found in calmodulin. Furthermore, one of the proposed chelating atoms in the mammalian clathrin light chain loops is the hydroxyl oxygen of tyrosine 89. Tyrosine residues have also been found in chelating positions of calcium-binding EF-hand loops in other proteins (45-47). Due to interactions between the aromatic ring and the hydroxyl group in this side chain, the hydroxyl oxygen is expected to chelate the calcium ion less efficiently in comparison to the electron-donating carboxylate oxygen that is found in this position in the EF-hand loops of calmodulin and parvalbumin. The dimensions of most EF-hand loops are too large to chelate magnesium (39) and even the lower affinity sites bind calcium in the presence of magnesium as found for the calcium-binding sites in clathrin light chains.

Clathrin light chains span the proximal portion of each arm of the triskelion (21) and are oriented with their C terminus toward the vertex (48). Thus, the calcium-binding sequence at the N-terminal side of the heavy chain binding region is likely to be near the "elbow" of the triskelion arm. In assembled clathrin coats, each vertex of a polyhedron (where three elbows meet) would be surrounded by three calcium ions, donated by one light chain from each of the three neighboring triskelions. This arrangement has potential for calcium to cause local conformational changes. Such changes influence exposure of the recognition sequence for the coated vesicle uncoating protein, hsc70. Recent data from our laboratory indicates that the region to the N-terminal side of the calcium-binding site in light chain LC3 is involved in binding hsc70 and stimulating ATP hydrolysis. Accessibility of this region is increased by calcium, demonstrating that calcium has local structural effects on the light chains.

Calcium binding by free light chains and whole triskelions has been demonstrated at 1 μM calcium and 3 mM magnesium (6). This is the upper range of physiological calcium concentrations (0.1-1 μM) (49) and exceeds intracellular magnesium concentrations (0.5 mM) (49). It has also been shown that the calcium concentration close to the plasma membrane or membranes of other cellular compartments can be higher than the average cytoplasmic calcium concentration (50, 51). Since clathrin operates in close proximity to the plasma membrane it could experience fluxes of calcium concentration that would be sufficient to regulate clathrin assembly or disassembly through binding of calcium to light chains. Calcium binds clathrin light chains with a KD of 25 μM, which means 50% of clathrin light chains would have calcium bound at 25 μM. Thus, at physiological concentrations of calcium at least 1-5% of light chains have calcium bound, depending on local calcium concentrations. The average clathrin-coated vesicle is composed of 60-140 clathrin triskelions (52) with 180-420 light chains. Statistically, some calcium would always be bound by the triskelions in a clathrin-coated structure and may expose enough recognition sites to initiate the action of the hsc70 uncoating protein. It is also possible that the affinity of assembled clathrin for calcium is higher than that measured for triskelions and free light chains, increasing the chances that assembled clathrin would bind calcium at physiological concentrations and stimulate the uncoating ATPase. Although the degree to which calcium controls the assembly state of clathrin in situ has yet to be defined, the localization of the calcium-binding site between the light chains of the light chain molecule that influence clathrin assembly and disassembly is strongly indicative of its regulatory role.

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