

Short Conceptual Overview

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Novel clathrin activity: developments in health and disease

Abstract: Clathrin self-assembles into a coat around vesicles filled with cargo such as nutrients, hormones, and proteins destined for degradation. Recent developments indicate clathrin is not a specialist, but is involved in different processes relevant to health and disease. Clathrin is used to strengthen centrosomes and mitotic spindles essential for chromosome segregation in cell division. In Wnt signaling, clathrin is a component of signalosomes on the plasma membrane needed to produce functional Wnt receptors. In glucose metabolism, a muscle-specific isoform, CHC22 clathrin, is key to the formation of storage compartments for GLUT4 receptor, and CHC22 dysfunction has been tied to type 2 diabetes. The activity of clathrin to self-assemble and to work with huntingtin-interacting proteins to organize actin is exploited by *Listeria* and enteropathic *Escherichia coli* in their infection pathways. Finally, there is an important connection between clathrin and human malignancies. Clathrin is argued to help transactivate tumor suppressor p53 that controls specific genes in DNA repair and apoptosis. However, this is debatable because trimeric clathrin must be made monomeric. To get insight on how the clathrin structure could be converted, the crystal structure of the trimerization domain is used in the development of the detrimimerization switch hypothesis. This novel hypothesis will be relevant if connections continue to be found between CHC17 and p53 anti-cancer activity in the nucleus.

Keywords: clathrin in disease; novel clathrin function in the nucleus; trimerization domain.

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Introduction

Over 35 years ago, clathrin was found to self-assemble into a lattice coat over budding vesicles in receptor-mediated

endocytosis (1, 2). However, evidence has come to light that clathrin is a multi-talented protein involved in activities that go beyond conventional endocytosis. The purpose of this mini-review was to discuss some of these novel clathrin functions relevant for health and disease. It is now evident that clathrin is suited to do work in cell division by enhancing the integrity of centrosomes and mitotic spindles essential to carry out chromosome segregation. Clathrin also plays a key role in Wnt signaling that mediates a broad range of processes from embryonic development, organogenesis, and stem cell biology to homeostasis and metabolism. Recently, pathogenic bacteria were found to exploit clathrin and huntingtin-interacting proteins (HIPs) as vehicles for infection. Lastly, there is growing linkage between clathrin and human malignancies. Clathrin heavy chain fragments fused to segments of specific proteins are strong markers for specific human cancers. In the nucleus, clathrin has been reported to help activate tumor suppressor p53 that regulates genes involved in DNA damage repair or apoptosis. This particular activity is controversial because, to participate, trimeric clathrin must somehow be made monomeric. There is currently no defined pathway in cells to do this. To address this issue, my laboratory solved the crystal structure of the isolated clathrin trimerization domain without light chain (PDB code: 3QIL). The X-ray model is intriguing because it suggests each heavy chain leg has its own on-board detrimimerization switch. It will be important to test the clathrin detrimimerization hypothesis summarized here to expand our understanding of what clathrin is fully capable of beyond what we know.

Clathrin structure

The *CLTC* gene on human chromosome 17 (at 17q.23.2) encodes the clathrin heavy chain (CHC17). Identical heavy chains associate to form the characteristic three-legged pinwheel (triskelion) (2). The N-terminal domain (NTD) of CHC17 heavy chain is folded into a seven-bladed β propeller (3). So far, four distinct protein interaction sites have

been catalogued in NTD (4). Specifically, clathrin binding and accessory proteins such as AP1, AP2, AP3, and GGA adaptor proteins contain clathrin box motifs recognized by a specific sequence in the N-terminal β propeller domain of clathrin (4). A short flexible segment joins the propeller to the long filamentous segment of the heavy chain. There is a right-handed bend or knee in this leg between the distal and the proximal domains. The crystal structure of the isolated proximal domain (PDB 1b89) predicted that the whole filamentous leg was composed of seven clathrin heavy chain repeats (CHCR1-CHCR7) (5). Later, the number of CHCRs was revised to eight to include CHCR0, the segment that is part of the ankle that also includes CHCR1 and CHCR2 (2).

The pinwheel shape is key to the ability of trimeric clathrin to interlock into a polyhedral lattice. The triskelion facilitates efficient self-assembly because every radiating leg serves to seed lattice growth in a different direction. Low-resolution structures of assembled clathrin baskets (6, 7) show that a main feature of the trimerization domain is a helix tripod (one long helix contributed by each leg) protruding downward like a handle (7, 8). Atomic details of the trimerization domain without the influence of bound CLC shown in Figure 1 and discussed later on provide a unique opportunity to view clathrin structure and function in a new light.

There are two types of clathrin light chains (CLC) [LCa and LCb (9)] that have neuronal splicing variants that bind

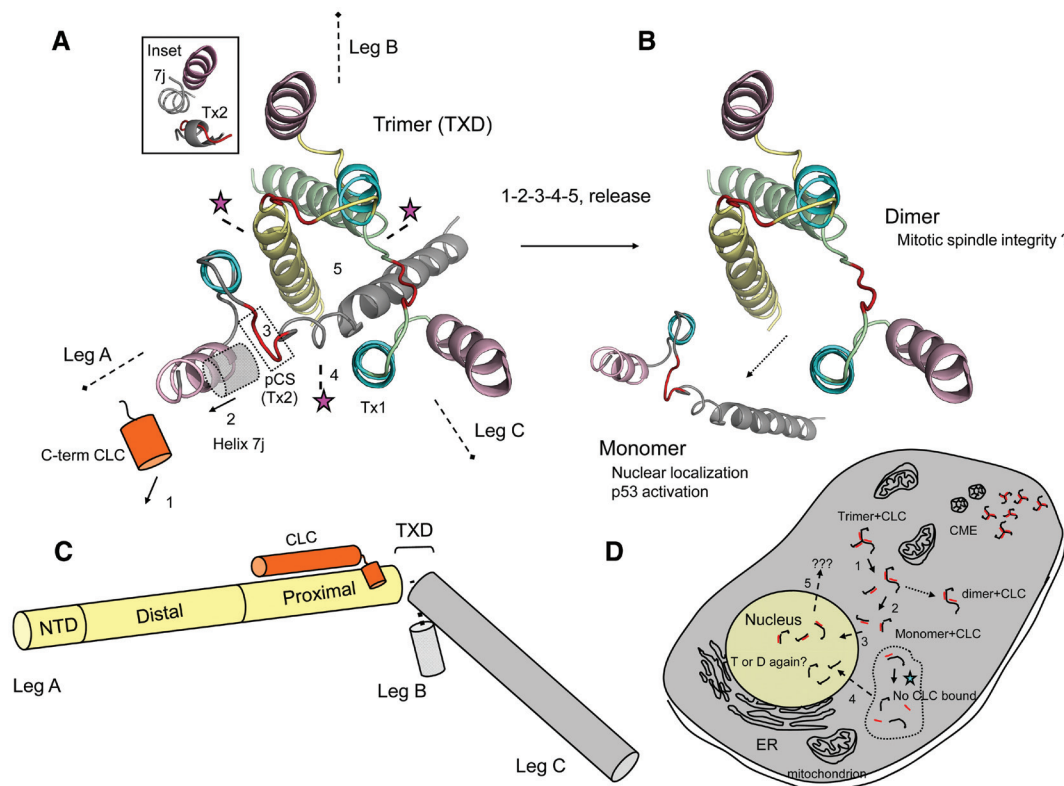


Figure 1 Proposed clathrin detrimerization switch hypothesis and nuclear localization.

(A) Top view shows the interaction interface (marked by dashed lines and magenta stars) between legs A (gray), B (yellow), and C (green). To throw one switch, the C-terminus of CLC is dislodged from its normal position (step 1), which enables helix 7j to move (step 2) away from pCS (red). The position of helix 7j in clathrin structures with bound CLC is indicated (light gray cylinder), and the dashed box is where Tx2 would be. Inset: Helix 7j (7j) is shifted and the short Tx2 helix is disrupted in TXD (PDB 3QIL and 1X14 comparison, only leg B shown). The resulting helix-to-coil transition (step 3) that pCS undergoes breaks the productive contact with Tx1 (teal, step 4). The last step (5) to release is to disrupt the tripod. This proposed mechanism indicates that the production of the monomer in panel (B) needs the switch in leg B to be activated simultaneously. (C) Diagram of the clathrin triskelion shows the N-terminal domain (NTD), distal and proximal domains, and the trimerization domain structure (TXD) that includes some helices from the proximal domain. Clathrin light chain is depicted in orange (on all three legs) showing how its C-terminus may interact with the vertex (see also panel A). (D) Schematic of a eukaryotic cell shows clathrin-mediated endocytosis (CME) at the plasma membrane and clathrin detrimerization to produce monomeric molecules destined for the nucleus. A pool of trimeric clathrin is detrimerized (1 and 2), and monomeric clathrin with CLC enters the nucleus (3). Monomeric clathrin may shed CLC (turquoise) and naked heavy chain trafficked inside (4). In this proposed model, it may be possible to return detrimerized clathrin to the cytoplasm (5). It is intriguing to speculate that trimeric and/or dimeric clathrin can be reformed in nuclear spaces.

to the proximal domain in vertebrates and invertebrates, but yeast clathrin has only one type of light chain (10). CLC is reported to modify clathrin knee geometry to control the formation of clathrin lattices (8). An important function of CLC is to interact with HIPs (HIP1 and HIP1R) in mammals or with the yeast homolog Sla2p to connect the clathrin lattice to the actin cytoskeleton (11, 12). The atomic model of HIP1 (PDB codes: 2NO2 and 2QA7) revealed that key CLC binding determinants are imbedded in a particularly flexible segment of the HIP1 coiled coil (12, 13). The clathrin/HIP interaction is essential for endocytosis to occur properly in membranes under physical tension (14). The CLC/Sla2p association permits endocytosis in yeast to proceed in the face of plasma membrane turgor pressure. Additionally, the CLC/Sla2p complex is an important component of the contractile vacuole in *Dictyostelium* (15–17).

Clathrin, HIP, actin, and infection

For cells to stick to each other, the actin cytoskeleton must organize at the adherens junction (AJ). A critical step in this process is to concentrate CHC17 to AJs so that HIPs can be recruited to modulate the spatial organization of actin (18). *Listeria monocytogenes* infection hijacks the normal work of clathrin and HIPs in cell-cell adhesion. To bring CHC17 to AJs to bind cadherin, clathrin must be phosphorylated (19). CHC17 phosphorylation is induced by the binding of internalin A protein of *Listeria* to cadherin. These steps organize actin cytoskeleton around CHC17 to internalize cell-bound *Listeria* (18, 19). It is important to point out that this internalization step is uniquely different from conventional clathrin vesicle formation. Enteropathic *Escherichia coli* (EPEC) has also developed an infection pathway that takes advantage of CHC17/HIP function. The phosphorylation of CHC17 at the EPEC/host interface induces clathrin-associated actin to form a pedestal structure into the host to import pathogenic molecules from attached EPEC (19).

Centrosome and mitotic spindle integrity

To divide without mishap, cells rely on the ability of clathrin to bolster the integrity of centrosomes and mitotic spindles. At the onset of mitosis, membrane trafficking is inhibited (20, 21) and a proportion of CHC17 clathrin is recruited to the kinetochore fibers (K-fibers) of mitotic

spindles to enhance integrity (22–24). In the M phase, microtubule-stabilizing protein TACC3 is phosphorylated by Aurora kinase A (25, 26). Activated TACC3 associates with the ankle of clathrin and combines with chTOG to form the CHC17-TACC3-chTOG complex (27). Clathrin must play an important role in the CHC17-TACC3-chTOG complex because K-fibers develop defects when CLC is cross-linked in S phase to inactivate CHC17 (26). Furthermore, clathrin must be trimeric for the CHC17-TACC3-chTOG complex to be active (28). The CHC17-TACC3-chTOG complex also plays a role in maintaining the stability of the centrosome in mammalian cells (26, 28). The removal of CHC17 from the CHC17-TACC3-chTOG complex is devastating, ending in centrosome fragmentation (26).

The importance of clathrin to centrosomes and mitotic spindles suggests CHC17 dysfunction could be a route to disease. For example, the depletion of CHC17 clathrin causes spindle morphology defects (28) and leads to chromosome dysfunction (24).

Cell signaling

CHC17 clathrin has a role in the wingless-type MMTV integration site family, member 1 (Wnt) signaling pathway (29). A crucial step in Wnt signaling is the interaction of low-density lipoprotein-related protein 5 and 6 (LRP5/6) with the negative regulator axin (30–32). The binding of LRP5/6 to axin is triggered by specific phosphorylation of LRP6 that is dependent on the formation of LRP6 aggregates or signalosomes on the cell surface. Clathrin was found to play a structural role in stabilizing the LRP6 signalosome (29). To concentrate CHC17 at Wnt signaling sites, clathrin interacts with AP2 adaptors that are bound to PtdIns(4,5)P₂ produced in the early stages of the Wnt signaling cascade. Based on size (100–200 nm) and morphology, the majority of clathrin patches are identified to be clathrin-coated pits (29). However, larger patches (>200 nm) have been visualized that are thought to be clathrin-coated plaques previously described (33). It will be important to delineate the details of how clathrin-coated pits and clathrin-coated plaques contribute to signalosome genesis.

Any dysfunction in the Wnt signaling pathway that involves clathrin has implications for disease. Improper Wnt signaling has the potential to adversely impact the signaling molecule β -catenin that functions in cell-cell adhesion and takes part in the transcriptional activation of specific target genes. Indeed, deregulation of β -catenin signaling is correlated with a host of human cancers, such as colon cancer, ovarian cancer, prostate cancer,

melanoma, hepatocellular carcinoma, endometrial cancer, and medulloblastoma pilomatricomas (34).

Glucose metabolism

Clathrin isoform CHC22 is involved in glucose metabolism. CHC22 arose from gene duplication and is preferentially expressed in skeletal muscle [for review, see Ref. (2)]. The *CLTCL1* gene encoding the CHC22 heavy chain is located on human chromosome 22 (at 22q11.21), but is only a pseudogene in mouse (35). CHC22 is trimeric, but does not bind any light chain (36). CHC22 is involved in establishing the intracellular storage compartment for the insulin-responsive GLUT4 glucose transporter in adipose and muscle tissues (37). The GLUT4 storage compartment is a complex system of tubule structures and vesicles found only in muscle, fat, and neurons of the cerebellum (38, 39). In response to an insulin signal, GLUT4 glucose transporter-containing vesicles become fused to the plasma membrane to modulate the distribution of glucose from blood to waiting tissues. The role of CHC22 is demonstrated by the fact that GLUT4 compartments cannot form when CHC22 is depleted (37). Although not yet fully understood, CHC22 has been observed to concentrate on expanded GLUT4 storage compartments in muscle cells of people with type 2 diabetes (37).

Human malignancies

The growing evidence that CHC17 clathrin is involved in a variety of human cancers strengthens the connection between clathrin and disease.

Oncogenic fusions

CHC17 clathrin has been reported to form two types of gene fusions in human cancers. First, chromosomal translocation t(2;17)(p23;q23) fuses CHC17 with an intact trimerization domain (aa1–1634) to the C-terminus (aa1058–1620) of anaplastic lymphoma kinase (ALK) (40). The CHC-ALK fusion is often detected in non-Hodgkin's lymphomas and has been reported in anaplastic nul/T-cell lymphoma (41, 42), large B-cell lymphoma (43–50), and the rare inflammatory myofibroblastic tumor (51, 52). To initiate ALK-mediated lymphomagenesis, Stat3 must be activated (53). It is not yet clear whether CHC-ALK is involved in activating Stat3 (54, 55). So far, the possibility that CHC-ALK

transforms cells by disrupting clathrin-mediated endocytosis or negatively impacts mitotic spindle stability is not supported by experimental evidence (40).

In the different kinds of fusion, chromosomal translocation t(X;17)(p11.2;q23) joins the C-terminal segment of transcription factor binding to IGHM enhancer 3 (TFE3, aa295–575) to CHC17 fragment aa1–932 (40). Since the clathrin trimerization domain is absent, it is not surprising the CHC-TFE3 cannot be trimeric. Instead, an intact dimerization motif in TFE3 can drive CHC-TFE3 to be dimeric (40). This may be relevant for better understanding CHC-TFE3 because dimeric CHC-TFE3 is also functional, capable of enhancing the stability of mitotic spindles by forming a bridge across K-fibers (40).

Clathrin and p53 activation

The decision to arrest growth or initiate apoptosis in response to DNA damage is the responsibility of p53. There are reports that clathrin is involved in activating p53 by entering the nucleus to present histone acetyltransferase p300 to p53 (56, 57). However, this novel cancer connection is debatable because luciferase-based detection assays show that the functional form of CHC17 is the monomer that must somehow localize to the nucleus. A proteasome-mediated pathway that involves Mdm2 (E3 ubiquitin ligase specific for p53) controls the level of p53 in healthy cells. When Mdm2 is bound, p53 is degraded to reduce the amount present (58, 59). However, if cells experience genotoxic stresses, the levels of p53 are increased by specific post-translational modifications that ultimately dissociate the Mdm2/p53 complex (60–62). These modifications spawn a cascade of activities that result in the transcription of specific genes involved in DNA repair (*p53R2* and *GADD45*), growth arrest (*p21^{waf1}*), and apoptosis (*Bax*, *Noxa*, *Puma*, and *p53AIP1*) (60, 63–66). In the case of apoptosis, a p53 mutation (S46F) was reported in a bladder carcinoma that strongly enhanced the transactivation of the *p53AIP1* promoter (67). The activities of p53-responsive promoters *p53AIP1*, *P21^{waf1}*, *p53R2*, and *Noxa* are increased when CHC17 is exogenously expressed and are reduced by RNAi silencing (56).

Clathrin detrimers and nuclear localization

A number of years ago, CHC17 was reported to take part in the activation of p53 to regulate specific genes involved in DNA repair and apoptosis (56). About 5% of CHC17

was found by nuclear fractionation and immunoelectron microscopy to be in nuclear spaces (56). Luciferase-based reporter assays indicate that CHC17 nuclear localization is increased by artificially deleting the trimerization domain (57). However, the proposal that clathrin must be monomeric to help activate p53 has been controversial because there is no defined mechanism to alter clathrin structure. To get insight into what structural elements held the triskelion together, my laboratory solved the crystal structure of the trimerization domain with a segment of proximal domain that could not bind CLC (CTXD) (see Figure 1). The structure showed that the molecules in the asymmetric unit were trimeric, but threefold symmetry was disturbed (68). This observation supports previous findings that CLC contributes to the net stability of trimeric clathrin (69–71).

The global impact of CLC on trimeric structure is seen when CTXD is compared to assembled clathrin (7) (PDB code: 1XI4) and unassembled clathrin hub (8) (PDB code: 3LVG) that both have bound CLC. Superpositions show that helix 7j in CTXD is not in the same spot, but is shifted by about 10 Å away from the vertex of the trimerization domain (68). This is informative because helix 7j contains a conserved cysteine (aa1573) that we previously found to contribute to the stability of the clathrin trimer (69, 72). This same cysteine was also demonstrated by multi-angle light scattering to cause bovine clathrin hub (aa1074–16750) to detrimmerize and to influence the cellular distribution of mutant clathrin constructs (68). Confocal microscopy shows that a proportion of the C1573A clathrin hub expressed in HEK293T, HeLa, H1299 (lung cancer), and MCF7 (breast cancer) localizes in nuclear spaces (68). A limitation of this work is the fact that the C1573A hub cannot interact with membranes because the N-terminal portion is absent. Therefore it is possible that C1573A hub gets inside the nucleus because there is no interaction with membranes to impede entry. To address this issue, it will be important to determine the subcellular localization of full-length C1573A clathrin.

The short Tx2 helix in assembled clathrin and clathrin hub models is not folded in CTXD (see inset, Figure 1A). This is significant for structure because Tx2 contributes to the interface between neighboring legs. The lost helical structure is also intriguing when you consider that Tx2 is predicted to transition between a helix and a coil as a conformational switch (labeled pCS in Figure 1A) (68). Figure 1A shows the structural layout of the detrimmerization switch that includes pCS, Tx1, helix 7j, the tripod core in heavy chain and a C-terminal portion of the light chain subunit. The red/turquoise daisy-wheeled interactions (Tx1/Tx2 contact) stitch adjacent legs together (see magenta star, Figure 1A) to support the tripod core.

The detrimmerization switch hypothesis proposes that Tx2 is helical only when helix 7j is close by. When Tx2 is unfolded, the red/turquoise interface is disturbed and the trimer becomes distorted (this is evident in the top view of CTXD in Figure 1A). As the crystal structure shows, the trimer is deformed but not destroyed because the contacts in the tripod are unaffected. Therefore, both red/turquoise and tripod interactions must be simultaneously broken to completely free a leg. The fact that helix 7j is shifted when CLC is absent raises the possibility that light chain determines the orientation of helix 7j. The space between helix 7j and 7h that is filled by light chain density in assembled clathrin and clathrin hub is not occupied in the CTXD model (68). In the proposed switch model, CLC plays a major role because helix 7j cannot move away from pCS unless the tail of the light chain is first repositioned. Additional work will be needed to sort out whether the position of helix 7j depends at all on the C-terminus of CLC. The fact that CLC knockouts in *Saccharomyces cerevisiae* destroy the trimeric clathrin structure (70, 71), while the clathrin structure in *Dicytostelium* is unaffected by knocking down CLC (73), suggests that any CLC role will likely be organism specific.

Outlook

Evidence now points to the fact that clathrin, long recognized to specialize in endocytosis, can wear a variety of different hats. In cell division, trimeric CHC17 clathrin forms a structural scaffold to stabilize centrosomes and mitotic spindles to ensure the faithful segregation of sister chromatids. Novel CHC17 activity extends to the Wnt signaling pathway, where clathrin is needed to properly form signalosomes. This has implications for disease because dysfunction in associated β -catenin signaling is implicated in a whole host of human malignancies. In blood glucose homeostasis, clathrin isoform CHC22 that does not participate in conventional clathrin-mediated endocytosis is involved in the formation of storage compartments for GLUT4 receptors. These findings extend our understanding of the role of clathrin that can potentially open the way to new approaches to control type 2 diabetes. The natural activity of clathrin to self-assemble and to work with HIPs to organize the actin cytoskeleton is co-opted by *Listeria* and EPEC in their infection pathways. This discovery is timely because the information could be used to address the ever-increasing problem of bacterial drug resistance to traditional antibiotics. We will have to wait to see whether mechanistic insights from *Listeria* and EPEC can be used

to develop anti-infection strategies that target clathrin, HIPs or both. Finally, the detramerization switch hypothesis in Figure 1 offers a way to begin to think about how trimeric clathrin may be detramerized for nuclear entry. This structure-based concept will become highly significant if future studies continue to support the link between CHC17 clathrin and p53-mediated gene regulation in cancer.

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