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Current Opinion in Cell Biology

Clathrin's life beyond 40: Connecting biochemistry with physiology and disease

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Abstract

Understanding of the range and mechanisms of clathrin functions has developed exponentially since clathrin's discovery in 1975. Here, newly established molecular mechanisms that regulate clathrin activity and connect clathrin pathways to differentiation, disease and physiological processes such as glucose metabolism are reviewed. Diversity and commonalities of clathrin pathways across the tree of life reveal species-specific differences enabling functional plasticity in both membrane traffic and cytokinesis. New structural information on clathrin coat formation and cargo interactions emphasises the interplay between clathrin, adaptor proteins, lipids and cargo, and how this interplay regulates quality control of clathrin's function and is compromised in infection and neurological disease. Roles for balancing clathrin-mediated cargo transport are defined in stem cell development and additional disease states.

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Current Opinion in Cell Biology 2020, **65**:141–149

Edited by **Frances M. Brodsky** and **Jennifer L. Stow**

This review comes from a themed issue on **Membrane Trafficking**

For a complete overview see the Issue and the Editorial

Available online 21 August 2020

https://doi.org/10.1016/j.ceb.2020.06.004

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Keywords

Clathrin, Clathrin-mediated endocytosis, Membrane traffic, Clathrin-actin interaction, Clathrin-microtubule interaction.

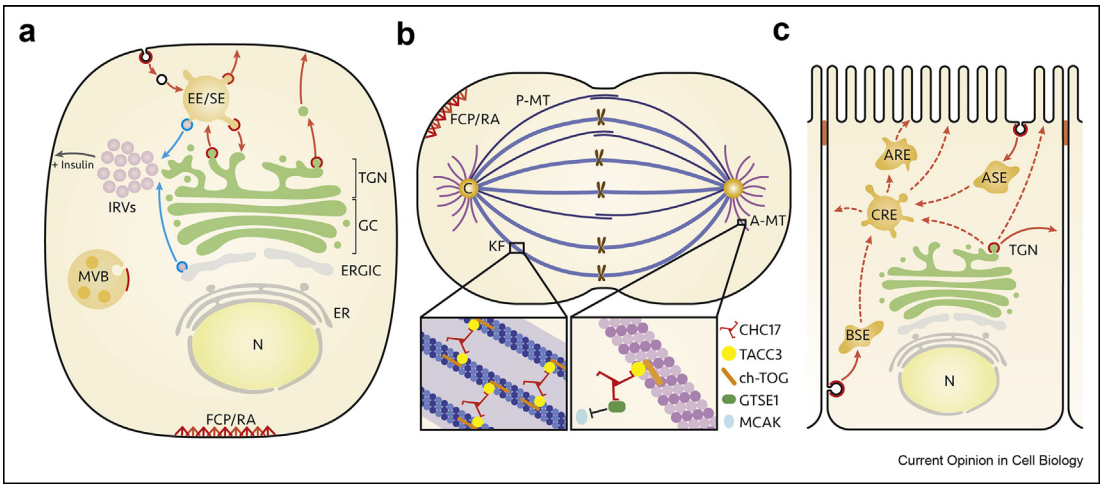
Introduction

The fortieth anniversary of Barbara Pearse's 1975 identification [1] and naming of clathrin for its clathrate or lattice-like morphology generated a number of thoughtful reviews covering milestones in the field [2–4]. These milestones created a framework for subsequent studies that have enabled mapping of diverse clathrin-mediated pathways in different cell types (Figure 1) and organisms. The fundamental process by which clathrin-coated vesicles (CCVs) capture cargo for intracellular transport through self-assembly at the membrane has been embellished with many regulators (Figure 2) and better understanding of clathrin interactions with the actin cytoskeleton. In some organisms, clathrin activity includes non–membrane traffic functions for clathrin-microtubule interactions during cytokinesis (Figure 1). These advances in molecular understanding are starting to reveal how diversification of clathrin's intracellular roles influences tissue development, tissue structure, metabolic pathways, and disease in both animals and plants. These effects of clathrin-mediated pathways beyond the interior of the cell result from genetic diversity of clathrin itself, differentially regulated interactions between clathrin pathways and cytoskeletal components, and tuning the balance of clathrin-mediated pathways for cargo transport to push cells into different fates.

Phylogenetic and population diversity: tissue- and species-specific functions

The assembled clathrin lattice comprises a triskelion at every vertex (Figure 3). The triskelion is formed by three clathrin heavy chain (CHC) subunits that trimerise at their C-termini and extend into 'legs' that bend at the 'knee', each with an N-terminal domain (TD) that interacts with cargo-recognising adaptor protein complexes. In humans and most other vertebrates, there are two genes encoding CHCs resulting in two types of clathrin (named for the encoding human chromosomes) CHC17 and CHC22. The gene encoding CHC22 has been completely lost in five vertebrate lineages leading to the absence of CHC22 from mice, rats, sheep, cows, pigs and a few other species [5]. CHC17 and its homologues have associated clathrin light chain (CLC) subunits that stabilise the trimerisation domain and extend to the knee where they can influence lattice formation [6]. In vertebrates, there are two CLC-encoding genes producing CLCa and CLCb subunits, which in cells bind only to CHC17 not CHC22. Yeast, flies and worms each have one type of clathrin, comprising products of single CHC- and CLC-encoding genes. In *Arabidopsis thaliana* there are two CHC-encoding genes and three CLC-encoding genes, suggesting there are two plant clathrins that associate

Figure 1

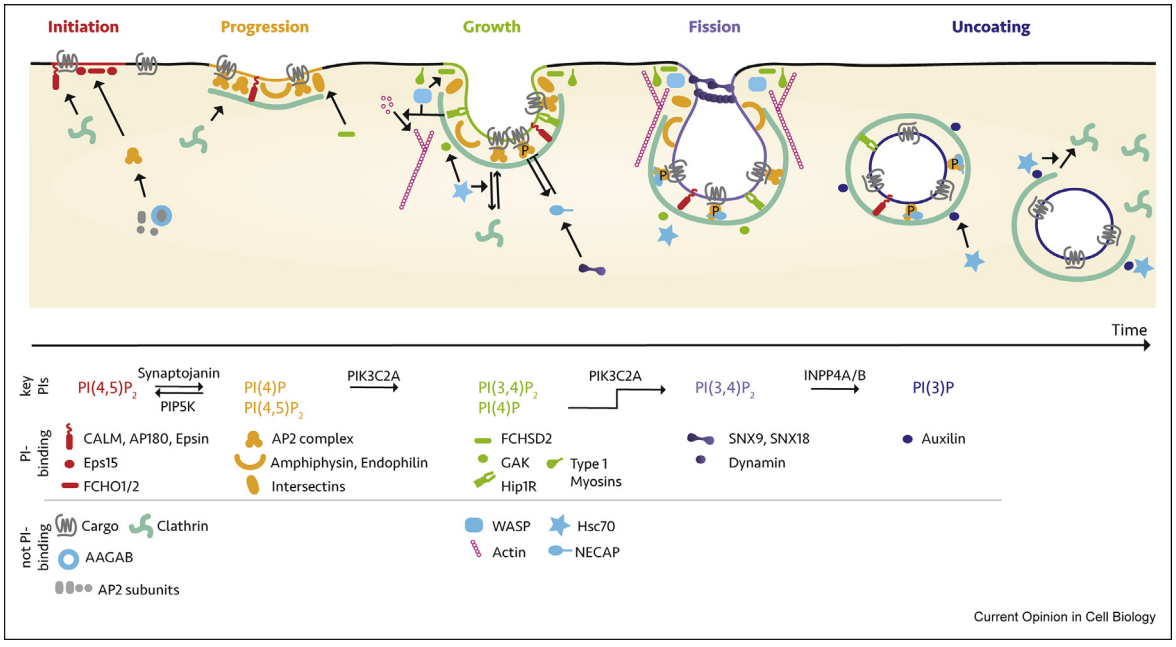


Pathways and functions mediated by CHC17 (red) and CHC22 (cyan) clathrins during interphase (a), mitosis (b) and within polarised epithelial cells (c). (a) Clathrin-mediated endocytosis (CME) by CHC17 clathrin at the plasma membrane (PM) transports cargo to the endolysosomal system (early and sorting endosomes, EE/SE). CHC17 clathrin also mediates intracellular sorting and transport in the endosomal system and trans-Golgi network (TGN), including maturation of secretory vesicles, and contributes to ESCRT sorting of cargo for inclusion in multivesicular bodies (MVBs). Flat CHC17 clathrin plaques/reticular adhesions (FCP/RAs) form at sites of αVβ5 integrin–extracellular matrix (ECM) adhesion [28,30]. CHC22 clathrin (in human skeletal muscle and adipose tissue) mediates traffic of recycled GLUT4 from endosomes and newly synthesised GLUT4 from the ERGIC to the intracellular pathways that package GLUT4 into insulin-responsive vesicles (IRVs, lavender) [12]. GLUT4 is re-internalised by CME (CHC17) following fusion of IRVs. **(b)** The CHC17/TACC3/ch-TOG complex binds to microtubules (MTs) during mitosis to stabilise both kinetochore fibres (k-fibres, KF, blue) and astral MTs (A-MTs, purple). This complex cross-links MTs within k-fibres, and at A-MTs recruits GTSE1, which in turn inhibits the MT depolymerase MCAK [23,24]. αVβ5 integrin–dependent FCP/RA complexes persist during mitosis and interact with retraction fibres to provide spatial memory to daughter cells [31]. Mitotic spindle components, polar MTs (P-MT, dark blue) and centrosomes (C) are shown. **(c)** CHC17 clathrin, in concert with AP1, is critical for polarised sorting of a range of newly synthesised and recycling apical and basolateral proteins in the epithelial endomembrane system [55–57]. Solid red arrows represent established CHC17 clathrin-mediated pathways, and dotted arrows represent pathways where CHC17 clathrin may also be acting. The endosomal system of polarised epithelia comprises the common recycling endosome (CRE), apical recycling endosome (ARE), apical sorting endosome (ASE) and basolateral sorting endosome (BSE). N, nucleus; ER,endoplasmic reticulum; ERGIC, ER to Golgi intermediate compartment; GC, Golgi complex; TACC3, transforming acidic coiled-coil protein 3; ch-TOG, colonic hepatic tumour overexpressed gene; GTSE1, G2 and S phase-expressed protein 1; MCAK, mitotic centromere-associated kinesin.

with three types of CLC [7]. Clathrin adaptors show considerably more phylogenetic variability than clathrin itself, with inferred origins before eukaryotic diversification but convergent loss of select complexes in several eukaryotic lineages (reviewed in[8]).

Clathrin-mediated endocytosis (CME), endosomal sorting to lysosomes or for recycling, and sorting at the trans-Golgi network to generate regulated secretory vesicles are the classical functions associated with CHC17 clathrin (Figure 1) [6]. CHC17 associates with tetrametric adaptor complexes AP2 at the plasma membrane (PM) and AP1 for intracellular sorting, as well as a number of monomeric adaptors, to regulate cargo recruitment at specific intracellular locations (see [8] for adaptor diversity). CHC22 clathrin expression is highest in skeletal muscle and testis, mostly low in other cell types, and shows transient upregulation during brain development [9,10]. In muscle and adipose tissue, CHC22 mediates membrane traffic that sequesters the GLUT4 glucose transporter intracellularly where it is packaged into insulin-responsive vesicles. These vesicles fuse with the PM upon insulin signalling, delivering GLUT4 for post-prandial glucose clearance from blood into these tissues (Figure 1) (see [11], this issue). Following GLUT4's consequent insertion in the PM, it is efficiently internalised by CHC17 for endosomal sorting that returns it to an intracellular GLUT4 pool for regeneration of insulin-responsive vesicles. This endosomal sorting is mediated by CHC17 in mice and CHC22 in humans. In humans, CHC22 also diverts newly synthesised GLUT4 for intracellular sequestration directly from the ERGIC in a pathway that operates at a low level in species without the CHC22 coat [12]. Thus, the presence of CHC22 enhances the ability of human cells to sequester GLUT4 intracellularly, and CHC22 mediates a trafficking step in the early secretory pathway that is distinct from classical CHC17-mediated traffic and endosomal sorting of GLUT4 [12]. The gene encoding CHC22 in humans (*CLTCL1*) has two major variants, differing by a methionine to valine substitution at residue 1316, both present at high frequencies across multiple ethnic groups. The CHC22-1316V variant arose in ancient humans and increased in population frequency after the advent of cooking and farming, suggesting dietary influence on its selection. Consistent

Figure 2

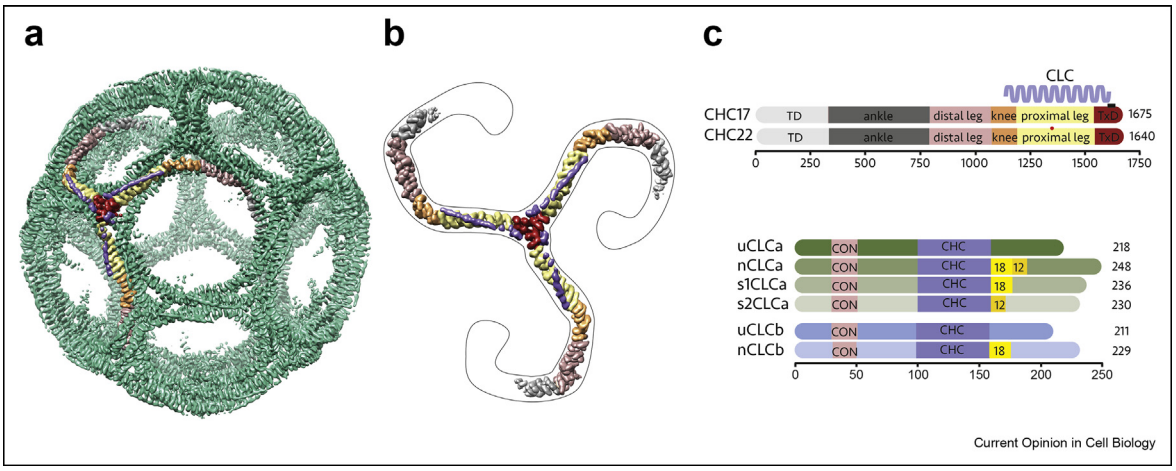


Protein recruitment and phospholipid conversion during clathrin-coated pit (CCP) maturation at the vertebrate plasma membrane. Key phosphoinositides (PIs), respective PI-binding and non–PI binding protein components (legend at the bottom) are mapped to five distinct stages of endocytic CCV formation. Initiation (red): PI(4,5)P_2, FCHO1/2, Eps15 and clathrin adaptors (chaperoned by AAGAB [40]) begin to recruit clathrin (CHC17 in humans), bind cargo and initiate CCP formation. Progression (orange): Increased clathrin and adaptor co-assembly promotes curvature, stabilised by N-BAR proteins amphiphysin and endophilin. Intersectins are recruited and bind the flat BAR protein FCHSD2 [72]. Growth (green) involves lattice rearrangement, potentially facilitated by GAK/Hsc70 recruitment [13,35]. NECAP is recruited via AP2 phosphorylation (indicated by 'P') [41–43]. Assembly of an actin-binding protein network including type 1 myosin initiates from FCHSD2, WASP, Hip family proteins and other components [17,72]. Fission (purple): Actin assembly facilitates progression of CCV formation through the late stages, where dynamin, SNX9 and SNX18 promote CV fission. Uncoating (dark blue): The GAK-auxilin/Hsc70 complex mediates uncoating [36]. Key PIs and PI-binding proteins are colour-coded based on CCP stages. Lipid kinases and phosphatases involved in interconversion of PIs are highlighted in the legend (bottom), and these conversions contribute to progression of protein recruitment and uncoating [46]. N-BAR, N-terminal amphipathic helix BAR; BAR, Bin/Amphiphysin/Rvs domain; WASP, Wiskott–Aldrich syndrome protein; HipR, huntingtin-interacting-protein-related protein.

with this hypothesis, several properties of CHC22-1316V suggest it would be more permissive for GLUT4 mobilisation and carbohydrate clearance [5]. A role for CHC22 trafficking in endosomal sorting was also observed in a neuronal cell line during investigation of a rare recessive genetic disorder of nonfunctional CHC22 that causes a defect in pain sensing [10]. Thus the different CHC22-mediated pathways may be more or less accentuated in different tissue types, perhaps dependent on the presence of different cargo.

Differential functions of the two vertebrate CLCs, which share ~60% sequence identity, have emerged in cargo selection [13], adhesion [14], vesicle formation [15] and in cytoskeletal interactions [16]. CLCa is preferentially recruited to focal adhesions [14], and CLCa, but not CLCb, binds an epithelia-specific splice variant of myosin VI, a type 1 myosin [16]. Disruption of myosin VI–CLCa interaction affects clathrin-coated pit formation at the apical membrane of polarised intestinal epithelial cysts. Experiments in both yeast and mammalian cells revealed that the actin-interacting proteins Las17/Wiskott–Aldrich syndrome protein (WASP) are spatially controlled during initiation of CME, forming a ring-like anchoring structure for actin assembly and recruitment of type 1 myosins, further supporting a role for these myosins in CME [17,18]. A comparison of CME between budding yeast (*Saccharomyces cerevisiae*) and fission yeast (*Schizosaccharomyces pombe*), as evolutionarily distant from each other as from humans, showed conservation of molecular players and a role for actin in CME in the two yeasts but differences in the timing and involvement of actin activity during endocytic budding [19]. Given that the requirement for actin has been linked to membrane tension in both yeast and animal cells [20,21], it was unexpected that pioneering electron microscopy (EM) of CME in *A. thaliana* revealed an absence of actin at clathrin-coated structures at the PM of root protoplasts, which also lacked large patches of assembled clathrin (plaques). Actin was found dispensable for CME in these cells, in spite of their cell wall-like cuticle [7], but needed for endosomal membrane traffic. However, analysis of actin-mediated autophagosome formation in *A. thaliana*, which

Figure 3



Structure of the clathrin basket highlighting clathrin subunits and their functional domains. (a) Cryo-EM model of the CHC17 clathrin minicoat (green) and one individual triskelion within the assembly showing the clathrin light chain (CLC, purple) and CHC17 domains colour-coded as in (b) and (c) (generated from EMD: 0114, [27]). **(b)** Structure of a single triskelion extracted from (a)(generated from EMD: 0114, [27]), formed from three CLCs (purple) bound to three trimerised CHC17 subunits (with colour-coding of domains from ankle to TxD as delineated in (c). Structural information for the CHC terminal domains (within the extended black outline) and the largely unstructured CLC N-termini are missing from this model, although TD structure has been solved at crystallographic resolution [73]. **(c)** Domain boundaries and alignments of CHC17 and CHC22 (top) and CLC isoforms (bottom). CHC domains: Terminal domain (TD, residues 1–330, light grey), ankle domain (residues 331–838, grey), distal leg (residues, 839–1073, rose), knee (residues 1074–1197, orange), proximal leg (residues 1198–1575, yellow) and trimerisation domain (residues 1576–1675 for CHC17, residues 1576–1640 for CHC22, TxD, red). For CHC17, the location of the alternatively spliced muscle insert in TxD (black bar) and the CLC-binding site (purple helix) are shown. For CHC22, the location of the M1316V polymorphism is highlighted by a red dot. CLC domains: CLCa (green) and CLCb variants (blue) share a 22-residue consensus sequence near the N-terminus (CON, rose), and sequence similarity in other domains is highest in the clathrin heavy chain binding domain (CHC, purple). One or two exons can be included by alternative splicing and produce inserts near the CLC C-termini (yellow and orange, numbers indicate number of residues) in neurons (nCLCa and nCLCb) and during heart development (s2CLCa). Expression of s1CLCa is not clarified. For both alignments, amino acid numbers are indicated at the bottom and the total number of amino acids for each subunit shown on the right.

occurs at ER–PM contacts, showed a role for CME protein AtEH (Eps15, Pan1 homologue) and potentially clathrin and AP2 in organising actin at these structures [22]. Comparative studies are therefore revealing differences in how clathrin-actin interactions are orchestrated in different species. Some of these likely represent convergent evolution to co-opt actin for clathrin pathways, but it is also possible that highly differentiated animal cells may use related diverse pathways to handle tissue-specific needs.

Clathrin interaction with the microtubule (MT) cytoskeleton also displays phylogenetic variation. Although the basic principles of mitotic regulation are highly conserved, there are many species-specific variations in this process, which include nontrafficking roles for clathrin in MT binding during cytokinesis (Figure 1). Interaction of the CHC17 TD with the MT-binding complex TACC3/ch-TOG stabilises kinetochore MTs and is induced by mitotically active Aurora A kinase phosphorylation [23]. CHC17 TD also binds GTSE1 at conventional adaptor-binding sites, and during cytokinesis this interaction stabilises astral microtubules through GTSE1 inhibition of the MT depolymerase MCAK [24]. Astral MTs are also stabilised by GAK, a clathrin-binding protein that participates in uncoating CCVs. Clathrin or GAK depletion affect spindle positioning [25]. These newly-defined roles for clathrin in astral MT stability may contribute to the fragmentation of centrosomes previously observed upon clathrin downregulation [26]. Phylogenetic analysis shows that clathrin interactions with GTSE1 arose in the vertebrate lineage, whereas TACC3 interactions are apparently more ancient, but it is not known whether these interactions are present in yeast or outside opisthokonts [24].

Lattice formation, uncoating and adaptor roles: quality control for cargo capture

Clathrin coats were amongst the first membrane traffic components to be visualised by cryo-EM and X-ray crystallography. Recent models of clathrin baskets formed from purified clathrin plus a fragment of the β-subunit of AP2 have now achieved local 4-5 Å resolution, a level at which amino acid contacts between adjacent CHC legs can be identified [27]. This model opens up the prospect of understanding regulation of lattice assembly and disassembly in molecular terms, and enables predictions about the effects of CHC17 mutations associated with neurological disease [27] and the variants of CHC22 associated with nutrition [5].

Extended hexagonal clathrin lattices are observed in many cell types, and these plaques play important biological roles as signalling platforms [28] and mechanical biosensors (see [29], this issue). They are also implicated in adhesion [30] and contribute to mitosis by anchoring daughter cells [31]. The existence of clathrin plaques, the inherent 'pucker' of the clathrin triskelion, and the mathematical principle that twelve pentagons are required to induce curvature for a complete, closed clathrin coat, have led to long-standing discussion in the field regarding the relationship between flat and curved clathrin structures. Some clarity in this discussion is now emerging (see the review by Sochacki and Taraska [32]). As might be expected, there appear to be multiple routes to curvature, some involving growth of structures with constant curvature from the outset and others involving flat lattice rearrangement. Alternative splicing of CHC17 in skeletal muscle, introducing seven residues near the trimerisation domain, promotes flat lattice formation and loss of this splicing event is associated with myotonic dystrophy in humans [33] and muscle hypertrophy in mice [34]. In tissues without this splicing event, flat lattices may be actively maintained by accessory proteins, cargo interaction, membrane tension and cytoskeletal interactions.

Lattice growth with constant curvature would be predicted given the amount of energy required to break bonds between multiple CHCs to introduce curvature through flat lattice rearrangement. However, dynamic triskelion exchange within the lattice may be promoted by the early functioning of uncoating proteins Hsc70 [35] and auxilin [13] during CCV formation, though visual detection of auxilin is only achieved at the final stages [36]. Assessing the involvement of AP2 adaptors in the flat to curved transition, it was observed by single-molecule fluorescence that the AP2 to clathrin ratio decreases as curvature occurs [37]. Two new models, based on cryo-EM structures of both natural and reconstituted clathrin coats, reveal that AP2 is primarily associated with hexagonal faces of closed clathrin structures, suggesting a role for AP2 interactions in enabling flat to curved lattice transition (posted on bioRxiv [38,39]). Recent advances define regulated steps for adaptor participation in CCV formation. Assembly of AP2 (and possibly also AP1) relies on the chaperone AAGAB, which binds to the α- and γ-subunits in their monomeric form [40]. In addition, regulation of AP2 by phosphorylation plays a key role in progression to a CCV. When cargo binds the open conformation of AP2, it exposes a phosphorylation site that, when modified, recruits NECAP which then recruits SNX9, a late stage promoter of budding. Although this modification is not essential to CCV completion in mammalian cells, inhibition slows the process [41]. In parallel observations, it was reported that NIMA-related kinases are essential for CME in *C. elegans* and that loss of NECAP binding prolongs AP2 residence in coated pits [42,43].

Conversion of phosphoinositide head groups plays a key role in coat nucleation, adaptor and accessory protein recruitment and uncoating (reviewed recently [44–46]) and summarised here for mammalian CCVs (Figure 2). These multicomponent interactions combine to generate forces required for membrane bending during CME (see [45], this issue). A relevant model from Maib et al. [13] suggests that clustering of GPCR cargo at the outset of coated pit formation increases local membrane rigidity and that areas of high rigidity require CLCb phosphorylation to drive uncoating-mediated clathrin turnover to produce curvature. Thus the presence of particular cargoes or high membrane tension, which oppose membrane deformation, could promote shallow clathrin structures that require CLCs and/or recruitment of GAK/auxilin to increase curvature through coat remodelling. In contrast, under less mechanically demanding circumstances CLCs are not required for CME, and some cargoes may be sequestered in CCVs that form by constant curvature growth. CLCs also influence lattice flexibility and AP2 adaptors add stiffness to the coat [47], defining additional roles for coat components in influencing the efficacy of coat contribution to bending membrane for cargo sequestration.

Cargo transport pathways: roles in development and disease

The balance of cargo sequestration into CCVs can affect cell fate, linking clathrin-mediated pathways to disease and tissue differentiation. Two studies from the Hurley laboratory [48,49] demonstrate how targeting cargo-adaptor interactions has been exploited by HIV for immune response invasion. It has been known for some time that the HIV nef protein prevents surface expression of class I histocompatibility molecules (MHCI) and the host restriction factor tetherin, affecting their respective ability to stimulate an immune response against HIV and block virion progeny release. Morris et al. [48] show that HIV nef binds directly to multiple cargo-binding sites on the AP1 adaptor and to the cargoes themselves. These interactions alter the conformation of AP1, resulting in interference in host trafficking pathways. The relatively weak interaction between nef and tetherin reduces AP1's ability to recruit clathrin, leading to retention of tetherin at the trans-Golgi network, whereas the strong nef-MHCI interaction promotes AP1-clathrin binding that subsequently targets MHCI for lysosomal degradation [48]. Supporting this model, Tavares et al. [50] reported that HIV nef interacts with both γ1 and γ2 forms of AP1 to deplete MHCI from the PM.

Further examples of defective cargo-specific CME in disease are emerging, continuing on this classical theme pioneered by the discovery of LDL receptor mutations in familial hypercholesterolaemia that affect its CME (recognised in the 1985 Nobel Prize to Brown and Goldstein [51]). Through systematic analysis of a number of disease-associated mutations, Meyer et al. [52] found that the introduction of LL motifs into disordered regions of the cytosolic domains of membrane proteins is surprisingly common, naming them 'dileucineopathies'. For three of these diseases, they show that the mutants have enhanced clathrin association, suggesting they become CCV cargo and are hence mislocalised. For the mutation that causes GLUT1 deficiency syndrome, they were able to reverse excessive CME of GLUT1 by AP2 knockdown. Also relevant to glucose transport, mutations in ankyrin B are associated with obesity and ankyrin B depletion was found to reduce CME of GLUT4 in murine adipocytes, leading to the discovery that ankyrin B acts as a specific adaptor between GLUT4 and CHC17 during GLUT4 uptake from the PM following insulin stimulated release [53]. Further demonstrating the disease relevance of specific adaptor-cargo interactions, it was observed that pharmacologically diverse antidepressants (e.g. fluoxetine) decrease the interaction between the BDNF receptor TRKB and AP2, thereby increasing surface levels of TRKB available for activation by BDNF, which is thought to be important for neuronal plasticity to counter mood disorders [54].

Cellular balancing of clathrin-mediated cargo transport pathways is emerging as an important influence on normal differentiation and development, as well as in oncogenesis. Revisiting the role of clathrin in development of epithelial cell polarity, two groups have independently established that clathrin-mediated pathways involving AP1 play a role in endosomal sorting of both newly synthesised and recycling cargo to the apical membrane, in addition to being required for basolateral sorting, as previously shown (Figure 1) [55–57]. The relative roles of the epithelia-specific μ1B subunit and the ubiquitous μ1A subunit in basolateral and apical targeting seem to vary with the cargo type [55,56]. Although clear that AP1-mediated sorting actively populates both polarity domains, loss of AP1 (surprisingly) did not affect epithelial tight junction formation in tissue culture models (MDCK cells) [56]. In contrast, stem cell differentiation was shown to be highly sensitive to the balance of clathrin-mediated pathways. Narayana et al. [58] showed that clathrin plays a key role in the fate of murine embryonic stem cells, as depletion of CHC17 reduces PM levels of E-cadherin (pro-pluripotency) while increasing levels of TGFβR1 (pro-differentiation), upsetting the balance of their antagonistic signalling. In another demonstration of the importance of CME in maintaining signalling balance via cargo control, Caballero-Diaz et al. [59] suggest that disruption of the balance between pro- and anti-apoptotic signals from TGFβR and EGFR signalling pathways in liver cancer may be potentiated by increased CHC17 levels. This observation complements the concept that 'adaptive endocytosis' upsets signalling receptor balance and contributes to oncogenesis [60]. In a related mechanism of oncogenesis, loss of PTEN in the SUM149 cell line derived from an aggressive breast cancer increases short-lived clathrin-coated pits that are capable of EGFR signalling and reduces formation of long-lived coated pits capable of receptor down-regulation [61].

Outstanding questions in the field: the devil (disease) is in the details

Linking clathrin-associated defects to vertebrate phenotypes remains a challenge. Clathrin-mediated pathways are so critical for cellular function, there appear to be many compensatory mechanisms, should one player in these pathways be defective. Phenotypes for clathrin-associated defects have, so far, been detected mainly in specialised cells with highly differentiated membrane traffic such as neurons, muscle cells, lymphocytes and epithelia. Mutation in the AAGAB protein affecting AP2 assembly manifests as the human skin disease punctate palmoplantar keratoderma type 1 [40], and AP1 mutations are associated with congenital enteropathy [62]. Deficiency of the CME-nucleating protein FCHO1 causes a T-lymphocyte defect [63], and loss of the assembly regulator AP180 affects murine cochlear inner hair cells [64] in addition to neuronal function, and the latter is seen for many other adaptor and assembly factor defects [65]. Interestingly, degenerative diseases, in which protein aggregates (Tau or α-synuclein) sequester the Hsc70 clathrin uncoating protein, have phenotypes resulting from disrupted CME that can be rescued by excess Hsc70 [66,67]. Likewise, dysregulated phosphorylation of the uncoating cofactor auxilin by mutant LRRK2 disrupts CME in dopaminergic neurons of patients with Parkinson's disease [68].

Despite identification of neuronal and lymphocyte phenotypes for CLCa or CLCb depletion [15,69], understanding the range of functions of the CLCs is not yet complete. Recent studies reinforce a role for CLCs in connecting CHC17 clathrin to the actin cytoskeleton [16] and underscore the fact that CLCs are needed for effective internalisation of some but not all CCV cargoes [13,69]. Distinct activities for CLCa and CLCb are also emerging [14,16], but evidence for these is still limited and does not yet fully explain the strong evolutionary conservation of the differences between the two CLCs nor the conservation of their variation by alternative splicing (Figure 3). From our studies [15], it appears that a combination of the neuronal splice variants nCLCa and nCLCb is important for CHC17 function in regeneration of synaptic vesicle (SV) pools after nerve stimulation. In mice, loss of nCLCb leads to dysregulation of

this pathway, producing more SVs, and loss of nCLCa leads to reduced SV production. These findings correlate with measured biophysical properties of CHC17 with single and mixed nCLCs showing that mixed nCLC-clathrin is more efficient at budding, and support an emerging concept of CLCb as a regulatory CLC, whereas CLCa sustains basic CHC17 functions. Notably, alternative splicing of both CLCs [15] and CHC17 [33] introduces sequences at the triskelion vertex that influence lattice morphology, but further elucidation of the roles of alternative splicing of CLCs is needed.

Outlook

Forty-five years after clathrin's discovery, elucidating its function in the context of differentiated tissues is the challenge of the future. Advances in single molecule dynamics and cryo-EM have enabled substantial recent progress in understanding the molecular details of clathrin-mediated membrane traffic, which in turn are paving the way to defining clathrin's influence on cell and organismal physiology. However, details are still missing in several areas of note, such as the molecular regulation of CCVs at internal membranes formed by AP1, GGA adaptors and CHC22 clathrin and roles of nonconventional cargo adaptors at the PM. Generating further versatile tools to effect specific and acute disruption of clathrin-mediated function in differentiated cell models, perhaps exploiting transient expression of dominant-negative fragments [70] or auxin-based degron approaches [71] may prove crucial to understanding the multiple and diverse functions of the CLC subunits, as well as facilitating the link between clathrin biochemistry and physiology.

Author contributions

Kit Briant: Conceptualisation, writing – original draft, writing – review and editing, visualisation

Lisa Redlingshöfer: Conceptualisation, writing – original draft, writing – review and editing, visualisation

Frances M. Brodsky: Conceptualisation, writing – original draft, writing – review and editing, project administration, funding acquisition

Conflict of interest statement

Nothing declared.

Acknowledgements

The authors thank Alexander Bird and George Bates for helpful discussion. The authors were supported by Wellcome Trust Investigator Award 107858/Z/15/Z and MRC-UKRI grant MR/S008144/1 while writing this review.

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- •• of outstanding interest

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