

Review

The cellular functions of clathrin

S. J. Royle

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH (United Kingdom), Fax: +44 1223 402310, e-mail: sjr51@mrc-lmb.cam.ac.uk

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Abstract. Membranes and proteins are moved around the cell in small vesicles. A protein coat aids the budding of such vesicles from donor membranes. The major type of coat used by the cell is composed of clathrin, a three-legged protein that can form lattice-like coats on membranes destined for trafficking. In this review, I out-

line what we know about clathrin and discuss some recent advances in understanding the basic biology of this fascinating molecule, which include building a molecular model of a clathrin lattice and discovery of a new function for clathrin that occurs during mitosis.

Keywords. Adaptor, cancer, clathrin, dynamin, endocytosis, mitosis, TIRFM.

Introduction

Endocytosis is crucial to cellular function. For example, the cell uses endocytosis to control the density of receptors on the cell surface and to acquire nutrients. Moreover, specialised cells use endocytosis to enable synaptic transmission or to present antigens on the cell surface. Because of these indispensable functions, endocytosis has received intense research effort for many years. This review focuses on clathrin, a protein that is essential for clathrin-mediated endocytosis (CME) in mammalian cells. Besides clathrin, there are other coat-forming proteins, such as COP I and COP II, that mediate intracellular traffic [1, 2], and there are clathrin-independent endocytic pathways which mediate internalisation of a variety of cargo [3]. Here, the role of clathrin in two cellular processes is described: the function of clathrin in forming lattices during clathrin-mediated membrane traffic and the recently discovered role of clathrin in mitosis.

Brief history of clathrin

The study of clathrin began over 40 years ago when Roth and Porter, who were studying the uptake of yolk pro-

tein in mosquito oocytes, described what we now know as clathrin-coated pits and vesicles [4]. Using electron microscopy (EM), they observed bristle-like coats at the borders of invaginations of the plasma membrane and of vesicles in the cytosol. In some glancing EM sections, the bristle-like coat could be seen to be composed of a lattice-like polygonal array, the geometry of which was similar to that of the leather seams on a football [5]. These observations led to the idea that membranous vesicles are contained within a basket and that this basket could actually drive deformation of the membrane into a pit and vesicle (see [6] for a detailed historical review).

Ten years on, the purification of coats and coated vesicles from bovine brain allowed Pearse to identify the major protein component, the clathrin heavy chain (~190 kDa) [7, 8]. In addition to the heavy chain, clathrin light chains of two classes (LCA and LCB, each ~25 kDa) were also isolated [9, 10]. Clathrin was found to be a trimeric assembly of three heavy chains each with an associated light chain, and it is this three-legged structure, or triskelion, that is the assembly unit of a clathrin coat.

EM and biochemical studies in a variety of tissues have shown that endocytosis proceeds as follows: the nucleation of a clathrin-coated pit (CCP) involving cargo cap-

ture and multimerisation of clathrin; propagation of the coated pit involving membrane invagination; budding or scission of the clathrin-coated vesicle (CCV) by completion of the clathrin cage and the action of dynamin; once inside the cell, the coat disassembles and the uncoated vesicle is trafficked to its destination (Fig. 1). More detailed information about the stages of endocytosis is available in other reviews [1, 11, 12].

Clathrin is involved in coating membranes that are endocytosed from the plasma membrane and those that move between the *trans*-Golgi network (TGN) and endosomes [11]. When coating membranes, clathrin does not link to the membrane directly, but does so via adaptor proteins. The main class of adaptors are the adaptor protein (AP) complexes [6]. AP-2 and AP-1 are members of this family and mediate endocytosis at the plasma membrane and clathrin-mediated traffic from the TGN to endosomes, respectively. In addition, there are several examples of other, alternative adaptors, e.g. β -arrestins, ARH, dab2 and numb [13]. More detailed information on adaptors and their role in membrane trafficking can be found elsewhere [14–16].

Clathrin genes

In humans, two isoforms of clathrin heavy chain exist that have been termed CHC17 and CHC22. CHC17 is a ubiquitous 1675-residue protein that is involved in membrane trafficking and mitosis (see below), whereas CHC22 is a 1640-residue protein expressed in skeletal muscle that is not thought to be involved in endocytosis, but may play a role in the organisation of membranes [17]. These proteins are encoded by two genes, *CLTC* and *CLTD*, at 17q23.2 and 22q11.21 and are 85% similar at the amino acid level. In this review, CHC17 will be referred to as CHC and only as CHC17 when it is necessary to distinguish it from CHC22.

Humans also have two clathrin light chains, a and b (LCA, 218 residues, and LCB, 211 residues) that are much more divergent (60% amino acid identity). LCA and LCB are encoded by two separate genes, *CLTA* and *CLTB*, at 9p13.3 and 5q35.2. There are neuronal-specific splicing variants of both light chains that involve the insertion of a 30- and 18-residue segment in LCA and LCB, respectively. Both LCA and LCB can associate with CHC17 but not with CHC22. Clathrin proteins are incredibly well conserved. For example, human CHC17 shares 70% amino acid similarity to clathrin heavy chain from yeast. Recently, Wakeham *et al.* performed a comparative analysis of clathrin genes from genomes sequenced so far and found that the reason we have two genes for CHC and two for light chain may be the result of independent gene duplication which occurred somewhere between 510 and 600 million years ago in our evolution [18].

The structure of clathrin

The clathrin triskelion has three kinked ‘legs’ that radiate from a central vertex (Fig. 2) [11]. The CHC leg is divided up into three regions: a proximal region that is close to the vertex and is where the light chain attaches, a distal region and the N-terminal domain. This arrangement was first visualised by EM studies of isolated triskelia [9, 10, 19]. In these micrographs, the N-terminal domain appears as a globular region at the end of a ~45 nm leg. Using cryo-electron microscopy (cryo-EM), Crowther and coworkers were able to generate the first three-dimensional maps of clathrin assemblies at low resolution

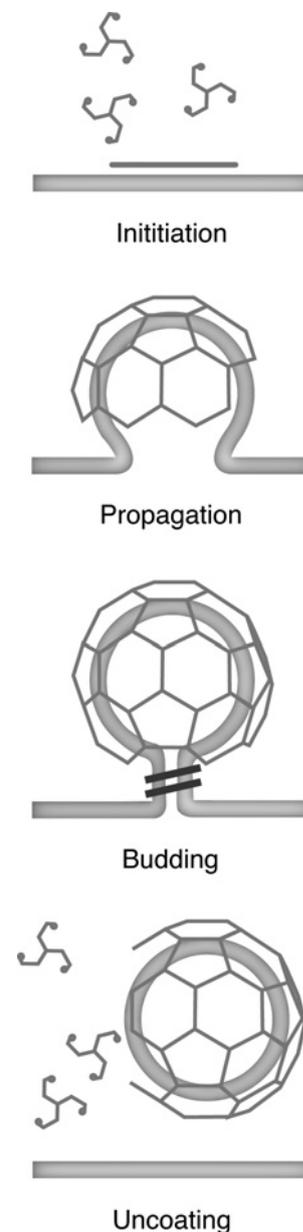


Figure 1. Schematic diagram of clathrin-mediated endocytosis (CME). CME proceeds in four stages: initiation, propagation, vesicle budding and uncoating [1].

[20, 21]. They visualised clathrin cages (geodesic assemblies of clathrin) and clathrin coats (cages with adaptor proteins). The maps showed the basic geometry of the cage and that the coat consisted of an outer density of CHC proximal and distal domains, a middle density of the N-terminal domains and an inner region comprising the adaptors. Three common types of clathrin cages are shown in Figure 2a. The mini-coat has tetrahedral symmetry and is probably too small to accommodate a vesicle. Hexagonal barrels and footballs have D₆ and icosahedral symmetry, respectively [22]. Each of these assemblies has 12 pentagons that are necessary for curvature of the clathrin lattice and a differing number of hexagons. Coats are typically >90 nm in diameter, whereas coats derived from the Golgi apparatus or those in the synaptic terminal are 75–100 nm in diameter. The size of the vesicle that is accommodated can vary according to the size of the coat, while coat thickness remains relatively constant at ~22 nm [22].

Improvements in cryo-EM led to a 21 Å resolution map [23]. This later map allowed the individual triskelia to be resolved, revealing how triskelia interact via the proximal and distal leg regions to form the polyhedral outer cage. A polygonal edge comprises four clathrin leg sections from different triskelia: an anti-parallel pair of proximal regions from triskelia with vertices at opposite ends of the edge and an anti-parallel pair of distal regions from triskelia with vertices one edge away (see Fig. 2b). So a triskelion leg runs from one vertex along two neighbouring polygonal edges and then turns inward, with its terminal domain extending into an inner network to contact the adaptor layer [24].

X-ray crystallographic analysis of CHC fragments expressed in bacteria detailed two main features of CHC at atomic resolution (Fig. 2c). First, crystallisation of residues 1–494 showed that the globular N-terminal domain is a seven-bladed β -propeller structure [25]. This domain is suited to interactions with clathrin box motifs

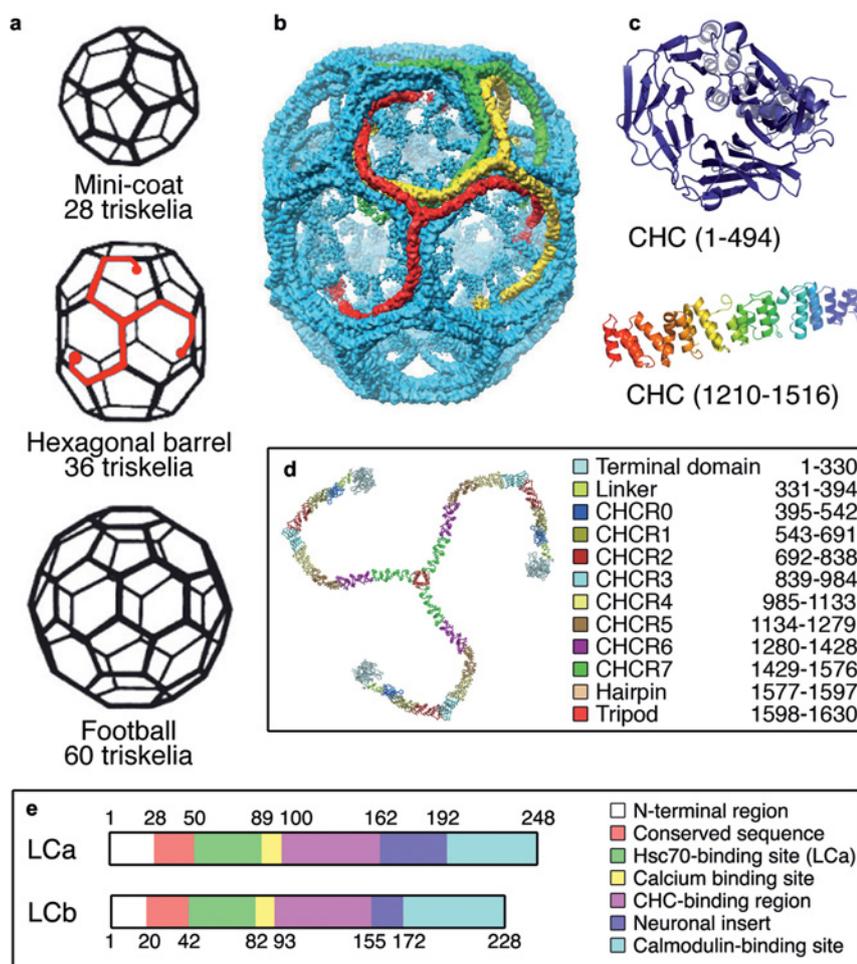


Figure 2. Molecular details of clathrin. (a) Three examples of clathrin cages with different geometry (after [22]). (b) Clathrin hexagonal barrel at 7.9 Å (CHCs only). Three triskelions are highlighted to show how triskelia interact in the cage. Taken from [29], copyright © 2004 Nature Publishing Group. (c) Structure of CHC N-terminal domain and linker (above) and superhelix (below) determined by X-ray crystallography. The N-terminal domain is in complex with a peptide containing a clathrin-box motif. Generated using PyMol and PDB files 1BLO and 1C9L. (d) Molecular model of CHC showing the structural features of the triskelion. (e) Schematic representation of the clathrin light chains a and b to indicate the linear domain structure.

(L Φ X Φ [DE], single amino acid code, where Φ is a bulky hydrophobic amino acid, X is any amino acid and brackets enclose alternatives) such as those present in AP-1, AP-2 and β -arrestin [26]. There is a second interaction site on this domain that can accommodate W-box motifs (PWXXW) such as those found in amphiphysin [27]. Second, X-ray analysis of crystals of part of the proximal leg (residues 1210–1516) revealed a right-handed superhelix of short α -helical zigzags [28]. By alignment, a motif termed the clathrin heavy chain repeat (CHCR) was identified consisting of five α -helix zigzags. As the linker in the distal leg also consisted of α -helical repeats [25], it was proposed that CHCRs extend over the length of the leg [28]. Recently, Fotin *et al.* revisited the cryo-EM method to resolve 3D maps of several different clathrin assemblies, including a hexagonal barrel and a mini-coat [29, 30]. The 7.9 Å resolution of the new maps was sufficient to allow placement of these crystal structures within the map and the remaining regions to be modelled (Fig. 2c, d). The resulting molecular model consists of the seven-bladed β -propeller domain positioned at 180° to a previous placement [24]; a short linker; 8 CHCRs (CHCR0–7); a final α -zigzag hairpin and then a ‘tripod’ region which mediates trimerisation (Fig. 2d) [29]. The molecular model of clathrin provides a clear picture of triskelion structure and also allows for prediction of residues involved in interactions with other triskelia [31].

How does a clathrin lattice disassemble? The answer may come from structural analysis of auxilin in clathrin cages. Auxilin is a co-chaperone of Hsc70, a protein involved in uncoating [12, 32]. Within the clathrin coat beneath each vertex, there is a convergence of three ankle regions from triskelia that are centred two vertices away [29]. The proximity of the C-terminal tripod regions with ankles of distinct triskelia is thought to stabilise the clathrin cage. Cryo-EM of clathrin coats with auxilin bound to them showed that auxilin was positioned between the tripod and the ankle and terminal domains of the other triskelia, causing a distortion of the coat. This suggests a possible mechanism for uncoating where auxilin may weaken the tripod-ankle interaction to destabilise the lattice [30, 33, 34], and allow Hsc70 to bind to the released C-terminus of CHC. In theory, a small, clockwise twist of a triskelion should be sufficient to disengage it from the cage [29].

Despite this wealth of information about CHC, we know much less about the structure and function of the light chains. The light chains bind to the proximal leg of CHC with high affinity (Kd $\sim 10^{-9}$) [35], and suppressor mutagenesis has suggested that there are two residues on CHC (K1326 and K1415) which contact LCa (W107 and W130) [36]. The light chains adopt α -helicity upon binding to CHC [36]. In the cryo-EM study, difference maps allowed the central portion of each light chain to be modelled as a 71-residue α -helix [29]. The absence of density

for N- and C-termini indicates that they may be mobile. The mutagenesis results allowed the central portion of the light chains to be oriented with the N- and C-terminal portions directed towards the knee and vertex, respectively. This positioning agrees with earlier work which showed that only the central portion of the light chains contacts CHC [37] and antibody localisation of light chain domains on isolated triskelia [38–40]. Together these results virtually rule out an alternative model where the light chain is bound to CHC in a U-shaped configuration with both N- and C-termini directed towards the vertex [41].

Clathrin light chains consist of what has been described as a linear array of domains: regions of protein discernable from the primary sequence or with distinct biochemical properties [42]. These are an N-terminal segment, a region that is 100% conserved between LCa and LCb, a portion to which Hsc70 binds (in LCa only), a calcium-binding domain, a region which binds the heavy chain, a site for neuronal-specific splice inserts and then finally a calmodulin-binding domain at the C-terminus (Fig. 2e). These various regions have been proposed to modulate clathrin assembly by interacting with other factors, such as calcium, although this has been difficult to address experimentally. Moreover, the functional relevance of the binding site for Hsc70 is unclear, because clathrin coats which are free of light chains *in vitro* are able to form and disassemble normally. So what is the function of the light chains? One idea, based on *in vitro* studies, is that clathrin light chains can negatively regulate clathrin assembly [43]. In this scheme, the light chains are thought to act as a ‘brake’ to slow down non-productive assembly of clathrin at physiological pH. Because adaptor protein complexes have been observed to stimulate clathrin assembly [44], the braking action of the light chains suggests that clathrin assembly may be adaptor-dependent. Consequently, only productive clathrin assemblies will arise at sites that contain cargo and are specified by the adaptors.

In neurons CME accounts for a significant amount of synaptic vesicle reuptake [45, 46]. It has been widely assumed that neuron-specific inserts in clathrin light chains somehow modulate the function of triskelia found in neurons to aid rapid uptake of synaptic vesicles by CME [47, 48]. Currently, there is no experimental evidence to support this. It is also unclear whether clathrin-mediated endocytosis at neuronal synapses is faster than in non-neuronal cells. The time constant for clathrin-mediated endocytosis of synaptic vesicles is between 10 and 20 s [45, 46], but there has yet to be a comparable measurement made in non-neuronal cells. The neuron-specific light chains have been shown to interact more efficiently with calmodulin [49], but whether there is a functional consequence of this interaction at synapses is unknown.

Whatever the precise function of the light chains, they have proven incredibly useful in live imaging studies, where they can be tagged with fluorescent proteins to allow us to examine clathrin dynamics [50–54].

Imaging clathrin

Immunocytochemistry studies have shown that clathrin in mammalian cells is found in numerous puncta at the plasma membrane, in the cytoplasm and in an accumulation at the Golgi apparatus (for example see Fig. 3a) [55, 56]. The advent of green fluorescent protein (GFP)-tagged LCa (GFP-LCa) allowed the visualisation of the dynamics of clathrin-coated structures in living cells [50]. Using standard epifluorescence (Epi) microscopy, fluorescent puncta corresponding to clathrin-coated structures (CCSs; either CCPs or CCVs) were found to be either stationary or dynamic. Some spots disappeared with a half-life on the order of tens of seconds, potentially representing CCVs that had been internalised and then uncoated. This study also found that CCPs formed at preferential sites on the membrane [50]. Interestingly, GFP-LCa in these CCSs exchanges fairly rapidly with a time course of ~10 s, suggesting there is a substantial amount of exchange between free clathrin and these stationary CCSs [57, 58].

Application of total internal reflection fluorescence microscopy (TIRFM) improved the imaging of clathrin-mediated endocytosis [51, 52]. This imaging method selec-

tively illuminates the bottom 50–150 nm of the cell in the vicinity of the cover slip [59], allowing CCSs on the plasma membrane to be imaged selectively and distinguished from GFP-LCa-positive structures deep within the cell. Combining TIRFM with Epi imaging allowed the visualisation of a budding CCV and tracking of the vesicle as it disappeared inside the cell [53]. Dual-colour TIRFM imaging has led to an understanding of the recruitment of other proteins such as dynamin and β -actin to the CCP [53]. Despite these advances, there was a lack of direct proof that CCSs were actually CCVs that contained cargo. A recent study demonstrated the uptake of fluorescently-tagged cargo (LDL, transferrin and reovirus particles) in relation to clathrin using dual-colour confocal imaging [54]. They found that a quarter of CCSs disassembled without incorporating cargo, suggesting that the capture of cargo may stabilise the developing CCP. However, this may not be a general rule, as other studies have found that cargo can be incorporated into pre-existing CCPs [51].

Merrifield and colleagues used an ingenious TIRFM-based approach to visualise the moment of scission. A pH-sensitive GFP (pHluorin) was fused to the extracellular/intravesicular aspect of the transferrin receptor and expressed in fibroblasts that were stably expressing LCa-dsRed [60]. Extracellular pH was stepped repeatedly from 5.5 to 7.4 in synchrony with image capture, allowing visualisation of the moment of vesicle scission: the point when the pHluorin could no longer be quenched by acidic solution. Dual-colour imaging confirmed that the pH-sensitive cargo was taken up in CCVs. This innovative approach is conceptually similar to biochemical experiments that used chemical accessibility to the budding clathrin-coated pit as a readout of scission [61], but allows subcellular spatial resolution together with a vast improvement in time resolution.

A question remains of whether or not large clathrin patches on the plasma membrane are functional and represent sites for preferential budding. A number of studies have found that new CCVs bud from large clathrin patches found on the plasma membrane. These studies are in keeping with deep-etch EM studies by Heuser showing large, flat clathrin lattices with CCVs budding laterally [62]. Another school of thought, however, holds that such large, flat lattices are non-functional and are actually an artefact of cell-coverslip contact. Ehrlich *et al.* found that the larger clathrin patches did not bud any new vesicles and that there were no preferred sites of initiation [54]. This is in contrast to the TIRFM/pHluorin study which reported that half of the CCVs budded from large, stationary clathrin patches and half were formed *de novo* [60]. Clearly, more work is needed to resolve this point; the work thus far has been at the limit of spatial resolution of the light microscope, and perhaps new methods are required to discern whether or not the flat clathrin patches can transform into coats.

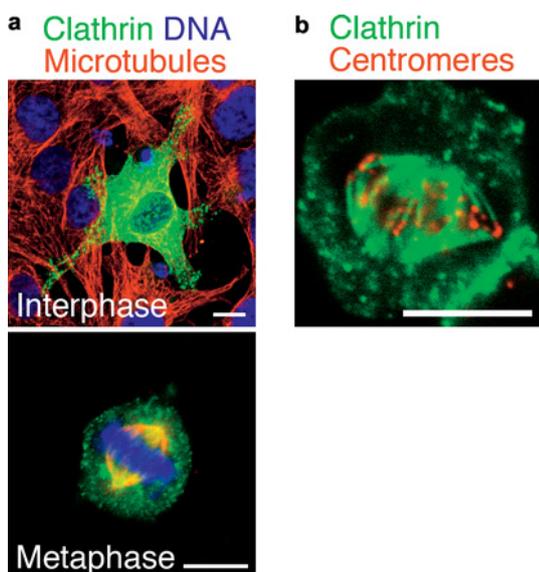


Figure 3. Clathrin localises to kinetochore fibres of the mitotic spindle during mitosis. (a) Distribution of GFP-LCa and α -tubulin in normal rat kidney (NRK) cells at interphase and metaphase. (b) GFP-LCa on the stable kinetochore fibres that remain after chilling the cell. Taken from [77], copyright © 2004 Nature Publishing Group. Scale bar, 10 μ m.

Clathrin knockouts

When the interactions between the various proteins involved in a biological process are mapped out, a protein ‘network’ can be established. Each protein in this network is known as a ‘node’, and those proteins with a significantly larger number of interactions are known as ‘hubs’. When looking at proteins involved in endocytosis (Fig. 4), one can see that there are several hubs, including clathrin. This is because clathrin interacts with numerous accessory proteins and adaptors to participate in membrane trafficking [11, 12, 15]. To impose some kind of directionality and order to endocytosis, there is obviously some temporal and spatial organisation to this confusing web of protein interactions. However, because of the key place that a hub has within a network, if a hub were to be deleted, a dramatic impact on endocytosis could be predicted. Clathrin knockouts have now been generated in a number of organisms. These studies largely underline the importance of clathrin in endocytosis and show that clathrin is essential in higher organisms.

When the CHC homologue gene from yeast (*Saccharomyces cerevisiae*) was disrupted, the cells survived albeit with slowed growth and limited endocytosis [63, 64]. However, in yeast it appears that endocytosis is mainly actin-dependent [65] and clathrin has a modulatory role, whereas the converse is true in mammalian cells [66]. Deletion of clathrin in *Dictyostelium discoideum* by antisense RNA or gene replacement resulted in slow growth and decreased endocytosis [67, 68]. It was also found that clathrin-null *D. discoideum* cells had defects in cytokinesis [69] but only when the cells are in suspension

[70]. In *Caenorhabditis elegans*, depletion of clathrin by RNA interference (RNAi) resulted in decreased yolk uptake by endocytosis in oocytes and dead progeny [71]. In *Trypanosoma brucei* [72], depletion of clathrin by RNAi dramatically reduced endocytosis and resulted in a ‘big-eye’ phenotype due to the coalescence of many intracellular vesicles. In *Drosophila melanogaster*, deletion of its CHC homologue by ethylmethane sulphonate mutagenesis (EMS) resulted in embryonic lethality [73]. These results, together with the observation that deletion of two of the other hubs in the network (AP1 and AP2 [74, 75]) are embryonic lethal in the mouse, suggest that deletion of CHC in mammals would also be incompatible with life.

At the single-cell level, chicken DT40 lymphocytes have been used as a system to conditionally knock out CHC by homologous recombination [76]. This study found decreased endocytosis but normal lysosomes in this cell type. More recently, RNAi has also been used in various mammalian cell types [77–79] to deplete levels of CHC. Such depletions have resulted in both decreased endocytosis and multiple defects in mitosis.

A new function for clathrin

Membrane trafficking only occurs during interphase. As the cell enters mitosis, clathrin-mediated membrane traffic is rapidly shut down and only resumes in late telophase [80]. This observation raises the possibility that clathrin may have a separate function that is distinct from membrane trafficking, which operates during mitosis.

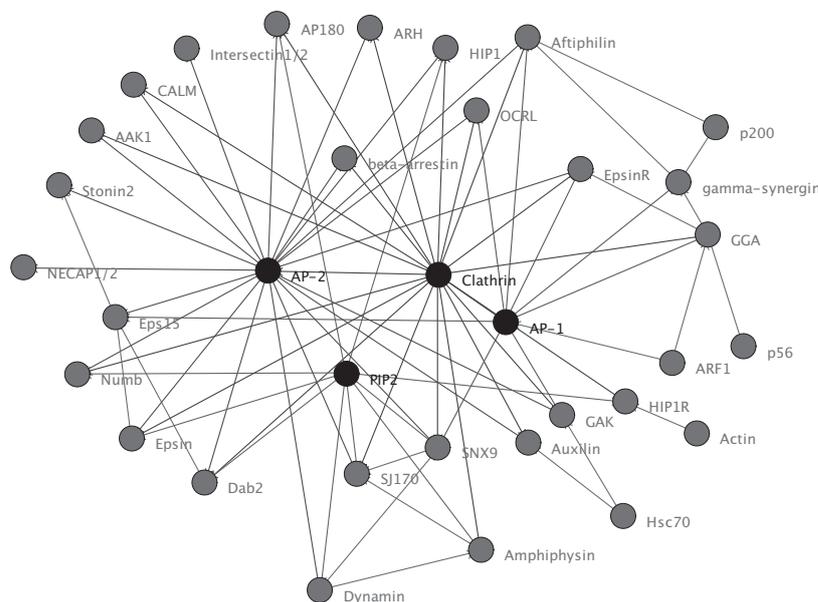


Figure 4. Clathrin is a hub in a network of proteins involved in membrane trafficking. Interactions of proteins involved in endocytosis and membrane trafficking drawn using Biolayout Java v.1.21. The intention here is not to provide an exhaustive illustration of all interactions but to illustrate the importance of clathrin in the network.

Early immunocytochemical studies showed that during mitosis, the subcellular distribution of clathrin is changed [56, 81]. During interphase, clathrin is found in numerous puncta at the plasma membrane, on endosomes and in an accumulation at the Golgi apparatus. These puncta correspond to clathrin-coated pits and vesicles. Maro and colleagues demonstrated that some clathrin is localised to the mitotic spindle in the developing mouse embryo [81]. Tissue culture cells in telophase also had an altered clathrin distribution [56]. These findings were interpreted at the time as an accumulation of coated vesicles in the mitotic spindle and were also largely ignored because of the possibility that the antibodies used in these studies had erroneously detected an unrelated mitotic protein. A later study by Okamoto and coworkers revisited this localisation of clathrin during mitosis using several different monoclonal antibodies and confirmed that clathrin was indeed found on the mitotic spindle [82]. Around the same time, two 'blind' approaches identified clathrin as a component of the mitotic spindle. First, a gene trap for nuclear proteins showed that clathrin was targeted to the spindle [83]. Second, clathrin was amongst a number of proteins that were identified by mass spectrometry from purified mitotic spindles [84], although with no known mitotic function assigned to clathrin, the authors suggested that clathrin could represent a contamination. Together these studies suggested that clathrin is targeted to the spindle apparatus, and they hinted that clathrin could even have a new function during mitosis.

The development of GFP-tagged clathrin light chains [50] and CHC [77] meant that it was possible to test whether clathrin itself was targeted to the mitotic spindle and not a related protein that had been detected by immunocytochemistry. In interphase, clathrin is distributed in numerous CCSs throughout the cytoplasm (Fig. 3a). During early prometaphase, clathrin is recruited to microtubules as they invade the nuclear lamina. Clathrin remains associated with spindle microtubules throughout congression, metaphase (Fig. 3a) and the segregation of chromosomes in anaphase. During telophase, clathrin becomes dissociated from the microtubules as the Golgi apparatus begins to reform.

Clathrin at the mitotic spindle does not represent a store of CCVs, as previously suggested. It is not associated with membranes, and moreover, immunoelectron microscopy showed that clathrin is intimately associated with spindle microtubules and away from membranes [77]. Clathrin is found on kinetochore fibres (Fig. 3b); these are the bundles of microtubules that connect the spindle pole to the kinetochore of the chromosome. During prometaphase, these fibres control movement (i.e. congression) of the chromosomes to the cell equator (metaphase plate) [85]. Clathrin associates with the spindle via the N-terminal domain of CHC [77]. When CHC is depleted from human or rat cell lines by RNAi, a number of mitotic de-

fects result. These include misaligned chromosomes and destabilisation of kinetochore fibres. Such defects result in prolongation of mitosis due to continued signalling of the mitotic spindle checkpoint. This led to the proposal that the function of clathrin in mitosis is to stabilise kinetochore fibres. The trimeric structure of clathrin could actually lend itself to stabilisation of spindle fibres: because the N-terminal domain (the foot of each leg) binds to the spindle, we suggest that clathrin may act as a bridge between two or three microtubules within a spindle fibre to increase its stability [77]. Alternatively, stability may be imparted by a clathrin lattice forming within the spindle. In EMs of spindle microtubules, however, evidence for such a lattice is lacking. Instead, intertubule bridges have been reported to span between microtubules within spindle fibres [86], supporting the bridge hypothesis.

It has been known since the 1960s that CME and other forms of membrane traffic are shut down during mitosis [80, 87], but the mechanism for this is unclear [88, 89]. From EM studies it appeared as though CME could not proceed due to a reduction in free clathrin triskelia [80]. In light of the compelling evidence for the recruitment of clathrin to microtubules during mitosis, we must now consider whether the simple mechanism of a change in the subcellular localisation of clathrin might be responsible not only for a role for clathrin in mitosis but also for the shutdown of clathrin-mediated membrane traffic during mitosis. In other words, is the shutdown of CME during mitosis an indirect effect of clathrin becoming absorbed in a new task?

So clathrin is a moonlighting protein: during interphase its function is in membrane trafficking and during mitosis it has a role in stabilising spindle fibres. Are there any other examples of moonlighting molecules? Proteins involved in nuclear transport during interphase, such as Nup107, Nup133 [90], Nup358 [91] and RanGAP [92], localise to kinetochores or to the mitotic spindle during mitosis, and this has been proposed to ensure orderly mitotic progression [91]. Dynamin-2, a large GTPase involved in membrane scission and actin reorganisation, is also found at the spindle apparatus during mitosis, but its distribution is different from that of clathrin. Dynamin-2 becomes concentrated at the midzone during telophase, where it may play a role in cytokinesis [93]. Dynamin-2 is also localised to the centrosomes and may contribute to centromere cohesion [94].

A number of questions arise from this work [77]. First, what molecule(s) recruit clathrin to the mitotic spindle? Clathrin may bind directly to the microtubules or it may bind via an intermediary protein. Second, how does clathrin switch between its function in interphase and that in mitosis? Clathrin may be modified in some way (by phosphorylation, for example) preventing it from working in membrane trafficking and allowing its mitotic function. Perhaps clathrin is modified by the numerous kinases that

are active periodically during the cell cycle. Third, what relevance does the function of clathrin in mitosis have on animal development or disease? The mis-segregation of chromosomes during mitosis is a potential source of aneuploidy, a form of genetic instability that is thought to lead to cancer or birth defects [95]. Because clathrin depletion resulted in misaligned chromosomes [77], the perturbation of clathrin function in disease is worthy of further investigation.

Gene fusions involving clathrin are found in a number of human cancers. For example, a fusion of clathrin heavy chain and anaplastic lymphoma kinase (CHC-ALK) has been reported in non-Hodgkin's lymphomas (anaplastic large-cell lymphomas) and in inflammatory myofibroblastic tumours [96–101], as well as the fusion of clathrin with the transcription factor gene *TFE3* in renal adenocarcinomas [96]. These fusion proteins may result in either the compromised function of clathrin in mitosis, the targeting of fusion partners to the spindle by clathrin or the misregulation of fusion partners by the addition of CHC to their N-termini.

Conclusions

We have come a long way in understanding the basic biology of clathrin in the time since this protein was first espied in the EMs of Roth and Porter. We now have a well-developed structural knowledge of clathrin and many of its interacting partners. A dizzying array of molecules that participate in endocytosis have been identified and their interactions mapped out. We can now visualise biological processes with much higher spatial and temporal resolution than previously; this will allow us to better understand the cellular functions of the myriad of endocytic proteins. Finally, the identification of a new function for clathrin provides an exciting new avenue for cell biological research involving clathrin.

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